

Evolution of the microbiological profile of vacuum-packed *ricotta salata* cheese during shelf-life

Daniele Casti,¹ Christian Scarano,¹
Carlo Pala,¹ Francesca Cossu,¹
Sonia Lamon,¹ Vincenzo Spanu,¹
Michela Ibba,¹ Anna Maria Mocci,¹
Francesco Tedde,¹ Gavino Nieddu,²
Carlo Spanu,¹
Enrico Pietro Luigi De Santis¹

¹Department of Veterinary Medicine,
University of Sassari, Sassari;

²Cooperativa Allevatori Ovini, Fenosu
(OR), Italy

Abstract

Ricotta salata cheese 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. *Ricotta salata* cheese is very critical for the possible growth of pathogenic and spoilage microorganisms. Sporadic cases of listeriosis associated with *ricotta salata* cheese have been reported over recent years. The objective of the present study was to assess the evolution of spoilage and pathogen microorganism of vacuum-packed *ricotta salata* cheese during the entire product shelf-life. The durability study was conducted on 18 vacuum-packed *ricotta salata* cheese samples analysed at the beginning of the shelf-life and after 60 and 90 days of refrigerated storage. Pathogens as *Listeria monocytogenes* and *Bacillus cereus* were never detected. During shelf-life total bacterial counts ranged between 7.90 ± 0.64 and 9.19 ± 0.58 CFU g⁻¹ on the rind and between 2.95 ± 0.68 and 4.27 ± 1.10 CFU g⁻¹ in the inner paste, while *Enterobacteriaceae* ranged between 4.22 ± 0.66 and 5.30 ± 0.73 CFU g⁻¹ on the rind and 3.13 ± 1.80 and 2.80 ± 0.88 CFU g⁻¹ in the inner paste. By considering the technology used, the intrinsic properties and the almost total absence of competing microflora, *ricotta salata* cheese can support the growth of spoilage and pathogen microorganisms originating from the processing environment. The high level of total bacterial counts and *Enterobacteriaceae* observed both on the rind and in the inner paste suggests contamination of the product from the processing environment. Therefore, a strict implementation of hygiene during processing is essential in order to reduce the load of environmental contaminants that may grow during refrigerated storage.

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*, *Anthotyros* and *Manouri* (Greece), *Anari* (Cyprus), *Requesón* (Spain), *Requeijao* (Portugal), *Brocciu* (France), *Urdă* (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 pre-heated 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 (a_w) 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

Correspondence: Carlo Spanu, Department of Veterinary Medicine, University of Sassari, via Vienna 2, 07100 Sassari.
Tel: +39.079.229454 - Fax: +39.079.229458.
E-mail: cspanu@uniss.it

Key words: Whey cheese; Sheep; Composition; Shelf life.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: this work was funded by *Programma di Sviluppo Rurale Sardegna 2007-2013 Misura 124 Cooperazione per lo Sviluppo di Nuovi Prodotti, Processi e Tecnologie nei Settori Agricolo Alimentare e in quello Forestale*, project ID: H78F13000050007. The authors are grateful to all the members of the joint dairy industry consortium *Associazione Temporanea di Scopo - Aziende Casearie Riunite* for their cooperation in the research.

Received for publication: 25 August 2015.

Revision received: 9 October 2015.

Accepted for publication: 9 October 2015.

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Italian Journal of Food Safety 2016; 5:5501
doi:10.4081/ijfs.2016.5501

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 originates 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 ($4\pm 2^\circ\text{C}$) during the entire product shelf-life.

Materials and Methods

Ricotta salata cheese samples

The study was conducted on vacuum-packed ricotta salata cheese wheels (Criovac 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 ($4\pm 1^\circ\text{C}$) until analysis was performed.

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 (T_0), time 60 (T_{60}) and time 90 (T_{90}) 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, 2004a), *Enterobacteriaceae* (ISO 21528-2; ISO, 2004a), yeasts and moulds (ISO 6611/IDF 94; ISO, 2004c).

Physico-chemical properties and composition

pH and a_w were measured using pH meter GLP22 (Crison 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.

Statistical analysis

Differences in mean mesophilic bacteria counts (\log_{10} CFU g^{-1}), intrinsic properties [mean \pm standard deviation (SD)] and composition (% \pm 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_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 \pm SD) of the targeted microbial groups at the different sampling times.

