Shelf life extension of italian mozzarella by use of calcium lactate buffered brine

M. Faccia*, G. Gambacorta, G. Natrella, F. Caponio

Department of Soil, Plant and Food Sciences

University of Bari, Via Amendola 165/A, 70126 Bari (Italy)

ABSTRACT

Italian traditional mozzarella is a high moisture table cheese that is sold packaged in water for preserving freshness. Despite of the high foreign demand, high perishability limits export. For extending shelf life, the dairy industries have long been engaged in controlling the growth of spoilage microflora, which is the main responsible of alteration. The present paper describes the results of a study that aimed to assess if using acidified brine instead of water, the growth of these microorganisms could be delayed. A suitable brine was first developed, based on calcium lactate and lactic acid, that did not impair the sensory characteristics of the cheese. Then, the shelf-life study was carried out, and the results revealed a significant delay of the growth of total mesophilic bacteria, *Pseudomonas* spp. and *Enterobacteriaceae*. The sensory characteristics of the cheese remained within the acceptability limits until 21 days and, compared with the sample stored in water, the shelf life was extended of more than 50 % Very interestingly, the experimental brine also prevented the occurrence of the blue discoloration defect, known to be caused by *Pseudomonas fluorescens*. Even though further investigation is needed, the results obtained can open new marketing perspectives for producers of traditional mozzarella.

Keywords: mozzarella; spoilage microorganisms; shelf life; preserving brine.

* Corresponding author.

E mail address: michele.faccia@uniba.it

1. Introduction

Mozzarella is a pasta filata cheese born in Italy several centuries ago that has become one of the most consumed dairy products worldwide (Francolino et al., 2010). It is manufactured from water buffalo or cow milk, but the latter is much more produced because of the wide availability of bovine milk. Two types of bovine mozzarella exist: low moisture, mostly used for dressing pizza, and high moisture, used as a table cheese (McMahon, Oberg, & McManus, 1993). Traditional Italian mozzarella is a high moisture type (60-65% water content) with very soft body and milky flavour. Its export has strongly increased during the last decades, and the main reasons for success are ease of use, delicate taste and freshness. In order to preserve these characteristics during marketing it is sold packaged in water. Unfortunately, this accelerates perishability: shelf life commonly ranges from 1 to 2 weeks, depending on the moisture level, manufacturing procedures and storage conditions (Gammariello et al., 2010; Ricciardi et al., 2015). In general, the cause of mozzarella deterioration is excessive microbial growth, but also the mass transfer (i.e. migration of salt and water) between the product and the preserving liquid plays a role. Microbial growth is responsible of acidification, off-flavours and chromatic alterations, whereas mass transfer causes loss of taste and surface disruption due to lowering of the colloidal calcium bound to the protein matrix (Rondinini & Garzaroli, 1990; Kindstedt et al. 1996; Joshi et al., 2003; Faccia et al, 2012; Losito et al., 2014). Since perishability has high economic impact on the distribution logistic, the Italian dairy industry is strongly interested to get shelf-life extension. It is well know that any strategies for delaying microbial alteration of mozzarella must be primary addressed to inhibit the growth of psychrotrophic bacteria, among which *Pseudomonas* spp. plays a primary role. The involvement of *Pseudomonadaceae* in mozzarella spoilage has become strongly evident after a number cases of blue discoloration occurred in Europe ascribed to *Pseudomonas fluorescens* (Cenci-Goga, 2014; Chiesa et al., 2014; del Olmo, Calzada & Nunez, 2018). This bacteria species grows easily in high moisture foods that present slightly acid or neutral pH (Nychas et al., 2008; Remenant et al., 2015; Stellato et at., 2017). For delaying their growth maintenance of the cold chain during marketing has to be fully guaranteed, besides improving the general hygienic conditions of cheesemaking. Recently, several researches have dealt with an innovative strategy for prolonging shelf-life of Italian mozzarella, based on the addition of antimicrobial compounds (Gammariello et al., 2008; Laurienzo et al., 2008; Sinigaglia et al., 2008; Conte et al., 2009; Incoronato et al., 2011; Lucera et al., 2014; Gorrasi et al., 2016). Despite the interesting results obtained, such a strategy cannot be applied in the industrial practice due to high costs, impact on the sensory characteristics, or incompatibility with the EU legislation.

In previous papers we have dealt with the development of brines for traditional mozzarella preservation, based only on “natural”, low cost and legal substances. The most relevant results obtained regarded the prolonged maintenance of the organoleptic properties, whereas little effect was observed under the microbiological point of view (Faccia et al., 2011; Faccia et al., 2013). Unfortunately, the effect of brine pH on microbial growth was not fully explored. Acidification is a traditional method for long-term food storage, nevertheless, for all we know, no investigation has been carried out on the preservation of bovine mozzarella in acidified brine. The present paper reports the results of a new study that had the objective to verify if keeping pH of the brine below 5.0 contributes to extension of shelf-life of this cheese.

