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# Microbiological and chemical characteristics of *pasta filata* type cheese from raw ewe milk, using thermophilic and mesophilic starters

*Mikrobiologische und chemische Eigenschaften von Käse vom Typ „Pasta filata" aus roher Schafsmilch unter Verwendung von thermophilen und mesophilen Startern*

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**Summary**

The aims of this study were a) to produce *mozzarella* type cheese from raw ewe milk at both high and low temperatures; b) to verify the effect of vacuum packaging on the microbiological characteristics of a low moisture *pasta filata* cheese. In the colder season, the use of both thermophilic and mesophilic starters allowed the reduction of the curd acidification time as compared to the use of thermophilic starters. The microbiological characteristics were not affected by the different acidification times. Pathogens and hygiene markers microorganisms were undetected in both group of *mozzarella* cheese. No differences were found for *Enterobacteriaceae* and coliform counts. Instead, the proteolysis marker was higher at a longer curd acidification. The decreasing of microorganisms used as hygiene markers as well as *Enterobacteriaceae* and coliforms present in milk and curd was observed in both groups of *mozzarella* cheese. It could be a result of the thermal effect of the stretching phase. Low moisture *pasta filata* cheese upon storage under vacuum for 40 days, according to microbiological results, is free of risks for consumer health.

**Keywords:** raw ewe milk, mozzarella, starters, vacuum storage

**Zusammenfassung**

Die Ziele dieser Studie waren: a) Herstellung von Mozzarellakäse aus roher Schafsmilch bei hohen und niedrigen Temperaturen; b) Überprüfung der Wirkung von Vakuumverpackungen auf mikrobiologische Eigenschaften. In kälteren Jahreszeiten erlaubte die Verwendung von sowohl thermophilen als auch mesophilen Startern eine Verkürzung der Säuerungsdauer im Vergleich zu der Verwendung von thermophilen Startern. Mikrobiologische Eigenschaften wurden durch unterschiedliche Ansäuerungszeiten nicht beeinflusst. Pathogene und Hygienemarker Mikroorganismen wurden in beiden Mozzarellakäsegruppen nicht entdeckt. Für Enterobacteriaceae und coliforme Zählungen wurden keine Unterschiede gefunden. Es ergab sich dagegen, dass der Proteolysemarker höher ist, wenn die Ansäuerung des Quarks länger ist. Die Abnahme von Mikroorganismen, die als Hygienemarker verwendet werden, sowie von Enterobacteriaceae und Coliformen, die in Milch und Quark enthalten sind, wurde in beiden Gruppen von Mozzarellakäse beobachtet. Es könnte ein Ergebnis der thermischen Wirkung der Dehnungsphase sein. Pasta-Filata-Käse mit niedriger Feuchtigkeit, der nach 40-tägiger Lagerung unter Vakuum aufbewahrt wird, ist gemäß den mikrobiologischen Ergebnissen frei von Risiken für die Gesundheit der Verbraucher.

