

Occurrence and behavior of *Bacillus cereus* in naturally contaminated ricotta salata cheese during refrigerated storage

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**1 Occurrence and behavior of *Bacillus cereus* in naturally contaminated Ricotta salata cheese**  
**2 during refrigerated storage**

3 Carlo Spanu<sup>\*1</sup>, Christian Scarano<sup>1</sup>, Vincenzo Spanu<sup>1</sup>, Carlo Pala<sup>1</sup>, Daniele Casti<sup>1</sup>, Sonia Lamon<sup>1</sup>,

4 Francesca Cossu<sup>1</sup>, Michela Ibba<sup>1</sup>, Gavino Nieddu<sup>2</sup>, Enrico P. L. De Santis<sup>1</sup>

5

6 <sup>1</sup>Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100, Sassari, Italy

7 <sup>2</sup>Cooperativa Allevatori Ovini Formaggi Soc. Coop. Agricola, Loc. "Perda Lada" Fenosu, 09170,

8 Oristano, Italy

9

10 <sup>\*</sup>Corresponding author. Tel.: +39 079 229454; fax.: +39 079 229458. E-Mail address:

11 cspanu@uniss.it (C. Spanu); Via Vienna 2, 07100, Sassari, Italy.

12

**13 Abstract**

14 The present study shows the fate of *B. cereus* in refrigerated ricotta salata cheese during shelf-life.

15 144 ricotta salata cheese belonging to nine naturally contaminated batches were stored refrigerated

16 and analyzed at 24 h, 30, 60 and 90 days of storage. Total bacterial count, *B. cereus* spores and

17 vegetative forms, intrinsic properties and composition were determined. The presence of spores was

18 sporadic while the prevalence and the level of *B. cereus* vegetative cells decreased respectively

19 from 83.3% and  $4.65 \pm 0.74$  cfu g<sup>-1</sup> at the beginning of the observation period to 33.3% and

20  $1.99 \pm 0.55$  cfu g<sup>-1</sup> after 90 days. No information is currently available on the fate of *B. cereus* in

21 ricotta salata. The production process of ricotta salata includes steps such as whey heating followed

22 by slow cooling of clots, which expose to the risk of spore germination and successive growth to

23 levels compatible with toxins production. The prolonged refrigerated storage was not favorable to

24 sporulation, explaining the successive death of vegetative cells. The present study demonstrate the

25 potential risk of food poisoning as consequence of pre-formed emetic toxins in ricotta salata. Food

26safety of ricotta salata relies on the rapid refrigeration of the product during critical phases for  
27cerulide production.

28Keywords: *Bacillus cereus*; whey cheese; sheep's milk

29

### 301. Introduction

31The *Bacillus cereus* group includes Gram-positive rod shaped spore-forming bacteria, which are  
32widely distributed in the natural environment. Within the group, *B. cereus sensu stricto* is the most  
33important organism causing food spoilage and food-borne illness (Kramer and Gilbert, 1989). *B.*  
34*cereus* causes two clinical forms of foodborne illness: the emetic and the diarrheal syndrome  
35(Granum and Lund, 1997). A dose of  $10^5$ - $10^8$  cells or spores per gram is generally considered  
36necessary to cause illness (ICMSF, 1996; Granum and Lund, 1997). *B. cereus* is frequently isolated  
37from raw milk and dairy products thus, representing a serious concern for the dairy industry  
38(Svensson et al., 2006). Due to its ubiquitous nature and the extreme resistance of endospores to  
39several harsh conditions (Nicholson, et al., 2000), it is difficult to avoid the contamination of dairy  
40products. *B. cereus* can enter the dairy chain mainly through raw milk contaminated at farm level  
41(Heyndrickx, 2011). However, contamination may also arise from the food-processing environment  
42(da Silva Fernandes et al., 2014). Dairy products have been seldom associated with human illness  
43despite the frequent contamination with *B. cereus* (EFSA, 2005). Whey products processed at high  
44temperatures and successively stored refrigerated are particularly exposed to the risk of *B. cereus*  
45(Heyndrickx and Scheldeman, 2002). The endospores are activated by whey heating applied during  
46protein denaturation ( $>80^{\circ}\text{C}$ ) and vegetative cells are then facilitated in their growth by the absence  
47of competing microbiota, inactivated by the heat treatment (Scheldeman et al., 2006). *B. cereus*  
48psychotropic strains can grow to temperature as low as  $4-5^{\circ}\text{C}$  and during the refrigerated storage  
49can reach levels potentially harmful for human health (Huck et al., 2007). Ricotta salata is a  
50traditional dry and salted sheep's milk whey cheese produced in Sardinia (Italy). Technology and  
51microbiological profile of ricotta salata have been previously described (Spanu et al., 2015). The