2. Materials And Methods

2.1 Mozzarella samples and development of the preserving brine

The cheeses used in the experimentation were mozzarella knots taken from an industrial dairy, manufactured from pasteurized milk by direct acidification (lactic acid). They had been mechanically stretched and hand-knotted, and weighed about 70 g each. The cheeses were highly standardized as to the production technology and chemical composition. Nevertheless, in order to minimize the risk of day-to-day differences, in each experimental trial samples deriving from a same batch of milk were used. The cheeses were rapidly transported under refrigeration to the Department laboratory where they were immediately immersed in 200 mL chilled brine and mechanically packaged in plastic trays sealed by plastic film (2 pieces for tray). For developing the preserving brine, 20 different low pH solutions in total were prepared and tested, complessively. All solutions were prepared by using food grade ingredients complying the EU legislation for dairy products: calcium lactate (CaL), sodium chloride, calcium chloride, citric (CA) and lactic acid (LA), all purchased by Farmalabor Srl, Canosa, Italy. The brines differed among them as to pH, concentration and/or association of the compounds.

2.2 Experimental design

The experimentation was performed in two steps: development of the brine and shelf-life study. The preparative phase had the objective of preparing a suitable brine, able to maintain low pH over time without changing the organoleptic characteristics of the cheese. To this aim, a panel composed of 3 experts working at the Department and belonging to the Italian National Association of Cheese Tasters evaluated texture (integrity of cheese surface), aroma and taste of the cheeses after 5 days refrigerated storage. They used a mozzarella sample packaged in water for comparison, and judged the sensory parameters in a very simple way: better, the same or worse than the control. Two preparative trials were needed (2 replicates each), during which the composition of the brines were progressively adjusted. When a suitable result was obtained, the final brine was prepared and the shelf-life study was carried out, as follows: two series of sealed trays containing mozzarella immersed in brine or water (experimental and control) were stored at 8 ± 1°C for simulating the real conditions of marketing; one tray for each treatment was taken at 1, 2 and 3 weeks and aseptically opened; the cheeses were immediately submitted to chemical, microbiological and sensory analyses. The study was repeated 3 times (2 replicates each time).

2.3. Analyses

Cheese moisture was determined according to the IDF method (1986), pH was measured by means of a penetration pH meter Dualpore (Hamilton, Reno, NV). Primary proteolysis was investigated by polyacrylamide gel electrophoresis in the presence of urea (urea-PAGE) as reported by Andrews (1983). The cheese samples were dissolved in 9 M urea and loaded onto the electrophoretic system. The gel was stained with Blue Silver stain (Candiano et al., 2004) and subjected to image analysis and densitometry by using Quantity One software (BioRad, Hercules, CA). The microbiological analyses focused on counting the spoilage microorganisms and were performed according to the IDF standard protocol (2001). The media (Oxoid, Milan, Italy) and conditions used for the enumerations were as follows: Plate Count Agar incubated at 30 °C for 48 h for total mesophilic bacteria; *Pseudomonas* Agar Base with added CFC selective supplement, incubated at 25 °C for 48 h, for *Pseudomonas* spp.; Violet Red Bile Glucose Agar incubated at 37°C for 18–24 h for *Enterobacteriaceae*; Yeast Extract Dextrose Chloramphenicol Agar at 30°C for 48 h for yeasts and molds. The sensory analysis was performed by a panel composed of 8 trained assessors selected following international standards (ISO, 1993) that carried out a Quantitative Descriptive Analysis (QDA). The panel had three open training sessions on mozzarella samples of different age: fresh, half and end of shelf-life. During training each panelist indicated a series of sensory descriptors that were quantified on a 5-point scale (from 0 to 4) and selected based on weight percentage (frequency of citations × perceived intensity); only attributes with a weight percentage greater than 30% were considered (Trani et al., 2016). At the end of the training sessions the acceptability limit (AL) for each of the descriptors was established. The same 5-points scale was used for QDA, where 4 was the best score, except for the sour attribute (best score = 0).

2.4. Statistical analysis

All data were processed using Statistica 7.1 for Windows (StatSoft Inc., Tulsa, OK). Considering that the samples of the three shelf-life trials started from different microbiological conditions, each trial was elaborated separately. Least significant different analysis was used to determine differences between control and experimental sample. For sensory analysis the means of the scores were calculated.