**Schlüsselwörter:** rohe Schafsmilch, *Mozzarella,* Startern, Vakuumverpackung

## Introduction

Sheep production is widespread in the Mediterranean area, including the Center as well as the South of Italy, and is mainly oriented to milk production. Most of the milk is used to produce hard and semi-hard cheeses. Some cheese types are famous and recognized as Protected Designation of Origin (PDO) by the European Union: *Pecorino Romano, Pecorino Sardo, Canestrato Pugliese, Idiazabal, Manchego* and *Roquefort* (Scintu and Piredda, 2007). Recently, also *Vastedda della Valle del Belice,* made only from ewe milk, was recognized as PDO cheese. It is a so called *pasta filata* cheese, though in Italy, this type of cheese is mainly produced from cow and buffalo milk. Ewe milk, mixed with cow and goat milk, is also used in the production of *Kashkaval,* another *pasta filata* type cheese, very popular in Eastern Europe. *Pasta filata* cheese is characterized by the stretching phase, which follows the curd acidification. The stretching phase consists in submitting curd to elongation in presence of hot water (90–95 °C). Casein aggregates are bound and form long chains, which entraps fat and moisture (Addeo et al., 1996). The poor streching ability of ewe milk (Niro et al., 2014) is probably the main limit to the spread of pasta filata cheese type from ewe milk. Recently, increased interest for "pasta filata" cheese was made evident also for ewe milk. The cheese making process used with cow milk to produce *Scamorza* was transferred to ewe milk (Albenzio et al., 2013a; Albenzio et al., 2013b). Ewe milk was inoculated with starters and at pH of 5.7 liquid rennet was added. Indeed, the trend and the length of the acidification phase depend on the temperature that affects the growth of lactic acid bacteria (LAB). In small cheese plants not oriented to *pasta filata* type and not equipped with temperature-controlled vessels, it is more difficult to manage the temperature during curd acidification. In fact, the trend and the length of the acidification phase depend on the temperature that affects the growth of lactic acid bacteria (LAB). One of the methods used to improve the management of the acidification phase is the addition of starter cultures, which can grow at different temperatures, both at high temperatures during summer as well as at low temperatures during winter (Gaglio et al., 2014). According to Faccia et al. (2015), the most appropriate technology to produce soft *pasta filata* cheese from ewe and goat milk in small dairies is a combination of direct acidification and lactic fermentation.

The present work was developed to study how improve *pasta filata* cheese technology in small cheese plants which process ewe milk Regarding cheese packaging, the effects of vacuum packaging, the most common packaging system used in small cheese plants, was evaluated only on ripened cheese (Nuñez et al., 1986; AGRIS Sardegna, 2011; Salari et al., 2011). Therefore, studies on soft cheese are useful to evaluate the effect of the vacuum packaging on microbiological characteristics.

The aims of this study are therefore to evaluate the microbiological and chemical characteristics of a) *mozzarella* cheese produced from raw ewe milk, with inoculation of different types of starters and b) low-moisture *pasta filata* cheese during storage by vacuum packaging.

## Materials and methods

### Process

*Mozzarella* type cheese. The trial was carried out in a small cheese plant in autumn 2013. Four replicates per *mozzarella* processing were performed. Regarding to the Brucellosis disease, Lazio region was declared free from the infection of *Brucella melitensis* (Decision 2014/91 UE). Raw ewe milk (100 l for each replicate) was inoculated with two kinds of commercial starters. Starter 1: thermophilic (T) lactic acid bacteria (LAB) containing only *Streptococcus thermophilus* (STA IDC 11; Centro Sperimentale del Latte SpA, Vernate, Milano). Starter 2: thermophilic and mesophilic (TM) lactic acid bacteria (LAB) with *Streptococcus thermophilus, Lactococcus lactis, L. cremoris* and *L. diacetylactis* (STAR IDC 12; Centro Sperimentale Latte, Vernate, Milano). After the starter inoculation, milk was added with rennet powder (Bellucci S.R.L., Modena, Italy). After coagulation, curd was cut and let acidify under whey. The vessel containing curd under whey was put in handmade bath in order to maintain curd temperature not below 37 °C. In order to verify whether acidified curd was ready to be stretched, a "stretching" test was carried out on a piece of curd treated with hot water. At the same time, the pH of curd was measured. During the stretching phase, hot water at about 85–90 °C was used. Cheese was then molded in shape of about 200 g, firmed in cold water, salted in saturated brine for 2 hours and stored at 4 °C into governing liquid with 2–3 % of NaCl.

Low-moisture *pasta filata* cheese. Another *pasta filata* cheese was created in order to study the effect of storage by vacuum packaging. This product weighted about 50 g. The process was similar to the one described above and included TM starter. After salting, cheese was left at 4 °C for some hours to reduce the moisture content. Then, it was wrapped in a special paper (Arcadia SpA, Sedegliano, Udine) and put under vacuum. This paper adheres perfectly to the cheese, so reducing air and humidity areas that, during storage under vacuum, could favor the growth of moulds. For the vacuum packaging, an envelope-embossed polyamide/polyethylene has average gas barrier.

