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Evolution of microbial counts and chemical and physico-chemical parameters in high-moisture Mozzarella cheese during refrigerated storage

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ABSTRACT

The microbiological quality, pH, colour, proteolysis and head space composition (using an electronic nose) of several commercial brands of high-moisture Mozzarella cheese produced in Italy were evaluated at the beginning and at the end (5 days) of refrigerated storage in order to evaluate the effect of the acidification system (direct acid addition or use of starter cultures) and storage on the quality of the cheese. A high variability was found for most parameters. At the end of storage all parameters were affected by the mode of acidification and cheese produced by direct acid addition had a significantly lower microbiological quality; counts of psychrotrophs exceeded 10^7 cfu/g for most samples and microbial counts showed a significant correlation with the residual shelf life. Multivariate analysis confirmed that samples at the beginning and at the end of storage were clearly separated but no grouping based on the mode of acidification was found. The electronic nose was only partially successful (80% correct classification) in classifying the cheeses on the basis of storage time or of microbial counts. This is likely to be due to the variety of brands used in the analysis and to differences in the starter systems or acidification mode used.

1. Introduction

High-moisture Mozzarella cheese (HMMC) or "Fior di Latte" is a soft (50–60% moisture), unripened pasta filata cheese manufactured from cow's milk which, in contrast with low-moisture Mozzarella cheese is used as a table cheese. As low-moisture Mozzarella cheese (LMMC, Kindstedt, Caric, & Milanovic, 2004), it can be produced using a variety of methods, including direct acidification by addition of citric acid, or natural acidification by addition of thermophilic defined or undefined strain starters (De Angelis & Gobbetti, 2011). In the direct acid addition process citric acid (or more rarely lactic acid) is added to pasteurized milk before rennet addition, until a pH of 5.6–5.8 is reached, and the curd is ready for stretching soon after coagulation (Faccia, Trani, & Di Luccia, 2009). Lower pH (5.1–5.3) and a slower acidification are used for cultured HMMC (De Angelis & Gobbetti, 2011). Undefined whey or milk cultures are required by the standards of identity of Protected Designation of Origin Water-Buffalo Mozzarella cheese (De Filippis, La Storia, Stellato, Gatti, & Ercolini, 2014; Ercolini, Mauriello, Blaiotta, Moschetti, & Coppola, 2004) or Traditional Speciality Guaranteed cow's milk Mozzarella cheese (De Angelis et al., 2011) and are used for traditional Mozzarella cheese production (de Candia et al., 2007; Parente, Rota, Ricciardi, & Clementi, 1997). Thermophilic lactic acid bacteria (LAB), such as *Streptococcus thermophilus,* alone or in combination with thermophilic lactobacilli (*Lactobacillus delbrueckii*, *Lactobacillus helveticus*), dominate these cultures, but mesophilic (*Lactococcus lactis*, *Leuconostoc,* other *lactobacilli*) LAB and *Enterococcus* are often present (de Candia et al., 2007; De Filippis et al., 2014; Ercolini et al., 2004; Parente et al., 1997). Defined starters for Mozzarella cheese usually include *S. thermophilus* alone, or in combination with *L. delbrueckii* subsp. *bulgaricus* or *L. helveticus* (De Angelis & Gobbetti, 2011).

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HMMC is packaged in a conditioning liquid (water, whey, stretching water, brine) (Faccia, Luisa, Marianna, Amalia, & Del Nobile, 2013) to preserve its soft and springy texture, and stored under refrigerated conditions. It has a short shelf life (usually ≤5 days at 4 °C) because its a_W and pH are not limiting for the growth of spoilage organisms. Addition of coatings or of preservatives and the use of modified atmosphere packaging has been proposed to increase the shelf life of HMMC, with a variable degree of success (Conte, Scrocco, Sinigaglia, & Del Nobile, 2007; Del Nobile, Gammariello, Conte, & Attanasio, 2009; Gammariello, Di Giulio, Conte, & Del Nobile, 2008; Lucera et al., 2014; Sinigaglia, Bevilacqua, Corbo, Pati, & Del Nobile, 2008).

Spoilage is often caused by coliforms (Cantoni et al., 2006), by proteolytic psychrotrophs (Baruzzi, Lagonigro, Quintieri, Morea, & Caputo, 2012) or by discoloration (Andreani et al., 2014) and *Pseudomonas* spp. often dominate the microbiota at the end of shelf life (Baruzzi et al., 2012). In fact, *Pseudomonas* counts exceeding 10^6 cfu/g have been associated to reduced acceptability (Lucera et al., 2014). Physico-chemical phenomena due to salt diffusion contribute to the loss of quality during storage (Faccia, Luisa, Marianna, Amalia, & Nobile, 2013).