3. Results And Discussion

3.1. Development of the preserving brine

The main problem was to counter the increase of pH of the brines over time, caused by the mass transfer between liquid and product as a consequence of diffusive gradients (Kindstedt, 1995; Kindstedt et al., 1996). From the data of Table 1 it can be observed that only in a few cases pH remained under the target value (below 5.0). A second problem was the impairment of the sensory characteristics of the cheese, mainly as to texture. Preserving the integrity of the mozzarella surface is a primary goal, since it represents a barrier to the mass transfer, besides being an important and attractive feature for the consumers. All brines containing citric acid caused sloughing and solubilisation of the surface, probably because it sequestered the calcium bound to the casein network (Caric, Gantar, & Kalab, 1985). This hypothesis was confirmed by the observation that surface deterioration was less pronounced when calcium lactate was present at 1.0% concentration: the abundant presence of ionic calcium counterbalanced the sequestering action of CA. Better results were obtained when pH was lowered with lactic acid, but only the brine containing 1.0% CaL at pH 4 was judged as useful for our purposes. In fact, only the mozzarella sample kept in this liquid had the same texture and odour than control, and also the taste was good, even though slightly less salty. The experimentation continued using this brine with a few adjustments. First of all, considering that texture had proven to be firmer than control, pH was lowered to 3.7 in order to better counterbalance raising of pH over time. In addition, a second brine was prepared including sodium chloride at low level (0.25 %), with the expectation of improving taste. As shown in Table n. 2, both brines gave good results: after 5 days pH remained well below 5.0, probably in connection with the buffering properties of the pair calcium lactate/lactic acid. As expected, the taste was improved in the presence of NaCl, but texture was slightly impaired; on the contrary, when salt was not included the cheese surface was firmer and taste was close to the control. As a final decision, the brine without salt was chosen for performing the shelf-life study.

3.2. Shelf-life study

Fig. 1 shows the pH evolution of the preserving liquids and cheeses, and of the cheese moisture, over time. Due to the mass transfer, after 21 days pH of the liquid of the control sample dropped from about 7.0 to about 6.3, whereas it increased from 3.7 to just under 5 in the experimental brine. As expected, the values in the cheeses had opposite trends: pH increased from 5.9 to about 6.1 in the control, and decreased from 5.9 to about 5.4 in the experimental. Cheese moisture reached the same final value in the two samples, passing from about 64 % to about 58 %, but it evolved in a different way. The control cheese absorbed water during the first 7 days, in fact moisture content exceeded 70%, then decreased; differently, the experimental cheese did not absorbed water, and moisture decreased almost regularly over time. This result suggests a commercial consideration: if mozzarella is kept in water and sold after a few days, the weight gain determines an economic benefit for the producers.

As regard the microbiological results, the experimental brine proved to have some inhibitory effects (Table 3). In fact, even though the 3 shelf-life trials started from different values of the microbial counts, the growth of most microbial groups was delayed in all cases. The most relevant effect was exerted against *Pseudonomas* spp., but also TV and *Enterobacteriaceae* counts were lower in almost all the experimental samples. Yeasts and moulds were not influenced by the acidic conditions of the liquid, whereas the *Pseudomonas* growth was delayed of 1 to 2 logarithmic units. A similar inhibiting effect was reported by Quintieri et al., (2012) and by Gammariello et al. (2008) when pepsin-digested lactoferrin or vegetable extracts, respectively, were added to the preserving liquid. However, none of the two methods can be adopted by the dairies, due to unavailability of the active ingredients on the market or deep impact on flavour. The antimicrobial effect of the brine against *Pseudomonas* was confirmed by the fact that, differently from the experimental sample, the governing liquid of the control sample always became fluorescent (Fig. 2). Moreover, in two out of three trials after 21 days the defect of blue discoloration occurred in control cheeses. In our opinion the results obtained are really encouraging, also in consideration of the fact that the preserving effect of low pH was only partially exploited. In fact, pH of the brine remained below 4.5 for only one half of the storage period, and that of the cheese only reached a minimum of 5.4. It should be very interesting to succeed in developing a more highly buffered brine, able to keep pH at least under 4.5 for longer time. The results of the proteolysis study are shown in Fig. 3. It must be stated that, differently from the low moisture type, proteolysis in Italian traditional mozzarella is unwanted, since it accelerates texture weakening (Faccia et al., 2014). In this view, the electrophoretic analysis indicated that the brine was absolutely compatible, since it did not affect casein degradation. As it can be noted, the casein pattern of the experimental sample was completely identical to that of control, and was characterized by slow hydrolysis of both αs1- and β casein until 14 days. At 21 days storage proteolysis markedly increased, leading to degradation of about 50 % of both casein fractions.