### Microbiological and chemical analyses

*Mozzarella* type cheese. During the four replicates, milk and curd samples were collected and submitted to microbiological and chemical analyses. All microbiological analyses were performed in the laboratories of IZSLT (Istituto Zooprofilattico Sperimentale Lazio e Tioscana – Experimental Institute for Zooprophylaxis in Lazio and Tuscany), accredited according to International Organization for Standardization (ISO/IEC 17025:2005) by Accredia, (Laboratory number 0201), the Italian Accreditation Body. Samples of T and TM cheese were collected at day 1 (the day after processing) and day 5. Samples of milk, curd and cheese of day 1 were submitted to the following microbiological analyses: detection of *Salmonella* (ISO 6579:2002), and *Listeria monocytogenes* (ISO 11290-2:1998/Amd 1:2004), enumeration of coagulase-positive staphylococci (ISO 6888-2:1999/Amd 1:2003), ß-glucuronidase-positive *E. coli* (ISO 16649-2: 2001), *Enterobacteriaceae* (ISO 21528-2:2004), coliforms at 30 °C (ISO 4832: 2006) and total microbial count (TMC) at 30 °C (ISO 4833-2:2013), (Filippetti et al., 2008; Carfora et al., 2013). At day 5, coliforms at 30 °C analyses were performed on cheese samples.

The following chemical and technological analyses were carried out on milk samples: total nitrogen (TN) and fat by NIR (Milkoscan FT 6000 – Foss Electric, Hillerød, Denmark) calibrated for ewe milk; pH was measured with pH meter (Mettler Toledo Inc., Leicester, UK). Clotting properties (r = rennet clotting time, K20 = curd firming time, A30 = curd firmness at 30 min, A2r = curd firmness at 2r) by Zannoni and Annibaldi method (1981).

Cheese samples collected at day 1 and day 5 were submitted to the following chemical analyses: pH, moisture (IDF, 1986), total nitrogen (TN) (FIL-IDF, 1993), soluble nitrogen (SN) (FIL-IDF, 1991), fat (FIL-IDF, 2001), salt (IDF, 1988).

Low-moisture *pasta filata* cheese. Samples stored by vacuum packaging were collected at 0, 20 and 40 days of storage and submitted to the following analyses: *Salmonella, Listeria monocytogenes,* coagulase-positive staphylococci, ß-glucuronidase-positive *E. coli, Enterobacteriaceae,* coliforms at 30 °C, TMC, yeasts and moulds (ISO 21527-1: 2008), (Filippetti et al., 2008; Carfora et al., 2013). Moisture, protein and fat content were analyzed by NIR spectroscopy (FoodScan™, Foss Electric, Hillerød, Denmark) (Filippetti et al., 2008).

### Statistical analysis

All the microbiological data were log-transformed before statistical analysis. Samples showing no colonies on plates counts were converted to zero after log-transformation. The GLM procedure of SAS software (SAS Institute Inc. 2007) was used for statistical analysis of microbiological and chemical analyses of *mozzarella* type cheese. Factorial model including the fixed effect of the starter type and the storage time of cheese was used.