attributed shelf-life of ricotta salata is generally up to several months under refrigerated storage (Casti et al., 2016). The present study was conducted following a case of large *B. cereus* contamination of ricotta salata occurred in one sheep's milk cheese-making plant operating in Sardinia. During the period September-October 2014, a local food business operator observed the presence of *B. cereus* contamination in ricotta salata samples during routine microbiological testing conducted as part of their procedure based on HACCP principles. The mean level of contamination was  $5.57 \pm 0.15 \log_{10} \text{ cfu g}^{-1}$  in a batch. Although no food safety criteria for *B. cereus* are applicable to foodstuffs placed on the market during their shelf-life (EC Regulation No. 2073/2005), the food business operator as corrective action withdrew the entire batch of ricotta salata. The subsequent production batches positive for the presence of *B. cereus*, were destined to a durability study. The few published data existing on *B. cereus* contamination in ricotta salata produced in Sardinia reported a prevalence of ca. 15% and a contamination level ranging from 1 to 3  $\log_{10} \text{ cfu g}^{-1}$  (Cosentino, et al., 1997; De Santis et al., 2008; Fadda et al., 2012). Despite the reported contamination levels are below the dose necessary to cause illness, they demonstrate that *B. cereus* in ricotta salata represents a potential concern for consumer's health. No published reports are currently available on the fate of *B. cereus* in naturally contaminated ricotta salata stored under refrigerated conditions. The aim of the present study was to describe the evolution of *B. cereus* in naturally contaminated ricotta salata during shelf-life and to assess the potential health risk associated with the microorganism survival or growth.

71

## 722. Materials and methods

73

### 742.1. Ricotta salata batches and samples

75 Ricotta salata batches used in the study were selected based on the natural occurrence of *B. cereus*.

76 With this aim, during the period September-October 2014, ricotta salata production batches were

77 tested on a daily basis for the presence of *B. cereus*. From each positive batch were randomly

78 selected sixteen ricotta salata wheels. Samples were immediately vacuum packed in plastic bags,

79transported refrigerated to the laboratory and stored in cold room ( $4\pm2^{\circ}\text{C}$ ) until analyses were  
80performed.

81

## 822.2. *Experimental design*

83Ricotta samples were analyzed at four different times during the shelf-life. Sampling times were:  
84within 24 h after the arrival of ricotta salata wheels defined as time zero ( $T_0$ ), 30, 60 and 90 days  
85after the production defined respectively as time 30 ( $T_{30}$ ), time 60 ( $T_{60}$ ) and time 90 ( $T_{90}$ ). From each  
86of the nine different batches and at each sampling time, two samples were used for microbiological  
87analysis and two samples for physico-chemical determinations.