As to the organoleptic characteristics, Figure n. 4 shows the results of the sensory analysis. On the whole, the assessors proposed 21 attributes for describing the sensory characteristics of the cheeses, most of which corresponded to those proposed by Pagliarini, Monteleone & Wakeling (1997). However, only 10 were selected on the base of weight percentage: 5 concerned flavour, 4 texture and 1 the brine appearance. Detailed description of the attributes is given in Table 4. As it can be seen, the control and experimental samples were rather similar after 7 days storage, even though the latter showed a clearer brine, was more succulent and had higher flavour intensity. It should be noted that this latter results seems to conflict with the results obtained during the preparative phase, where flavour had been judged as slightly more intense in the control sample. A possible explanation is that the evaluation had been done after only 5 days storage, and the mass transfer phenomenon had not yet determined a relevant taste depletion. Nevertheless, both samples were widely within the acceptability limits for all parameters. After 2 weeks storage the quality of control mozzarella started to decline: several attributes approached the limits of acceptability, such as surface integrity, consistency, and odour and flavour intensities. Differently, the experimental sample was almost unchanged at this time, except slight decrease of flavour. After 21 days the control was no longer acceptable, both as to texture (mainly due to sloughing of the surface) and taste (very sour), whereas the experimental sample still was within the limits of acceptability. On the whole, all mozzarella stored in the experimental brine reached 21 days shelf-life, whereas those stored in conventional conditions barely reached 14 days. This results indicated a shelf-life extension of at least 7 days, much more than reported in the literature in the case of application of chitosan, active coating and/or modified atmosphere packaging (Conte at al., 2009; Del Nobile et al., 2009).

4. Conclusions

The results of the present study suggests that the acidification of the preserving liquid can help to extend shelf-life of traditional mozzarella, and opens new perspectives for enlarging the market. Such an approach, differently from other strategies recently proposed for longer preserving this cheese, can be easily applied in the dairy practice. Even though experimentation is in progress for further lowering pH, testing at industrial level has recently started.

Acknowledgements

The authors would like to thank Capurso Azienda Casearia dairy of Gioia del Colle (BA) for supplying mozzarella samples. Prof. M. Faccia was the responsible of the research, developed the brine and wrote the manuscript; prof. G. Gambacorta coordinated the laboratory analyses; dott. G.

Natrella performed the analyses and statistically elaborated the data; prof. F. Caponio collaborated to the discussion and gave suggestions for manuscript draft.

References

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. *Journal of Dairy Research*, 50, 45–55.

Candiano, G., Bruschi, M., Musante, L., Santucci, L., Ghiggeri, G. M., Carnemolla, B., Orecchia, P., Zardi, L., & Righetti, P. G. (2004). Blue silver: A very sensitive colloidal Coomassie G-250 staining for proteome analysis. *Electrophoresis*, 25, 1327–1333.

Cantoni, C., Marchisio, E., & Galli, M. (2000). Blu-green coloration of "mozzarella" cheese. *Industrie Alimentari*, 39, 586-588.

Caric, M., Gantar, M., & Kalab, M. (1985). Effects of Emulsifying Agents on the Microstructure and Other Characteristics of Process Cheese - A Review. *Food Structure*, 4, 297-312.

Cenci-Goga, B.T., Karama, M., Sechi, P., Iulietto, M.F., Novelli, S., & Mattei, S. (2014). Evolution under different storage conditions of anomalous blue coloration of Mozzarella cheese intentionally contaminated with a pigment-producing strain of*Pseudomonas fluorescens. Journal of Dairy Science,* 97, 6708-6718.

Chiesa, F., Lomonaco,S., Nucera, D., Garoglio, D., Dalmasso, A., Civera, T. (2014). Distribution of *Pseudomonas* species in a dairy plant affected by occasional blue discoloration. *Italian Journal of Food Safety*, 3, 245-248

Conte, A., Gammariello, D., Di Giulio, S., Attanasio, M., & Del Nobile, M.A. (2009). Active coating and modified-atmosphere packaging to extend the shelf-life of Fior di latte cheese. *Journal of Dairy Science,* 92, 887–894.

Conte, A., Longano, D., Costa, C., Ditaranto, N., Ancona, A., Cioffi, N., Scrocco, C., Sabbatini, L., Contò, F., & Del Nobile, M.A. (2013). A novel preservation technique applied to Fiordilatte cheese. *Innovative Food Science and Emerging Technologies*, 19, 158-165

Conte, A., Scrocco, C., Sinigaglia, M., & Del Nobile, M.A. (2013). Innovative active packaging system sto prolong shelf-life of mozzarella cheese. *Journal of Dairy Science*, 90, 2126-2131.

Del Nobile, M.A., Gammariello, D., Conte, A., & Attanasio, M. (2009). A combination of chitosan, coating and modified atmosphere packaging for prolonging Fior di latte cheese shelf life. *Carbohydrate Polymers*, 78, 151-156.

del Olmo, A., Calzada, J., & Nunez. M. (2018).The blue discoloration of fresh cheeses: A worldwide defect associated to specific contamination by Pseudomonas fluorescens. *Food Control*, 86, 359-366.