## Results and discussion

### *Mozzarella* type cheese – Process

During the four replicates carried out on *Mozzarella* cheese, both T and TM curds were successfully stretched at pH 5.0. The length of the acidification phase was affected by the kind of starter. In fact, TM curd was ready for stretching in four hours and a half on average, while T curd in seven hours on average. Curd acidification begins when the whey is drained off (McSweeney et al., 2004). At the conditions of the present trial (autumn), the use of starters based on the thermophilic and mesophilic LAB allowed to reach an optimal curd pH in about the same time currently required in the cheese-making of buffalo and cow *mozzarella,* 3–5 hours starting from whey draining (Mucchetti and Neviani, 2006). The necessary time to reach optimal curd pH depends mainly on curd temperature, recommended to be not less than 30 °C (Addeo et al., 1996; Salvadori del Prato, 2001). In the Mediterranean countries, ewe milk is produced also during cold seasons, when the management of the acidification phase is difficult due to the lack of specialized facilities in traditional or small cheese plants. The use of starters containing both thermophilic and mesophilic LAB could favor the acidification at different temperatures and allow to carry out the acidification phase in a reasonable time. *Vastedda della Valle del Belice* cheese was traditionally produced only in summer with raw milk and with no starters, but it is currently requested throughout the year. Recently, twelve thermophilic and mesophilic LAB strains isolated from PDO *Vastedda della Valle del Belice* cheese were studied for the full-year production of Vastedda-like cheese with raw milk and natural starters (Gaglio et al., 2014). In *Fior di Latte* from raw sheep milk processed by the direct acidification and lactic fermentation, time from whey draining to stretching was about 5 hours (Faccia et al., 2015).

### *Mozzarella* type cheese – Microbiological characteristics

Table 1 displays the microbiological characteristics of milk, curd and *mozzarella* type cheese at day 1 and day 5.

*Salmonella* and *L. monocytogenes,* pathogens considered as food safety criteria (Reg CE 2073/2005), were undetected by qualitative analyses in milk, curd and cheese, in accordance with the above Regulation.

In the cheese samples, counts of microorganisms considered as hygiene markers (Reg CE 2073/2005), ß-glucuronidase-positive *E. coli* and coagulase-positive staphylococci were lower than the detection limit of the method. According to Reg CE 2073/2005 the detection of staphylococcal enterotoxins was not carried out due to the absence of coagulase-positive staphylococci.

Higher counts of these microorganisms were found in milk and curd. The average coagulase-positive staphylococci in milk and curd samples, was, respectively, 2.0 log 10 cfu x $g^{-1}$ and 2.4 log 10 cfu x $g^{-1}$. These data are in accordance with regulatory limits and, according to italian legislation, the detection of staphylococcal enterotoxins was not carried out. The average ß-Glucuronidase-positive *E. coli* in milk and curd samples, was, respectively, 1.8 log 10 cfu x $g^{-1}$ and 3.3 log 10 cfu x $g^{-1}$.

In another *pasta filata* fresh cheese from raw milk as *Vastedda della Valle del Belice* coagulase-positive staphylococci and ß-Glucuronidase-positive *E. coli* counts resulted positive (Mucchetti et al., 2008). The same microorganisms were found to be lower than the detection limit of the method on *Mozzarella di Bufala Campana* from pasteurized milk, while on the curd their number was very variable according to the different trials (Zottola et al., 2009). In *Fior di Latte* from raw sheep milk, coagulase-positive staphylococci were detected (Faccia et al., 2015).

*Enterobacteriaceae* and coliform counts were positive in cheese samples. The average of *Enterobacteriaceae* resulted 1.7 log 10 cfu x $g^{-1}$ in T *mozzarella* type cheese and 1.3 log 10 cfu x $g^{-1}$ in TM ones. The average coliform was 1.5 log 10 cfu x $g^{-1}$ in T *mozzarella* like cheese and 1.4 log

**TABLE 1:** *Microbiological characteristics of milk, curd and mozzarella type cheese at day 1 and 5.*

| | Milk | Curd | day 1 mozzarella | | day 5 mozzarella | |
|---|---|---|---|---|---|---|
| | | | T | TM | T | TM |
| *Salmonella* spp. (in 25 g) | absent | absent | absent | absent | – | – |
| *Listeria monocytogenes* (in 25 g) | absent | absent | absent | absent | absent | absent |
| Coagulase positive staphylococci (log 10 cfu x $g^{-1}$) | 2.0 | 2.4 | 0.0 (*) | 0.0 (*) | – | – |
| Beta-glucuronidase-positive *E. coli* (log 10 cfu x $g^{-1}$) | 1.8 | 3.3 | 0.0 (*) | 0.0 (*) | – | – |
| *Enterobacteriaceae* (log 10 cfu x $g^{-1}$) | 4.9 | 5.2 | 1.7 | 1.30 | – | – |
| Coliforms (log 10 cfu x $g^{-1}$) | 5.0 | 5.1 | 1.5 | 1.4 | 2.2 | 1.3 |
| Total mesophilic count (log 10 cfu x $g^{-1}$) | 6.1 | 7.1 | 6.6 | 6.9 | 7.5 | 7.6 |

