Introduction

Ricotta salata cheese or Ricottone or Ricotta Toscanella is a salted variety of ricotta traditionally made in Sardinia (Italy) from the whey remaining after the production of Pecorino Romano protected designation of origin or other sheep milk cheeses. A wide variety of whey cheeses are manufactured from sheep milk in several European countries, especially in the Mediterranean basin: i.e. Mizithra, Anthoyros and Manouri (Greece), Anari (Cyprus), Requesón (Spain), Requeijão (Portugal), Brocciu (France), Ürdâ (Balkans region), each with its own distinctive technology. In most of the cases these are fresh and soft or semi-soft traditional products made in small-scale operations and sold in local markets. The manufacturing of ricotta salata cheese in Sardinian industrial cheese-making plants follows the traditional batch process (Laore, 2015). The whey is filtered and preheated at 60-70°C with the use of a plate heat exchanger. The whey can be added with pasteurised cream or milk to enrich the solid content. The blend is then transferred to large open kettles (1200-1500 L capacity) and heated to temperature above 85°C and held for 30 min. After the flocculated protein rises to the surface, clots are collected using perforated ladles and transferred into plastic cylindrical molds lined with cheese cloth. The curd is then pressed to enhance drainage for up to 24 h and transferred in cold room (10-12°C) for about 10 days. Salting can be made by adding directly sodium chloride to the whey or to the curd during molding or by drying salt during refrigerated storage or in brine after molding. Ricotta salata cheese is dried until the moisture content is ca. 50% if intended to be used for grating or of ca. 55-60% if used as is. After the production process the mean fat and protein content are respectively 28-33% and 14-23%, the pH ranges from 6.1 to 6.9, while the water activity (aw) from 0.940 to 0.970 (Spanu et al., 2012, 2013). The final product is a firm wheel of ca. 3 kg weight, which is individually packed in vacuum bags as a whole or after cutting into wedges. Ricotta salata cheese is stored at refrigeration temperature and the attributed shelf-life differs from three weeks up to several months, depending on the food business operators (Spanu et al., 2015b). Ricotta salata cheese is often used in salads or many other dishes sliced, crumbled or grated without further heating. In consideration of its physico-chemical properties and the presence of and edible rind, ricotta salata cheese is very critical for the possible growth of pathogenic and spoilage microorganisms. Pathogens of particular concern are those able to grow in ricotta salata cheese at refrigeration temperatures, i.e. Listeria monocytogenes and psychrotrophic Bacillus cereus strains. Outbreaks and sporadic cases of listeriosis associated with ricotta salata cheese have been reported over recent years (RASFF, 2008; CDC, 2012). Evidence that L. monocytogenes contamination of ricotta salata cheese origins from the processing environment and that this product supports the growth of the pathogen during refrigerated storage have been documented (Spanu et al., 2012, 2015a; Ibba et al., 2013). Despite the frequent contamination of ricotta salata cheese with B. cereus and the recovery of potentially pathogenic strain, no cases of B. cereus human illness have been associated with the consumption of ricotta salata cheese (De Santis et al., 2008). The high moisture content, the high pH and the availability of nutrients considered, such product is very susceptible to microbiological spoilage by Enterobacteriaceae, yeast, moulds and Pseudomonas. The presence of pathogens and contaminants such as L. monocytogenes and B. cereus has also been observed. However, little research has been conducted to describe the evolution of the microbiological profile of Ricotta salata cheese during the shelf-life (Spanu et al., 2013). The objective of the present study was to assess and characterise the evolution of
spoilage and pathogen microorganism of vacuum-packed ricotta salata cheese under refrigerated storage (4±2°C) during the entire product shelf-life.

Materials and Methods

**Ricotta salata** cheese samples

The study was conducted on vacuum-packed ricotta salata cheese wheels (Crovac Cook-In HT3000; Sealedair Ltd., St Neots, UK), which were provided by a local cheese-making plant using the whey remaining after the manufacturing of sheep milk cheeses (Table 1). The main steps of the process flow of ricotta salata cheese examined in the present study are summarised in the diagram in Figure 1. A total of 18 samples were randomly selected from 3 different batches (6 ricotta salata cheese for each batch) produced within one month. After a drying period of approximately 10-15 days in cold room, ricotta salata cheese samples were sent to the laboratory. Immediately after their arrival, samples were labelled according to their use for the experiment and stored at refrigeration temperature (4±1°C) until analysing.