Faccia, M., Trani, A., Loizzo, P., Gambacorta, G., & Di Luccia, A. (2011). Shelf-life della mozzarella vaccina conservata in soluzioni saline: primi risultati. *Scienza e Tecnica Lattiero-Casearia*, 62, 167-171.

Faccia, M., Mastromatteo M., Conte, A., & Del Nobile, M.A. (2012). Influence of the different sodium chloride concentrations on microbiological and physico-chemical characteristics of mozzarella cheese. *Journal of Dairy Research*, 79, 390-396.

Faccia, M., Angiolillo, L.,Mastromatteo M., Conte, A., & Del Nobile, M.A.(2013) The effect of incorporating calcium lactate in the saline solution on improving the shelf life of Fiordilatte cheese. *International Journal of Dairy Technology*, 66,373-381.

Faccia, M., Trani, A., Loizzo, P., Gagliardi, R., La Gatta, B, & Di Luccia, A. (2014). Detection of αs1-I casein in mozzarella Fiordilatte: a possible tool to reveal the use of stored curd in cheesemaking. *Food Control*, 42, 101-108.

Francolino S., Locci F., Ghiglietti R., Iezzi R., Mucchetti G. (2010). Use of milk protein concentrate to standardize milk composition in Italian citric Mozzarella cheese making. *LWT – Food Science Technology*, 43, 310-314.

Gammariello, D., Di Giulio, S., Conte, A., & Del Nobile, M.A. (2008). Effects of natural compounds on microbial safety and sensory quality of Fior di latte cheese, a typical Italian cheese. *Journal of Dairy Science*, 91, 4138–4146

Gammariello, D., Conte, A., Attanasio, M., & Alessandro Del Nobile, M. (2010). Study on the combined effects of essential oils on microbiological quality of Fior di Latte cheese. *Journal of Dairy Research,* 77, 144-150.

Gorrasi, G., Bugatti, V., Tammaro, L., Vertuccio, L., Vigliotta, G., & Vittoria, V. (2016). Active coating for storage of Mozzarella cheese packaged under thermal abuse. *Food Control*, 64, 10-16.

Incoronato, A.L., Conte, A., Buonocore, G.G., & Del Nobile, M.A. (2011). Agar hydrogel with silver nanoparticles to prolong the shelf life of Fior di Latte cheese. *Journal of Dairy Science*, 94, 1697-1704.

International Dairy Federation (1986). Cheese and Processed Cheese Products. Determination of dry matter. *FIL-IDF Standard No. 4*, Brussels, Belgium.

International Dairy Federation (2001). Milk and milk products -- General guidance for the preparation of test samples, initial suspensions and decimal dilutions for microbiological examination. *FIL-IDF Standard No. 122*, Brussels, Belgium.

International Standard Organization (1993). Sensory analysis: General guidance for the selection, training and monitoring of assessors. Part 1: Selected assessors. *ISO 8586-1*, Geneva, Switzerland.

Joshi, N.S., Muthukumarappan, K., & Dave, R.I. (2003). Understanding the role of calcium in functionality of part skim mozzarella cheese. *Journal of Dairy Science*, 86, 1918-1926.

Kindstedt, P.S. (1995). Factors affecting the functional characteristics of unmelted and melted mozzarella cheese. *Advances in Experimental Medicine and Biology*, 367, 27-41.

Kindstedt, P.S., Larose, K.L., Gilmore, J.A., & Davis L. (1996). Distribution of salt and moisture in mozzarella cheese with soft surface defect. *Journal of Dairy Science*, 79, 2278-2283.

Laurienzo, P., Malinconico, M., Mazzarella, G., Petitto, F., Piciocchi, N., Stefanile, R., & Volpe. M.G. (2008). Water Buffalo Mozzarella Cheese Stored in Polysaccharide-Based Gels: Correlation Between Prolongation of the Shelf-Life and Physicochemical Parameters. *Journal of Dairy Science*, 91, 1317–1324.

Losito, F., Arienzo, A., Bottini, G., Priolisi, F.R., Mari, A., & Antonini, G. (2014). Microbiological safety and quality of Mozzarella cheese assessed by the microbiological survey method. *Journal of Dairy Science*, 97, 46-55.

Lucera, A., Mastromatteo, M., Conte, A., Zambrini, A.V., Faccia, M., & Del Nobile, M.A (2014). Effect of active coating on microbiological and sensory properties of fresh mozzarella cheese. *Food Packaging and Shelf-Life*, 1, 25-29.

McMahon, D.J., Oberg, C.J., & McManus, W. (1993). Functionality of Mozzarella cheese. *Australian Journal of Dairy Technology,* 48, 99-104.