T: thermophilic lactic acid bacteria; TM: thermophilic and mesophilic lactic acid bacteria; (*) < 10 cfu x $g^{-1}$

10 cfu x $g^{-1}$ in TM ones. Significant differences between T and TM cheese were not found. *Enterobacteriaceae* found on Vastedda fresh cheese (6.3 log 10 cfu x $g^{-1}$) was higher if compared with our results (Mucchetti et al., 2008).

The average TMC increased from milk (6.1 log 10 cfu x $g^{-1}$) to curd (7.1 log 10 cfu x $g^{-1}$) and slightly decreased in T (6.6 log 10 cfu x $g^{-1}$) and TM cheese (6.9 log 10 cfu x $g^{-1}$). Regulatory limits are present only for raw milk, Regulation (EC) 853/2004, and are respected by samples. Table 3 displays microbiological characteristics of *mozzarella* cheese after 5 days of storage. The search for *L. monocytogenes* was negative. The average coliform count was 2.2 log 10 cfu x $g^{-1}$ in T *mozzarella* like cheese and 1.4 log 10 cfu x $g^{-1}$ in TM ones. The average TMC was 7.5 log 10 cfu x $g^{-1}$ in T *mozzarella* type cheese and 7.6 log 10 cfu x $g^{-1}$ in TM ones. These values of TMC could be attribued to starter cultures (Gaglio et al., 2014). No significant differences in coliforms were found between T and TM cheese. However, it is noteworthy that the coliforms in T cheese increased by 48 % from day 1 to day 5, while the values found in TM cheese were very similar (Fig. 1).

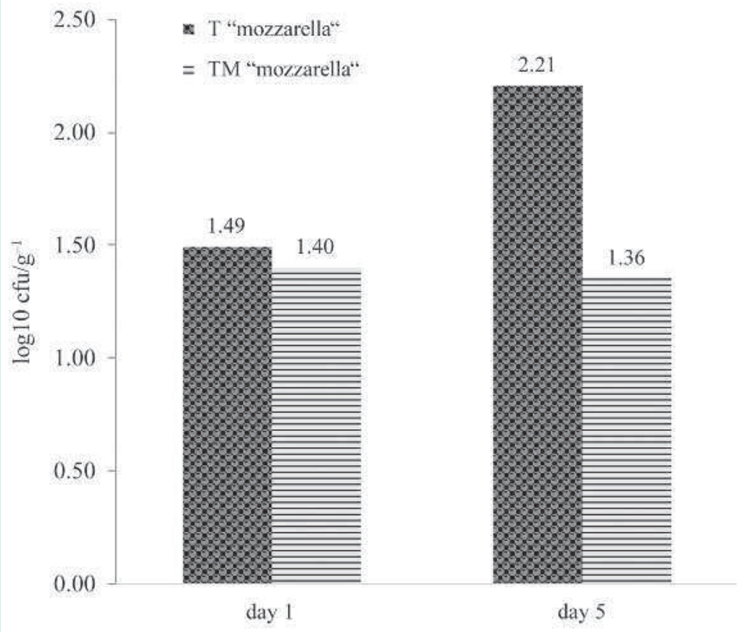


**FIGURE 1:** *Coliform count of T and TM "mozzarella" cheese at day 1 and 5.*

The mean values of coliforms in all cheese (T and TM), collected at day 1 and 5, were compared with milk ad curd (Fig. 2). Coliform count, similar in milk and curd, decreased by 72 % from curd to fresh cheese and increased during cheese storage (19 %). Similar decreasing from curd to fresh cheese was observed for Enterobacteriaceae (71 %).