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

Analysis	Sampling time		
	T_0	T_{60}	T_{90}
Microbiological profile	6	6	6
Intrinsic properties and composition	6	6	6

T_0 , 0 days after the arrival of the samples (packaging day); T_{60} , 60 days after the arrival of the samples; T_{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_0	T_{60}	T_{90}
TVC	Rind	7.90 \pm 0.64 ^a (n=6/6)	7.79 \pm 2.21 ^a (n=6/6)	9.19 \pm 0.58 ^a (n=6/6)
	Paste	2.95 \pm 0.68 ^a (n=5/6)	4.51 \pm 1.66 ^a (n=6/6)	4.27 \pm 1.10 ^a (n=6/6)
<i>Enterobacteriaceae</i>	Rind	4.22 \pm 0.66 ^b (n=4/6)	5.16 \pm 0.59 ^{ab} (n=5/6)	5.30 ^a \pm 0.73 (n=6/6)
	Paste	nd	3.13 \pm 1.80 ^a (n=3/6)	2.80 \pm 0.88 ^a (n=2/6)
Yeasts and moulds	Rind	3.02 \pm 0.36 ^b (n=3/6)	4.99 \pm 0.81 ^a (n=4/6)	4.01 \pm 1.03 ^{ab} (n=4/6)
	Paste	2.00 \pm 0.00 ^a (n=2/6)	1.67 \pm 0.58 ^a (n=3/6)	2.30 ^a (n=1/6)

T_0 , 0 days after the arrival of the samples (packaging day); T_{60} , 60 days after the arrival of the samples; T_{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 mean \pm standard deviation (\log_{10} colony forming units g^{-1}). Values within brackets indicate the prevalence of positive samples. ^{a,b}Means in the same row with different superscript letters are significantly different ($P < 0.05$).

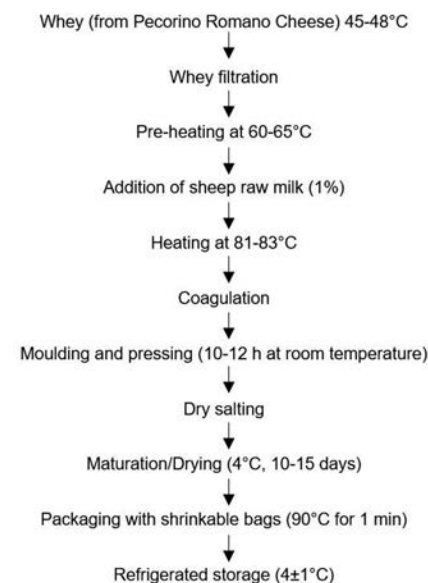


Figure 1. Process flow of the analysed *ricotta salata* cheese samples.

Intrinsic properties and composition

The pH of *ricotta salata* cheese during refrigerated storage under vacuum packing decreased from an initial level (T_0) of 6.54 ± 0.05 to a final level (T_{90}) of 5.68 ± 0.16 ($P < 0.05$). The a_w 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 \pm SD) and composition (% \pm SD) evolution during storage (T_0 , T_{60} , T_{90}) 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 a_w), 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 differs 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; Ibba *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 ($> 80-85^\circ C$) 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 $< 10^\circ C$, only few psychrotrophic strains grow at temperature as low as $4-5^\circ C$. 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.

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*

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

Parameters	T_0	T_{60}	T_{90}
pH	6.54 ± 0.03^a	6.04 ± 0.14^b	5.68 ± 0.18^c
a_w	0.973 ± 0.005^a	0.977 ± 0.008^a	0.978 ± 0.005^a
Moisture (%)	57.9 ± 1.85^a	56.3 ± 0.88^a	57.2 ± 1.81^a
Total solids (%)	42.13 ± 1.84^a	44.65 ± 3.75^a	43.33 ± 5.01^a
Fat (%)	19.55 ± 2.08^a	21.24 ± 0.94^a	20.08 ± 1.74^a
Protein (%)	14.84 ± 0.81^a	15.18 ± 0.52^a	15.17 ± 1.03^a
Salt (%)	3.42 ± 0.24^a	3.10 ± 0.34^a	3.38 ± 0.34^a

T_0 , 0 days after the arrival of the samples (packaging day); T_{60} , 60 days after the arrival of the samples; T_{90} , 90 days after the arrival of the samples. Values are expressed as mean or % (where specified) \pm standard deviation. **Means in the same row with different superscript letters are significantly different ($P < 0.05$).

salata cheese shelf-life. As far as control of contaminants originating from raw ingredients are concerned, which are not eliminated during processing (say sporeformers), it relies upon prevention of their growth through the maintenance of an unbroken chill chain during the storage.

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