Experimental design

The durability study was conducted analysing ricotta salata cheese samples at different time during the shelf-life. Sampling times were: within 24 h after the arrival of ricotta salata cheese wheels defined as time zero (T0), time 60 (T60) and time 90 (T90) respectively 60 days and 90 days after the arrival of the samples. In order to avoid temperature abuse during the storage period, the temperature of the cold room was monitored in continuous and recorded daily. Ricotta samples were analysed for the determination of the microbiological profile, physicochemical properties and composition. In Table 1 the number of samples and the analysis performed at each sampling time are reported.

**Microbiological profile**

From each ricotta salata cheese sample, 25 g aliquots of the rind (2 cm in depth) and of the inner paste were aseptically collected. Aliquots were analysed for the enumeration of total viable count (TVC) at 30°C (ISO 4833:2003; ISO, 2003), L. monocytogenes (ISO 11290-1:1996; ISO, 1996), B. cereus (EN/ISO 7932:2004; ISO, 2004b), Enterobacteriaceae (ISO 21528-2; ISO, 2004a), yeasts and moulds (ISO 6611/IDF 94; ISO, 2004c).

**Physico-chemical properties and composition**

pH and a\(\text{w}\) were measured using pH meter GLP22 (Crismon Instruments SA, Barcelona, Spain) and water activity meter Aqualab 4 TE (Decagon, Pullman, WA, USA), respectively. Near infrared transmittance (NIT) compositional analyser (FOSS, Eden Prairie, MN, USA) was used for the determination of centesimal composition which included analysis of moisture, fat, protein, salt and total solids.

### Table 1. Number of *ricotta salata* cheese wheels and analysis performed at each testing time.

| Analysis                          | Sampling time |
|----------------------------------|---------------|
|                                  | T0            | T60           | T90           |
| Microbiological profile          | 6             | 6             | 6             |
| Intrinsic properties and composition | 6             | 6             | 6             |

T\(\text{0}\), 4 days after the arrival of the samples (packaging day); T\(\text{60}\), 60 days after the arrival of the samples; T\(\text{90}\), 90 days after the arrival of the samples. Number of samples per each time refers to the three batches.

### Table 2. Mean log microbial counts of *ricotta salata* cheese units during shelf-life.

| Parameters              | Samples | T\(\text{0}\)          | T\(\text{60}\)        | T\(\text{90}\)        |
|-------------------------|---------|-----------------------|----------------------|----------------------|
| TVC                     | Rind    | 7.90±0.64\(\text{a}\) (n=6/6) | 7.79±2.21\(\text{a}\) (n=6/6) | 9.19±0.58\(\text{b}\) (n=6/6) |
|                         | Paste   | 2.95±0.68\(\text{b}\) (n=5/6) | 4.51±1.66\(\text{a}\) (n=6/6) | 4.27±1.10\(\text{a}\) (n=6/6) |
| *Enterobacteriaceae*    | Rind    | 4.22±0.66\(\text{a}\) (n=4/6) | 5.16±0.59\(\text{a}\) (n=6/6) | 5.30±0.73\(\text{a}\) (n=6/6) |
|                         | Paste   | nd                    | 3.13±1.80\(\text{a}\) (n=3/6) | 2.90±0.38\(\text{a}\) (n=2/6) |
| Yeasts and moulds       | Rind    | 3.02±0.36\(\text{a}\) (n=3/6) | 4.99±0.81\(\text{a}\) (n=4/6) | 4.01±1.03\(\text{a}\) (n=4/6) |
|                         | Paste   | 2.00±0.00\(\text{a}\) (n=2/6) | 1.67±0.58\(\text{a}\) (n=3/6) | 2.30\(\text{a}\) (n=1/6) |

T\(\text{0}\), 0 days after the arrival of the samples (packaging day); T\(\text{60}\), 60 days after the arrival of the samples; T\(\text{90}\), 90 days after the arrival of the samples; TVC, total viable counts; nd, not detected (below the detection limit of the method). Values are expressed as means ± standard deviation (log\_10 colony forming units g\(^{-1}\)). Values within brackets indicate the prevalence of positive samples. **Means in the same row with different superscript letters are significantly different (P<0.05).**

### Statistical analysis

Differences in mean mesophilic bacteria counts (log\_10 CFU g\(^{-1}\)), intrinsic properties [mean±standard deviation (SD)] and composition (%±SD) between sampling times were compared using Fisher’s least significant difference (LSD) test. All statistical analyses were performed with R Studio software, version 0.98.1103 (RStudio, Inc., Boston, MA, USA).