Raw milk cheese deserve greater attention since the final products can become contaminated by pathogenic microorganisms as a result of their presence in raw milk (Donnelly, 2004). The growth of milk pathogens in raw cheeses is highly dependent on the variety of cheese and on the involved technology. It is well documented that pathogens grow more easily in cheese with higher moisture, high pH and low salt content (Grappin and Beuvier, 1997).

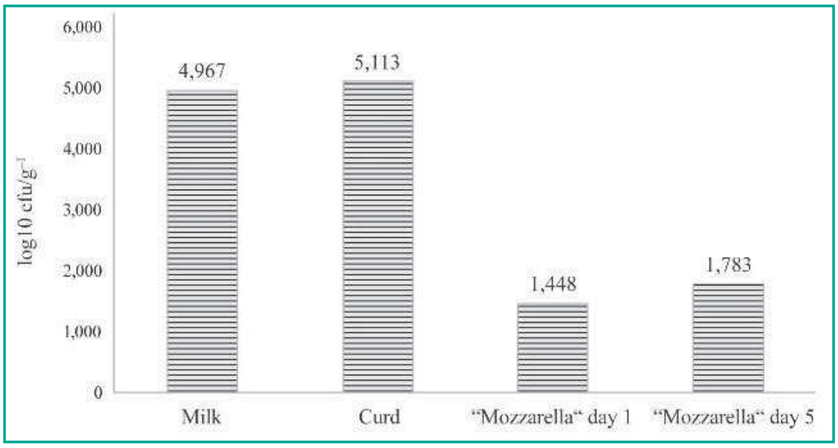


**FIGURE 2:** *Coliform count of milk, curd and "mozzarella" cheese (T and TM type) at day 1 and 5.*

The decreasing of microorganisms used as hygiene markers as well as *Enterobacteriaceae* and coliforms from milk and curd to cheese could be a result of the thermal effect of the stretching phase. The high temperatures reached during this phase of *pasta filata* processing contributes to reduce contaminating microbial flora and to the safety of the resulting products (Mucchetti and Neviani, 2006).

Based on all microbiological results, the higher acidification time of T cheese did not results in poor hygienic characteristics, also during cheese storage. During the trial, the applied process contributed to reduce the microbiological risk of cheese produced from raw milk. According to Johnson et al. (1990), the good management of curd acidification can contribute to reducing microbiological risk and/or increasing shelf life of cheese from raw milk. In the small cheese plants not equipped with temperature-controlled vessels, curd acidification could be managed by an appropriate use of starters (Niro et al., 2014, Settanni et al., 2012).

### *Mozzarella* type cheese – Physico-chemical characteristics

The mean physical, chemical and technological characteristics of the milk used in *mozzarella* type cheese trials were as follows: pH 6.72, total protein 5.64 %, fat 6.33 %, r 12.97 min, k20 8.25 min and A30 28.44 mm.

The chemical characteristics of the T and TM *mozzarella* type cheese are reported in Table 2. At day 1, the moisture of the T cheese was significantly lower ($P<0.05$) than the TM one (58.12 vs 61.27 %). The lower moisture of T *mozzarella* type cheese could be due to the longer acidification time, in which the higher rate of curd syneresis occurred. Rapid acidification allows the total manufacturing time to be shortened, because it reduces the total amount of syneresis during cheese making and enables a higher moisture content to be achieved in the final cheese (Barbano et al., 1994).