88

## 892.4. *Microbiological analysis*

90Ricotta salata samples were analyzed for the determination of total aerobic mesophilic bacteria (ISO  
914833, 2013) and enumeration of *B. cereus* (ISO 7932, 2004). The enumeration of both *B. cereus*  
92vegetative cells and spores was determined, respectively before and after heating at  $80^{\circ}\text{C}$  for 10 min  
93by plating two 0.1 mL aliquot on selective chromogenic culture media such as Mannitol Egg Yolk  
94Polymyxin agar (MYP, Biolife, Milan, Italy) and Polymyxin Pyruvate Egg-Yolk Mannitol  
95Bromothymol Blue (PEMBA, Oxoid) agar. Samples were incubated at  $30^{\circ}\text{C}$  in aerobic conditions  
96for 24 h. From each positive sample were picked five presumptive *B. cereus* colonies, transferred  
97onto Trypticase Soy Agar (TSA, Biolife) and incubated at  $37^{\circ}\text{C}$  for 24 h. Each isolate was  
98submitted to phenotypic identification and successively confirmed by PCR (Oh et al., 2012).

99

## 1002.5. *Intrinsic properties and composition*

101PH and  $a_w$  were measured using pH meter GLP22 (Crison Instruments SA, Barcelona, Spain) and  
102water activity meter Aqualab 4 TE (Decagon, Pullman, WA, USA), respectively. Determination of  
103centesimal composition (% of moisture, fat, protein, salt and total solids) was performed using the  
104Near Infrared Transmittance (NIT) compositional analyzer (FOSS, Eden Prairie, MN, USA).

105

## 1063. **Results**

107

### 1083.1. *Microbiological profile*

109The mean aerobic mesophilic counts ( $\log_{10}$  cfu g<sup>-1</sup>;  $\bar{x} \pm \text{SD}$ ) of ricotta salata analyzed at T<sub>0</sub>, T<sub>30</sub>, T<sub>60</sub>  
110and T<sub>90</sub> were 5.17±1.39, 5.69±0.54, 5.99±0.67 and 5.62±0.87, respectively. The prevalence of *B.*  
111*cereus* vegetative cells and the mean contamination level decreased during the refrigerated storage  
112( $P<0.05$ ). At T<sub>0</sub>, the prevalence was 83.3% with counts ranging from 3.45  $\log_{10}$  cfu g<sup>-1</sup> to 6.20  $\log_{10}$   
113cfu g<sup>-1</sup>, while at T<sub>90</sub> the observed prevalence was 33.3% with counts ranging from 1.30  $\log_{10}$  cfu g<sup>-1</sup>  
114to 2.56  $\log_{10}$  cfu g<sup>-1</sup> (table 1). The mean reductions over time ( $\Delta T$ ) in *B. cereus* vegetative cells  
115concentration ( $\log_{10}$  cfu g<sup>-1</sup>) were 0.38, 1.74 and 2.66 at T<sub>30</sub>, T<sub>60</sub> and T<sub>90</sub>, respectively. The detection  
116of *B. cereus* spores after heat activation was observed in two samples belonging to two different  
117batches, one at T<sub>30</sub> (2.30  $\log_{10}$  cfu g<sup>-1</sup>) and one at T<sub>60</sub> (2.0  $\log_{10}$  cfu g<sup>-1</sup>), respectively. Out of 49 total  
118positive samples (68.0%) were isolated 245 presumptive *B. cereus* strains of which 101 were  
119confirmed by molecular identification.

120  
1213.2. *Physico-chemical characteristics*  
122The pH values ranged between 6.23 and 6.67 at T<sub>0</sub> and between 5.30 and 6.32 at T<sub>90</sub>, while a<sub>w</sub>  
123values ranged between 0.964 and 0.986 at T<sub>0</sub> and between 0.976 and 0.983 at T<sub>90</sub>. The evolution of  
124the mean centesimal composition values (%;  $\bar{x} \pm \text{SD}$ ) at different sampling times is reported in table

1252.  
126

#### 1274. Discussion

128Despite raw milk is the main source of contamination of dairy product with sporeformers, their  
129level is generally low, <1-10<sup>2</sup>cfu mL<sup>-1</sup> (Vissers et al., 2007). Seasonal variation has been reported  
130with counts as high as 10<sup>4</sup>cfu mL<sup>-1</sup> (Slaghuis et al., 1997; TeGiffel et al., 2002; Coorevits et al.,  
1312008). The presence of *B. cereus* in ricotta salata is a rare finding, with maximum contamination  
132level of ca. 3  $\log_{10}$  cfu g<sup>-1</sup> (Cosentino et al., 1997; De Santis et al., 2008; Fadda et al., 2012; Spanu et  
133al., 2012). The high level of contamination, up to 8.33  $\log_{10}$  cfu g<sup>-1</sup>, observed in the present study  
134and the large number of positive batches (nine) over a limited period of time (three months), should