Spoilage of HMMC has been assessed by several methods. Microbial counts, sometimes coupled to modelling of microbial growth, have been used frequently (Baruzzi et al., 2012; Conte et al., 2007; Del Nobile et al., 2009; Faccia et al., 2013; Gammariello et al., 2008; Losito et al., 2014; Sinigaglia et al., 2008). Assessment of proteolysis has also been used as an indicator of spoilage (Baruzzi et al., 2012; De Angelis et al., 2008). Colour assessment has been used recently because of the frequent occurrence of blue discoloration of Mozzarella caused by *Pseudomonas fluorescens* strains (Andreani et al., 2014). Sensory evaluation has been used more rarely (De Angelis et al., 2008; Del Nobile et al., 2009; Lucera et al., 2014). However, head space fingerprinting by sensor arrays (electronic noses, EN) has never been used, although this technique has been proved effective for other soft cheeses (Benedetti, Sinelli, Buratti, & Riva, 2005; Kamleh, Toufeili, Ajib, & Kanso, 2012), and odour has been found as the limiting factor in determining HMMC acceptability during refrigerated storage (Faccia et al., 2013).

In most papers dealing with HMMC spoilage a limited variety of products (usually products obtained from a single producer or obtained in pilot plant trials) has been evaluated. The objective of this work was to evaluate the changes in microbiological quality, pH, proteolysis, colour, head space composition and proteolysis in a variety of commercial high-moisture Mozzarella cheese produced with different acidification methods during refrigerated storage.

2. Materials and methods

2.1. Sampling

Samples (20) of high moisture Mozzarella cheese belonging to 14 different commercial brands (A-N) were purchased over one month in local supermarkets the same day they were delivered from cheese making plants and were analysed immediately or stored for 5 days at 10 °C to simulate a temperature abuse before analysis. The shelf life duration indicated on the packages varied between 5 and 20 days, and 5 days was the most frequent consume by date. Of the 14 brands, five had been produced by industrial cheese plants and nine by artisanal cheese plants. For three brands (one industrial, two artisanal) cheeses were purchased on three different days during the sampling period. The cheeses were produced using different acidification systems: five brands declared the use of citric acid, four the use of starter cultures, while for the others no indication was provided on the labels. All samples were produced from cow's milk.

2.2. Microbiological analyses

Two replicate cheeses (125 g) obtained from two packages were used for each sample and each was analysed in duplicate. The first decimal dilution was carried out in sterile 2% trisodium citrate, while further dilutions were carried out in sterile quarter-strength Ringer solution. *Enterobacteriaceae* were enumerated by pour plating in Violet Red Bile Glucose Agar, with incubation at 30 °C for 24 h. Psychrotrophic contaminants and *Pseudomonas* spp. were enumerated by spiral plating (WASP Spiral Plater, bioMérieux Italia SpA, Bagno a Ripoli, Firenze, Italy) on Gelisate agar (Gelatin Peptone Agar, 21 °C, 25 h) and *Pseudomonas* agar base with CFC supplement (25 °C, 48 h), respectively. Colonies were enumerated using a digital colony counter (EasyCount 2, bioMérieux Italia).

2.3. Physico-chemical and chemical analyses

pH was measured using a spear-tip electrode (Hamilton Bonaduz AG, Bonaduz, Switzerland) and a pH-meter (Orion 420A plus, Thermo Fisher Scientific, Rodano, Italy) on both cheese and on the storage liquid. A ten-MOS (Metal Oxide Sensors) electronic device (PEN-3, AIRSENSE, Analytics GmbH, Schwerin, Germany) was used for head space analysis. The fluxed aroma was obtained using an output needle inserted into a Teflon 50 mL vial containing 5 g of cheese at 20 °C with an air flow of 400 mL/min. The sample run lasted 60 s and was followed by 300 s flush time. Each measurement, carried out in triplicate, was recorded in a text file by Win Munster v.1.6.2.2 software. Colour was measured in triplicate on both inside and outside of cheese using a Chroma Meter CR-300 Minolta, with the CIELAB (L*, a*, e b*) system, using a white tile for calibration. Proteolysis was assayed on the clear supernatant of the first decimal dilution from counts using the *o*-phtaldialdehyde method (Rohm, Tschager, & Jaros, 1996).

2.4. Statistical analysis

Statistical analysis (principal component analysis, discriminant analysis partial least square regression, nonparametric tests, mixed linear models) and graphics were performed using Systat 13 (Systat Software Inc., San Jose, CA, USA).

2.5. Reagents and media

Unless otherwise stated, all reagents were purchased from Sigma–Aldrich, Milan, Italy, while all microbiological media were obtained from Oxoid Ltd. (Basingstoke, UK).

3. Results

3.1. Evolution of microbial counts, proteolysis, pH and of head space composition during storage

Commercial samples of HMMC were analysed immediately and after five days of refrigerated storage. The microbiological quality of samples on the day of purchase varied greatly. Fig. 1 shows the distribution of counts, cheese pH, proteolysis and CIELAB colour parameters at days 0 and 5 for cheeses grouped by mode of acidification. All parameters varied significantly ($p < 0.05$) with time, with the exception of L*. No significant differences among products obtained with different acidification mode were found for *Enterobacteriaceae* at either 0 or 5 days, nor for psychrotrophs, *Pseudomonas* or a* at 0 days. However, cheese produced by direct acid addition had a significantly higher counts of psychrotrophs and *Pseudomonas* compared to cheeses obtained by addition of starter cultures and cheeses produced by unknown acidification mode.