The mean moisture content of both T and TM cheese at day 1 was 59.69 % and was slightly higher than the one of *mozzarella* from cow and buffalo milk, 58.8 and 55.5 %, respectively[1]) (1). Both *Vastedda della Valle del Belice* and *Scamorza* cheese have lower moisture content than the *mozzarella* type cheese, respectively, 46.7 % (Mucchetti et al., 2008) and 44.9 % (nut.entecra.it/646/tabelle_di_compo-

[1]) nut.entecra.it/646/tabelle_di_composizione_degli_alimenti.html, 2009

sizione_degli_alimenti.html, 2009) and they are included in the semi-hard cheese category of Codex Alimentarius. The *Mozzarella* type cheese produced in the present trial could be ascribed to the soft cheese category of Codex Alimentarius. In spite of the scarce ability of ewe milk to be stretched, it was possible, to obtain a product similar to the *mozzarella* obtained from cow and buffalo milk through bacterial acidification,

The moisture content of day 5 samples was higher on average than the one of day 1 cheese. Instead, salt content and, consequently, ash content, of whom NaCl represents the highest component, decreased from day 1 to day 5. During storage in governing liquid, cheese absorbed water while releasing salt into the governing liquid.

The values of the soluble protein showed that protein degradation was higher in the T cheese especially at day 5 (1.55 vs 1.13 %) (P<0.05). *Mozzarella* is a fresh cheese where the extent of proteolysis is very limited (Upadhyay et al., 2004). Proteolysis during cheese aging is a well-known biochemical reaction (Upadhyay et al., 2004), but it remains less known in *pasta filata* cheese such as *mozzarella* (Johnson and Law, 1999; Feeney et al., 2002). Moisture content generally affects the extent of proteolysis (Upadhyay et al., 2004). Out of the factors, which mostly affect *mozzarella* proteolysis, lactic acid bacteria havew to be focused (Barbano et al., 1993; Hutkins, 1993; Feeney et al., 2002). The process type and management of (Hynes et al., 2004) affect both the moisture content and lactic acid bacteria directly or indirectly. Some undesirable changes in the characteristics of *mozzarella* can be due to casein proteolysis (Rudan et al., 1999; Feeney et al., 2002). We can hypothesize that, in the trial conditions, a longer curd acidification could favor the proteolytic degradation.

The pH values of both kind of *mozzarella* decreased after 5 days of storage.

### Low-moisture *pasta filata* cheese – Microbiological and physico-chemical characteristics

Table 3 displays the microbiological characteristics of curd and low-moisture *pasta filata* cheese at day 0, 20 and 40. *Salmonella* and *L. monocytogenes* were both absent in 25 g. ß-Glucuronidase-positive *E. coli,* coagulase-positive staphylococci, *Enterobacteriaceae* and coliforms were detected in curd but not in cheese at 0, 20 and 40 days, where they were lower than the detection limit of the method. The mean total mesophilic count was lower in curd (7.0 log 10 cfu x $g^{-1}$) and increased during storage of cheese: day 0 (8.3 log 10 cfu x $g^{-1}$), day 20 (8,4 log 10 cfu x $g^{-1}$) and day 40 (9.1 log 10 cfu x $g^{-1}$). Yeasts, higher in curd, were lower than the detection limit of the method at day 0 and grew during cheese storage. Mould content, present in curd, was lower than the detection limit of the method at day 0 and day 40, detected at day 20.

**TABLE 2:** *Physico-chemical characteristics of mozzarella type cheese at day 1 and 5.*

| | day 1 | | day 5 | |
|---|---|---|---|---|
| | T | TM | T | TM |
| moisture (%) | 58.12[b] | 61.27[a] | 64.62 | 62.44 |
| protein (%) | 20.66 | 18.31 | 16.40 | 17.11 |
| soluble protein (%) | 1.38 | 1.26 | 1.55[a] | 1.13[b] |
| fat (%) | 17.23 | 15.89 | 15.87 | 15.34 |
| NaCl (%) | 0.80 | 0.77 | 0.60 | 0.67 |
| pH | 4.99 | 5.00 | 4.91 | 4.91 |

T: thermophilic lactic acid bacteria; TM: thermophilic and mesophilic lactic acid bacteria, [a, b]: P<0,05