135be considered as an event strictly associated with the late summer and early fall production period.  
136Microbiological testing of each production batch, conducted on a regular basis in the frame of the  
137food business operator's HACCP procedures, showed no occurrence of *B. cereus* contamination  
138during the rest of the year. This could be explained with the typical sheep's milk breeding systems  
139adopted in Sardinia. Milk production is seasonal, starting from December until July. The peak of  
140milk production is concentrated between January and May, with a decrease between June and  
141August, when the sheep start entering in the dry period. Cheese-making during the dry period relies  
142on the milk available provided by flocks adopting the out-of-season breeding system. Poor pasture  
143quality during this season determines a decline in milk yield and microbiological quality (Sitzia et  
144al., 2015). Due to economic reasons, raw milk is picked and transformed every three or five days  
145instead that daily. In addition, during winter and spring periods sheeps mainly graze on grass  
146pasture, while during the summer and fall periods on stubble with concentrate and feedstuff  
147supplement, which may increase the risk of transferring spores into raw milk. Total bacterial count  
148and yield records of the milk used to make the ricotta salata used in the present study, were obtained  
149by the food business operator. Data confirmed differences in milk yield and microbiological quality  
150over the milking season. In the period from January to June, the total bacterial count (geometric  
151mean) of raw milk was ca. 140,000 cfu mL<sup>-1</sup> with an average production of 2,150,000 l, while in the  
152out-of-season period the total bacterial count was ca. 1,100,000 cfu mL<sup>-1</sup> with an average milk yield  
153of 135,000 l. Therefore, the production of ricotta salata during the out-of-season period was  
154characterized by risk factors increasing the likelihood of a high initial level of *B. cereus*  
155contamination in the product. The greater relative decrease in *B. cereus* vegetative cells was  
156observed after 60 days (-1.36 log<sub>10</sub> cfu g<sup>-1</sup>) and after 90 days (-0.92 log<sub>10</sub> cfu g<sup>-1</sup>) of refrigerated  
157storage. Since psychrotrophic strains can grow at temperature as low as 4-5°C, contamination was  
158likely due to mesophilic strains which minimum growth temperature is 15°C (ICMSF, 1996).  
159Growth and survival characteristics of *B. cereus* vary widely between strains and depend upon a

complex series of interacting factors such as temperature, pH, water activity (NaCl concentration), nutrients and presence of competitive microbiota. *B. cereus* is generally a poor competitor in unpasteurized products (Andersson, Ronner, & Granum, 1995). The high total bacterial count (ca. 6 log), combined with the decrease of pH values (from 6.49 to 5.63) observed over time, suggest the possible presence of contaminants from the whey or the environment that may have exerted a possible competitive action. A previous study, conducted on vacuum packed ricotta salata, showed a mean aerobic mesophilic bacteria count ( $\log_{10}$  cfu g<sup>-1</sup>) after 2 and 4 months of refrigerated storage ranging from 7.56±0.85 and 4.57±0.62 on the rind and from 3.64±0.71 and 2.95±0.65 on the inner paste, respectively (Spanu et al., 2013). At the beginning of the ricotta salata storage *B. cereus* is mainly present in its vegetative form, as consequence of heat activation of spores occurred during whey heating. The successive phase of pressing of the warm clots, expose the product to temperature at risk for the growth of the vegetative forms to levels compatible with the emetic toxin (cerulide) production. The reduction in *B. cereus* vegetative cells count over the storage period suggests the death of the microorganism rather than sporulation, since no grow was observed in samples analyzed after pasteurization. The sporulation is a complex process which occurs as response to stress such as starvation, high cell density ( $10^6$ - $10^7$  cfu g<sup>-1</sup>) or DNA damage and it is regulated by hundreds of genes (Eichenberger et al., 2003; Piggot and Hilbert, 2004). Borge et al. (2001) concluded that vegetative cells are unlikely to develop endospores in refrigerated media. The high levels of ricotta salata contamination with *B. cereus* observed in the present study indicates that this product could represents a risk for human health due to the potential presence of pre-formed emetic toxins in the product. The low contamination level observed in the product after long refrigerated storage could lead to the wrong conclusion that the product is safe, while cerulide still persists. In fact, cerulide is highly resistant to heat, low pH, and proteolytic activity of pepsin and trypsin (Kramer and Gilbert, 1989; Rajkovic et al., 2008). Further investigation is needed in order to