### Results

**Microbiological profile**

Pathogen microorganisms such as *L. monocytogenes* and *B. cereus* were always below the detection limit (10 and 100 log\(_{10}\) CFU g\(^{-1}\), respectively) both on the rind and in the inner paste. The mean TVC at T\(\text{0}\) was ca. 8 log\(_{10}\) CFU g\(^{-1}\) on the rind and ca. 3 log\(_{10}\) CFU g\(^{-1}\) in the inner paste, no significant increase during the storage was observed. The mean *Enterobacteriaceae* count was always greater on the rind as compared to the inner paste, with maximum concentration of ca. 5 log\(_{10}\) CFU g\(^{-1}\) and 3 log\(_{10}\) CFU g\(^{-1}\) respectively. Yeasts and moulds ranged between 3 and 5 log CFU g\(^{-1}\) on the rind and were ca. 2 log CFU g\(^{-1}\) on the inner paste. Table 2 shows the results (log\(_{10}\) CFU g\(^{-1}\); mean±SD) of the targeted microbial groups at the different sampling times.

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**Figure 1. Process flow of the analysed ricotta salata cheese samples.**

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[pag] [Italian Journal of Food Safety 2016; 5:5501]
Intrinsic properties and composition

The pH of ricotta salata cheese during refrigerated storage under vacuum packing decreased from an initial level (T₀) of 6.54±0.05 to a final level (T₉₀) of 5.68±0.16 (P<0.05). The aw was stable for the entire duration of the study at level between 0.97±0.01 and 0.98±0.01 (P>0.05). Minor changes in composition (moisture, fat, proteins, salt and total solids) were observed during storage. Table 3 shows the intrinsic properties (mean±SD) and composition (%±SD) evolution during storage (T₀, T₆₀, T₉₀) of ricotta salata cheese wheels.

Discussion

The durability of ricotta salata cheese defined by food business operator is generally of several months under refrigerated storage. The shelf-life of ricotta salata cheese depends upon a number of interacting factors other than storage temperature such as packaging conditions, product composition, presence of preservatives and competitive microflora. The combination of production technology (whey heating which inactivates natural microflora and absence of starter cultures), intrinsic properties (high pH and elevated aw), determine the ability of the product to support the growth of pathogen and spoilage microorganisms. The origin of contamination can be from raw materials or from the environment and from one microorganism to another (Almeida et al., 2013). The production of ricotta salata cheese, especially in the traditional batch manufacturing system, includes manual manipulation of the curd after floating, the exposition to environmental contamination from several food contact surfaces (drainage tables, plastic moulds, pressing equipment, drying shelves) and non-food contact surfaces (floors, drains, walls and ceiling).