Nychas, G.J.E, Skandamis, P.N., Tassou, C.C., & Koutsoumanis, K.P. (2008). Meat spoilage during distribution. *Meat Science*, 78, 77-89.

Pagliarini, E., Monteleone, E. & Wakeling, I. (1997). Sensory profile description of mozzarella cheese and its relationship with consumer preference. *Journal of Sensory Studies*, 12, 285-301.

Quintieri, L., Caputo, L:, Monaci, L., Deserio, D., Morea, M., & Baruzzi, F. (2012). Antimicrobial efficacy of pepsin-digested bovine lactoferrin on spoilage bacteria contaminating traditional Mozzarella cheese. *Food Microbiology*, 31, 64-71.

Remenant, B., Jaffres, E., Dousset, X., Pilet, M.F., & Zagorec, M. (2015). Bacterial spoilers of food: behavior, fitness and functional properties. *Food Microbiology*, 45, 45-53.

Ricciardi, A., Guidone, A., Zotta, T., Matera, A., Claps, S., & Parente, E. (2015). Evolution of microbial counts and chemical and physico-chemical parameters in high-moisture Mozzarella cheese during refrigerated storage. *LWT – Food Science Technology*, 63, 821-827.

Rondinini, G. & Garzaroli, C. (1990). Microbiological aspects and spoilage of Mozzarella cheese processed via citric acid acidification. *Industrie Alimentari*, 29, 329-334.

Sinigaglia, M., Bevilacqua, A., Corbo, M. R., Pati, S., & Del Nobile, M. A. (2008). Use of active compounds for prolonging the shelf life of mozzarella cheese. *International Dairy Journal*, 18, 624-630.

Stellato, G., Utter, D.R., Voorhis, A., De Angelis, M., Murat Eren, A., & Ercolini, D. (2017). A few Pseudomonas oligotypes dominate in the meat and dairy processing environment. *Frontiers in Microbiology*, 8, 264.

Trani, A., G. Gambacorta, P. Loizzo, A. Cassone, & M. Faccia. 2016. Short communication: Chemical and sensory characteristics of Canestrato di Moliterno cheese manufactured in spring. *Journal of Dairy Science*, 99, 6080-6085

FIGURE CAPTIONS

Fig. 1. Evolution of pH in the preserving liquids and cheeses, and of the cheese moisture, over time.

Fig. 2. Occurrence of fluorescence in the preserving liquid of the control sample (C).

Fig. 3. UREA-PAGE patterns of control (C) and experimental mozzarella samples (A) at 0, 7, 14 and 21 days refrigerated storage.

Table 1. First preparative trial: evaluation after 5 days storage of the cheeses at 8 ± 1°C. SI=surface integrity; CaL=calcium lactate; CA=citric acid; LA=lactic acid. B=better than control; S=same as control; W=worse than control; NA= not acceptable.

	Cheese after 5 days			Brine after 5 days
Brine composition	Texture (SI)	Odor	Taste	pH
Control (water)	-	-	-	6.21
CaL 0.5%, $CaCl_2$ 0.1%, pH 4 with CA	W	W	W	5.76
CaL 0.5%, $CaCl_2$ 0.1%, pH 4 with LA	W	S	W	5.62
CaL 1.0 %, $CaCl_2$ 0.2%, pH 4 with CA	W	W	W	4.92
CaL 1.0 %, $CaCl_2$ 0.2% pH 4 with LA	W	S	W	4.83
NaCl 0.8%, $CaCl_2$ 0.4%, pH 4.5 with CA	NA	S	S	4.66
NaCl 0.8%, $CaCl_2$ 0.4%, pH 4.5 with LA	W	S	S	4.88
NaCl 0.4%, $CaCl_2$ 0.2%, pH 3 with CA	NA	S	W	5.46
NaCl 0.4%, $CaCl_2$ 0.2 %, pH 3 with LA	NA	S	W	5.17
CaL 0.5%, pH 4 with CA	NA	S	S	5.07
CaL 0.5%, pH 4 with LA	W	S	S	5.63
CaL 1.0 %, pH 4 with CA	W	S	W	4.63
CaL 1.0 %, pH 4 with LA	B	S	S	4.69

Table 2. Second preparative trial: evaluation after 5 days storage of the cheeses at 8 ± 1°C. SI=surface integrity; CaL=calcium lactate; LA=lactic acid. B=better than control; S=same as control; W=worse than control; NA= not acceptable.

	Cheese after 5 days			Brine after 5 days
Brine composition	Texture (SI)	Odor	Taste	pH
Control (water)	-	-	-	6.17
CaL 1.0 %, NaCl 0.25 %, pH 3.7 with LA	W	S	B	4.16
CaL 1.0 %, pH 3.7 with LA	B	S	S	4.18

Table 3. Mean microbial counts ($\log_{10}$ cfu g^{-1}) of microbial groups during storage of mozzarella samples at 8 ± 1°C. Data from each trials were elaborated separately.