184 assess whether the origin of the contamination is from ingredients, processing environment or from  
185 packaging materials and to determine the pathogenicity of the strains.

## 186 **5. Conclusion**

187 Ricotta salata production process includes critical phases such as heat coagulation and slow cooling  
188 of clots, which support the activation of *B. cereus* spores and the successive growth of vegetative  
189 cells, in the absence of competing microbiota. The present investigation demonstrates that the level  
190 of *B. cereus* vegetative cells in naturally contaminated ricotta salata decreases during refrigerated  
191 storage, while the presence of spores is a rare finding. The control of *B. cereus* in ricotta salata  
192 relies on one hand on limiting the level of spores in raw milk, and as consequence in the whey, and  
193 on the other hand in preventing germination and successive growth of vegetative cells.

194

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201

## 202 **References**

- 203 1. Andersson, A., Ronner, U., Granum, P. E. (1995). What problems does the food industry  
204 have with the spore-forming pathogens *Bacillus cereus* and *Clostridium perfringens*?  
205 International Journal of Food Microbiology, 28(2), 145-155.

- 206 2. Borge, G. I. A., Skeie, M., Sørhaug, T., Langsrud, T., Granum, P. E. (2001). Growth and  
 207 toxin profiles of *Bacillus cereus* isolated from different food sources. International Journal  
 208 of Food Microbiology, 69, 237-246.
- 209 3. Casti, D., Scarano, C., Pala, C., Cossu, F., Lamon, S., Spanu, V., Ibba, M., Mocci, A.M.,  
 210 Tedde, F., Nieddu, G., Spanu, C., De Santis, E.P.L. (2016). Evolution of the microbiological  
 211 profile of vacuum packed sheep's ricotta salata cheese wheels during shelf-life. Italian  
 212 Journal of Food Safety, *Accepted, in press*.
- 213 4. Coorevits, A., De Jonghe, V., Vandroemme, J., Reekmans, R., Heyrman, J., Messens, W., De  
 214 Vos, P., Heyndrickx, M. (2008). Comparative analysis of the diversity of aerobic spore-  
 215 forming bacteria in raw milk from organic and conventional dairy farms. Systematic and  
 216 Applied Microbiology, 31, 126-140.
- 217 5. Cosentino, S., Mulargia, A. F., Pisano, B., Tuveri, P., Palmas, F. (1997). Incidence and  
 218 biochemical characteristics of *Bacillus* flora in Sardinian dairy products. International  
 219 Journal of Food Microbiology, 38(2), 235-238.
- 220 6. da Silva Fernandes, M., Fujimoto, G., Schneid, I., Kabuki, D. Y., Kuaye, A. Y. (2014).  
 221 Enterotoxigenic profile, antimicrobial susceptibility, and biofilm formation of *Bacillus*  
 222 *cereus* isolated from ricotta processing. International Dairy Journal, 38(1), 16-23.
- 223 7. De Santis, E. P. L., Foddai, A., Viridis, S., Marongiu, P., Pilo, A. L., Scarano, C. (2008).  
 224 Toxin gene pattern in *Bacillus cereus* group strains isolated from sheep ricotta cheese.  
 225 Veterinary research communications, 32(1), 323-326.
- 226 8. Eichenberger, P., Jensen, S. T., Conlon, E. M., van Ooij, C., Silvaggi, J., Gonzalez-Pastor, J.  
 227 E., Fujita, M., Ben-Yehuda, S., Stragier, P., Liu, J. S., Losick, R. (2003). The sigmaE regulon  
 228 and the identification of additional sporulation genes in *Bacillus subtilis*. Journal of  
 229 Molecular Biology, 327, 945-972.