Contaminants may reach the product by means of aerosol, condenses, dripping or inappropriate practices of operators (Wiedmann, 2003). The batch production of ricotta salata cheese is an open system which exposes the product to environmental contamination. The enumeration of total viable count showed a total bacterial load ca. 4 log greater on the rind as compared to the inner paste. This is the result of the exposition of the rind to the processing environment. This is confirmed by the high contamination with Enterobacteriaceae especially on the rind. Although no microbiological criteria is defined by Regulation (EC) No 2073/2005 (European Commission, 2005) for Enterobacteriaceae in whey cheeses that undergone heat treatment, the presence of these important indicator microorganisms reveals that in the cheese-making plants good hygienic and good manufacturing practices should be improved. In particular, the presence of Enterobacteriaceae in the inner paste indicates poor hygienic conditions in the phase of ricotta salata cheese molding. This is a very critical step in ricotta production, where manual manipulation can transfer contaminants from the processing environment into the inner product. With reference to pathogenic microorganisms it is demonstrated that L. monocytogenes contamination origins from the processing environment. Food contact and non-food contact surfaces represent the main vehicles of contamination, mainly due to manipulation of the product after the production process. This explains the frequent recovery of L. monocytogenes on ricotta salata cheese rind (Spanu et al., 2012, 2015a; Iba et al., 2013). Contamination of the inner paste is a rare finding and is generally associated with poor hygienic procedures during manual molding (Spanu et al., 2015b). Previous studies reported a prevalence of contamination ranging from 0.0% up to 30.0% of the samples (Pilo et al., 2007; Spanu et al., 2015a). In the present study L. monocytogenes was below the detection limit (<0.04 CFU g⁻¹) both on the rind and the inner paste during the entire shelf-life. This further confirms the importance of the strict implementation of hygiene during the production and storage steps (Tompkin, 2002; Carpentier and Cerf, 2011). The pathways of contamination of ricotta salata cheese with sporeformers differs with respect to L. monocytogenes and other environmental contaminants. In fact, spore forming bacteria enter the dairy chain mainly with raw milk (Heyndrickx, 2011). The spores present in milk can resist to heat applied to milk such as thermisation and pasteurisation treatments (Scheldeman et al., 2006). High temperature reached during whey heating (>80-85°C) activates the germination of residual spores, which are facilitated in their growth by the absence of other competitive microflora eliminated by heat treatments (Griffiths and Phillips, 1990). Despite it is almost impossible to avoid the contamination of spores from the dairy industry, cases of human illness associated to the consumption of dairy products contaminated with B. cereus is a rare finding (EFSA, 2015). B. cereus strains are generally not able to grow at temperature <10°C, only few psychrotrophic strains grow at temperature as low as 4-5°C. Therefore, temperature abuse is necessary during storage to lead multiplication of the vegetative cells. In a product such as ricotta salata cheese, characterised by an extended shelf-life, sporeformers have the time to reach concentration potentially dangerous to human health (ICMSF, 1996). An essential strategy for the control of sporeformers is the strict cold-chain maintenance for the entire shelf-life of the product. During the 90 days of refrigerated storage B. cereus was never detected, indicating that a combination of the quality of raw material and temperature control during storage can contribute to the safety of ricotta salata cheese.

Table 3. Intrinsic properties and composition evolution during storage of ricotta salata cheese wheels.

| Parameters       | T₀         | T₆₀        | T₉₀         |
|------------------|------------|------------|-------------|
| pH               | 6.54±0.03  | 6.04±0.14  | 5.68±0.18   |
| aw               | 0.973±0.005| 0.977±0.008| 0.978±0.005 |
| Moisture (%)     | 57.3±1.85  | 56.3±0.88  | 57.2±1.81   |
| Total solids (%) | 42.13±1.84 | 44.05±3.75 | 43.33±5.01  |
| Fat (%)          | 19.55±2.08 | 21.24±0.94 | 20.08±1.74  |
| Protein (%)      | 14.84±0.81 | 15.18±0.52 | 15.17±1.03  |
| Salt (%)         | 3.42±0.24  | 3.10±0.34  | 3.38±0.34   |

T₀, 0 days after the arrival of the samples (packaging day); T₆₀, 60 days after the arrival of the samples; T₉₀, 90 days after the arrival of the samples. Values are expressed as mean or % (where specified)±standard deviation. *Means in the same row with different superscript letters are significantly different (P<0.05).