TVC= Total Viable; M&Y= Molds and Yeasts; Ps= *Pseudomonas* spp; Ent = *Enterobacteriaceae*. Value pairs bearing superscript asterisk are different at significant level.

	Days	TV	M&Y	Ps	Ent
Trial 1					
Control	0	3.04	1.90	3.51	2.93
Control	7	5.08	2.95	3.64	3.11
Experimental		4.77	3.00	3.81	2.98
Control	14	6.98*	3.83	7.04*	2.90*
Experimental		5.85*	3.13	5.97*	3.43*
p		*0.03*		*0.03*	*0.05*
Control	21	7.77*	3.11	6.95*	3.43
Experimental		6.83*	4.18	5.72*	3.29
p		*0.01*		*0.002*	
Trial 2					
Control	0	4.15	2.30	5.01	3.41
Control	7	6.97*	2.81	6.40*	4.21*
Experimental		5.68*	2.40	3.97*	3.26*
p		*0.01*		*0.002*	*0.01*
Control	14	8.03*	4.80*	7.78*	3.82*
Experimental		7.02*	3.53*	7.19*	3.45*
p		*0.03*	*0.001*	*0.03*	*0.05*
Control	21	9.21*	5.76*	8.19*	5.36*
Experimental		7.55*	4.00*	7.20*	3.32*
p		*0.002*	*0.001*	*0.002*	*0.01*
Trial 3					
Control	0	3.86	2.18	2.78	2.18
Control	7	5.24	5.30*	6.99*	4.26*
Experimental		5.11	4.29*	6.54*	2.47*
p			*0.01*	*0.02*	*0.05*
Control	14	7.03*	6.11*	8.21*	6.55*
Experimental		6.44*	5.31*	6.78*	4.11*
p		*0.05*	*0.03*	*0.002*	*0.04*
Control	21	9.33*	6.87	9.28*	8.04*
Experimental		7.41*	6.58	7.28*	5.34*
p		*0.01*		*0.01*	*0.03*

Table 4. Sensory attributes for mozzarella selected and corresponding description.

Attribute	**Description**
Brine clearness	Absence of turbidity and suspended solids in the preserving brine
Surface integrity	Presence of a very thin “skin” on the cheese surface, perfectly intact, without breakings and/or detaching
Elasticity	Tendency of the cheese to return to the original conditions after being slightly pressed with a fork
Consistency	Resistance during cutting with a kitchen knife
Succulence	Tendency to release water when cut and/or at chewing
Odour	Intensity of pleasant perceptions when sniffing
Flavour	Intensity of pleasant perceptions when chewing
Sour	The taste of acidified milk or whey
Bitter	The taste of a 0.2% calcium chloride solution
Aftertaste	Intensity of pleasant perceptions after swallowing