**TABLE 3:** *Microbiological characteristics of snack cheese at day 0, 20 and 40.*

| | Curd | Low-moisture *pasta filata* cheese | | |
|---|---|---|---|---|
| | | day 0 | day 20 | day 40 |
| *Salmonella* spp. (in 25 g) | absent | absent | absent | absent |
| *Listeria monocytogenes* (in 25 g) | absent | absent | absent | absent |
| Coagulase positive staphylococci (log 10 cfu x $g^{-1}$) | 3.1 | 0.0(*) | 0.0(*) | 0.0(*) |
| Beta-glucuronidase-positive *E. coli* (log 10 cfu x $g^{-1}$) | 2.3 | 0.0(*) | 0.0(*) | 0.0(*) |
| Enterobacteriaceae (log 10 cfu x $g^{-1}$) | 5.6 | 0.0(*) | 0.0(*) | 0.0(*) |
| Coliforms (log 10 cfu x $g^{-1}$) | 2.5 | 0.0(*) | 0.0(*) | 0.0(*) |
| Total mesophilic count (log 10 cfu x $g^{-1}$) | 7.0 | 8.2 | 8.4 | 9.1 |
| Yeasts (log 10 cfu x $g^{-1}$) | 5.5 | 0.0(*) | 3.3 | 2.9 |
| Moulds (log 10 cfu x $g^{-1}$) | 4.0 | 0.0(*) | 2.8 | 0.0(*) |

(*) <10 cfu x $g^{-1}$

The chemical analyses of the low-moisture *pasta filata* cheese, showed that the mean moisture content was 50.00 %, protein content 24.20 % and fat content 22.33 %, corresponding to 298 kcal/100 g of product and 149 kcal per portion.

Microbiological results of fresh cheese were satisfactory, if compared with those reported by other Authors. In fresh *Vastedda della Valle del Belice,* having the similar moisture content (53.30 %), coagulase-positive staphylococci, ß-Glucuronidase-positive *E. coli* and *Enterobacteriaceae* were always present (Mucchetti et al., 2007). The coliforms found in the *mozzarella* cheese stored under vacuum (Tanweer and Goyal, 2011) were always higher than 2.0 log 10 cfu x $g^{-1}$. Coliforms, *E. coli,* mould and yeasts were detected from day 1 to day 24, in mozzarella cheese, having about 50 % of moisture content and stored under vacuum (Fuentes et al., 2015).

The data collected in our trials showed that the storage of low-moisture *pasta filata* cheese at 4 °C, under vacuum, for 40 days is free of risks for the consumer health, according to the considered parameters. Indeed, the vacuum packaging resulted the best method to store *Vastedda della Valle del Belice,* if compared to the other packaging systems (Palmeri et al., 2015).

## Conclusions

To satisfy the market demand of new cheese types from ewe milk, and especillay fresh cheese, it is necessary to develop new cheese processes. The *pasta filata* cheese, like *mozzarella,* is a kind of cheese, which is highly requested by consumers, but, up to now, the poor stretching ability of ewe milkhas limited the application of this process with ewe milk. The use of a mix of microorganisms able to grow at different temperatures could favor curd acidification so reducing the acidification time in the colde season. The use of both thermophilic and mesophilic starters, in experimental trials, allowed to reduce the curd acidification time compared to the use of thermophilic starters. Based on the microbiological results obtained, the longer acidification time of the cheese inoculated with the thermophilic lactic acid bacteria did not results in poor hygienic characteristics. The microorganisms used as hygiene markers (*E. coli* and coagulase positive staphylococci) were not detectable in both kinds of fresh cheese. In the same way, *Enterobacteriaceae* and coliform counts were analogous in the two kinds of fresh cheese. Even after a few days of storage, the coliform count did not differ between the two kinds of cheese. The use of different starters affected some chemical characteristics: in the fresh cheese, in the products with a longer acidification time, the moisture content was lower, while, during storage, the proteolytic process was favoured. This could imply some undesirable characteristics of *mozzarella* cheese.

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The results of this work could be useful to promote technological innovation in small cheese plants processing ewe milk.

## Conflict of interest

The authors declare that there is no conflict of interest.

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