Conclusions

The batch production system adopted in Sardinian traditional sheep ricotta salata cheese production and the presence of manual manipulation make this product particularly exposed to environmental contamination. In addition, the absence of a competitive microflora, the richness in nutrient and the intrinsic properties are favourable conditions for growth of spoilage and pathogen microorganisms. The strategies for the control of environmental contaminants are based on their prevention through the strict implementation of hygienic conditions during production process. Superficial contamination of the products can be reduced by the adoption of post-lethality treatment (i.e. heat treatment) in vacuum-packed product, allowing to extend ricotta...
Ibba M, Cossu F, Spanu V, Virdis S, Spanu C, Griffiths MW, Phillips JD, 1990. Incidence, European Commission, 2005. Commission EFSA, 2015. The European Union summary De Santis EPL, Foddai A, Virdis S, Marongiu P, CDC, 2012. Multistate outbreak of listeriosis linked to imported Frescolina Martebrand ricotta salata cheese. Int J Food Microbiol 167:303-9. Campertin B, Cerf O, 2011. Persistence of Listeria monocytogenes in food industry equipment and premises. Int J Food Microbiol 145:1-8. CDC, 2012. Multistate outbreak of listeriosis linked to imported Frescolina Martebrand ricotta salata cheese (final update). Centers for Disease Control and Prevention, Atlanta, GA, USA. Available from: http://www.cdc.gov/listeria/outbreaks/cheese-09-12/ De Santis EPL, Foddai A, Virdis S, Marongiu P, Pilo AL, Scarano C, 2008. Toxin gene pattern in Bacillus cereus group strains isolated from sheep ricotta cheese. Vet Rec Commun 32:323-6. EFSA, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J 13:3991. European Commission, 2005. Commission Regulation of 15 November 2005 on microbiological criteria for foodstuffs, 2073/2005/EC. In: Official Journal, L 338/1, 22/12/2005. Griffiths MW, Phillips JD, 1990. Incidence, source and some properties of psychrotrophic Bacillus found in raw and pasteurised milk. Int J Dairy Technol 43:62-6. Ibba M, Cossu F, Spanu V, Virdis S, Spanu C, Scarano C, De Santis EPL, 2013. Listeria monocytogenes contamination in dairy plants: evaluation of Listeria monocytogenes environmental contamination in two cheese-making plants using sheep milk. Ital J Food Safety 2:e31. Heyndrickx M, 2011. The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. Appl Environ Soil Sci 2011:561975. ICMSF, 1996. International commission on microbiological specification for foods. Microorganisms in foods. 1st ed. Clays Ltd., Suffolk, UK. ISO, 1996. Microbiology of food and animal feeding stuffs-horizontal method for the detection and enumeration of Listeria monocytogenes. Part 1: detection method. ISO Norm 11290-1. International Standardization Organization ed., Geneva, Switzerland. ISO, 2003. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms: colony count technique at 30°C. ISO Norm 4833. International Standardization Organization ed., Geneva, Switzerland. ISO, 2004a. Microbiology of food and animal feeding stuffs. Horizontal methods for the detection and enumeration of Enterobacteriaceae. Part 2: colony-count method. ISO Norm 21528-1. International Standardization Organization ed., Geneva, Switzerland. ISO, 2004b. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive Bacillus cereus and colony-count technique at 30°C. ISO Norm 7932. International Standardization Organization ed., Geneva, Switzerland. ISO, 2004c. Milk and milk products. Enumeration of colony-forming units of yeasts and/or moulds. Colony-count technique at 25 °C. ISO Norm 6611:2004 (IDF 94:2004). International Standardization Organization ed., Geneva, Switzerland. Laore, 2015. L’agroalimentare a marchio di qualità. Formaggi. Laore-Agenzia regionale per lo sviluppo in agricoltura, Cagliari, Italy. Available from: http://www.sardegnaagricoltura.it/documenti/14_43_201410 06144130.pdf Pilo AL, Marongiu P, Corgiolu G, Virdis S, Scarano C, De Santis EPL, 2007. Listeria monocytogenes contamination sources in sheep cheese processing plants and strains virulence genes typing. In: Proceeding of the 5th IDF International Symposium on the Challenge to Sheep and Goats Milk Sectors, Alghero, Italy. RASFF, 2008. The Rapid Alert System for Food and Feed annual report. Available from: http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2008_en.pdf Scheldeman P, Herman L, Foster L, Heyndrickx M, 2006. Bacillus sporothermodurans and other highly heat-resistant spore formers in milk. J Appl Microbiol 101:542-55. Spanu C, Scarano C, Ibbi M, Spanu V, De Santis EPL, 2015a. Occurrence and traceability of Listeria monocytogenes strains isolated from sheep’s milk cheese making plants environment. Food Control 318:325-47. Spanu C, Scarano C, Spanu V, Pala C, Di Salvo R, Piga C, Buschettu L, Casti D, Lamon S, Cossu F, Ibbi M, De Santis EPL, 2015b. Comparison of post-lethality thermal treatment conditions on the reduction of Listeria monocytogenes and sensory properties of vacuum packed ricotta salata cheese. Food Control 740:747-50. Spanu C, Scarano C, Spanu V, Penna C, Virdis S, De Santis EPL, 2012. Listeria monocytogenes growth potential in Ricotta salata cheese. Int Dairy J 10:122-24. Spanu C, Spanu V, Pala C, Virdis S, Scarano C, De Santis EPL, 2013. Evaluation of a post-lethality treatment against Listeria monocytogenes on Ricotta salata cheese. Food Control 30:200-5. Tompkin RB, 2002. Control of Listeria in the food-processing environment. J Food Protect 65:709-23. Wiedmann M, 2003. ADSA Foundation Scholar Award. An integrated science-based approach to dairy food safety: Listeria monocytogenes as a model system. J Dairy Sci 86:1865-75.