Figure 1

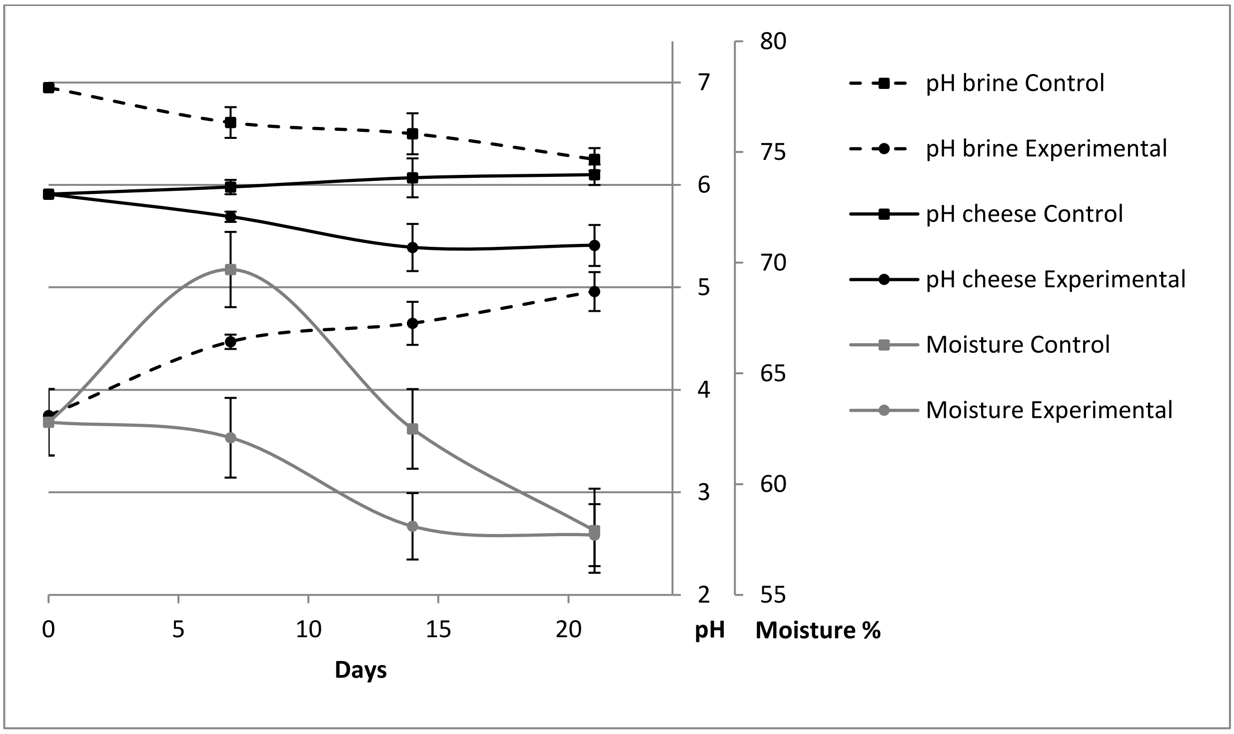

Figure n. 1

Figure 2

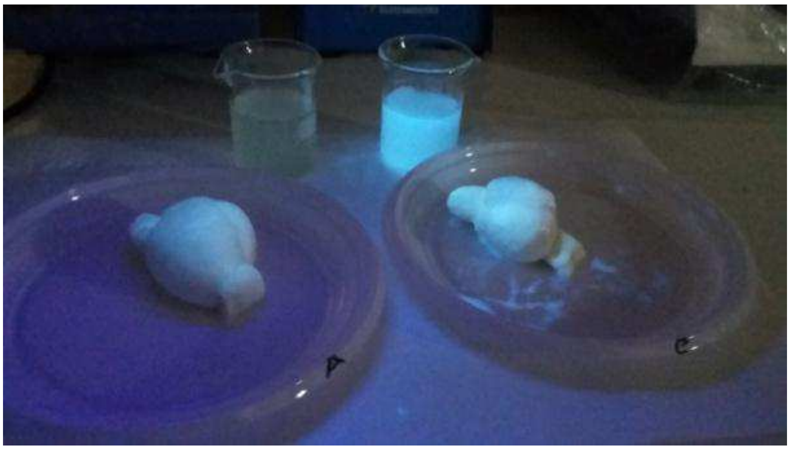

Figure n. 2

Figure 3

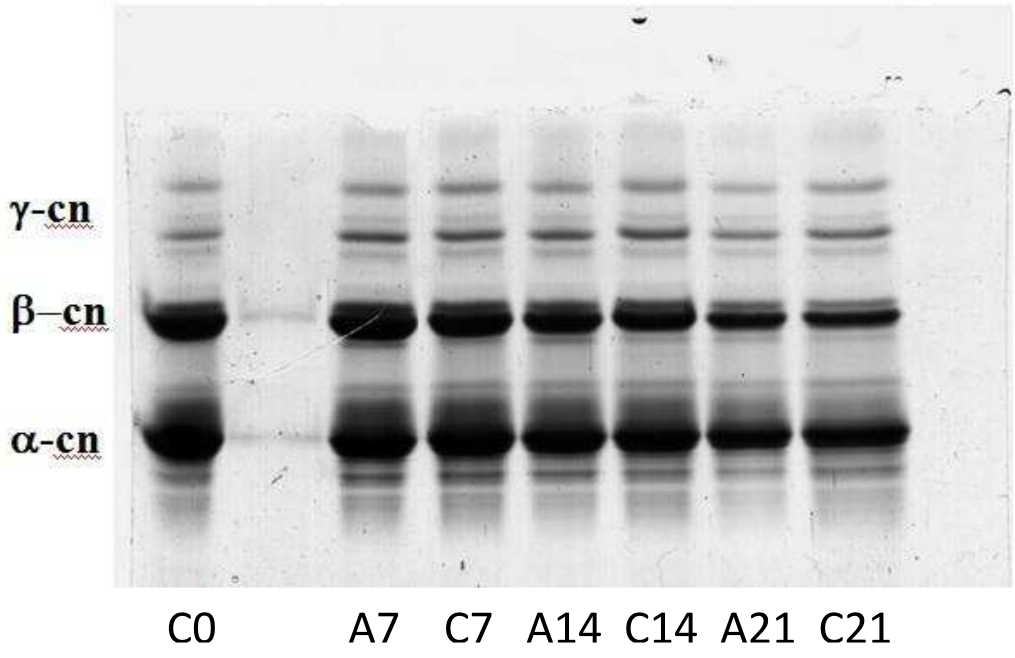

Figure n. 3