9. EFSA (2005). European Food Safety Authority. Opinion of the scientific panel on biological hazards of *Bacillus cereus* and other *Bacillus* spp. in foodstuff. The EFSA Journal, 175, 1-48.
10. Fadda, A., Delogu, A., Mura, E., Noli, A. C., Porqueddu, G., Rossi, M. L., Terrosu, G. (2012). Presence of *Bacillus cereus*, *Escherichia coli* and Enterobacteriaceae in fresh and salted Ricotta cheese: official controls in Sardinia during the period 2009–2012. Italian Journal of Food Safety, 1(5), 43-45.
11. Granum, P. E., Lund, T. (1997). *Bacillus cereus* and its food poisoning toxins. FEMS Microbiology Letters, 157(2), 223-228.
12. Heyndrickx, M., Scheldeman, P. (2002). Bacilli associated with spoilage in dairy and other food products. In: Berkely, R., Heyndrickx, M., Logan, N.A., De Vos, P. (Eds.), Applications and Systematics of Bacillus and Relatives. Blackwell Science, Oxford, UK, 64-82.
13. Heyndrickx, M. (2011). The importance of endospore-forming bacteria originating from soil for contamination of industrial food processing. Applied and Environmental Soil Science, Volume 2011, Article ID 561975, 11 pages, doi:10.1155/2011/561975.
14. Huck, J. R., Hammond, B. H., Murphy, S. C., Woodcock, N. H., Boor, K. J. (2007). Tracking spore-forming bacterial contaminants in fluid milk-processing systems. Journal of Dairy Science, 90, 4872-4883.
15. ICMSF, 1996. International Commission on Microbiological Specifications for Foods. Micro-organisms in Foods. Volume 5: Microbiological Specification of Food Pathogens. London, Blackie Academic and Professional, 514 pp.
16. ISO, (2004). Microbiology of food and animal feeding stuffs-horizontal method for the enumeration of presumptive *Bacillus cereus*: Colony-count technique at 30 °C. ISO 7932:2004. Geneva, Switzerland: International Organization for Standardization.

17. ISO, (2013). Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique. ISO 4833-1:2013. Geneva, Switzerland: International Organization for Standardization.
18. Kramer, J. M., Gilbert, R. J. (1989). *Bacillus cereus* and other *Bacillus* species, p. 21-70. In: M.P. Doyle (ed.), Foodborne Bacterial Pathogens. Marcel Dekker, New York.
19. Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., Setlow, P. (2000). Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiology and Molecular Biology Reviews, 64, 548-572.
20. Oh, M.-H., Ham, J.-S., Cox, J. M. (2012). Diversity and toxigenicity among members of the *Bacillus cereus* group. International Journal of Food Microbiology, 152, 1-8.
21. Piggot, P. J. Hilbert, D. W. (2004). Sporulation of *Bacillus subtilis*. Current Opinion in Microbiology, 7, 579-586.
22. Rajkovic, A., Uyttendaele, M., Vermeulen, A., Andjelkovic, M., Fitz-James, I., in't Veld, P., Denon, Q., Vêrhe, R., Debevere, J. (2008). Heat resistance of *Bacillus cereus* emetic toxin, cereulide. Letters in Applied Microbiology, 46, 536-541.
23. Scheldeman, P., Herman, L., Foster, S., Heyndrickx, M. (2006). *Bacillus sporothermodurans* and other highly heat-resistant spore formers in milk. Journal of Applied Microbiology, 101(3), 542-555.
24. Sitzia, M., Bonanno, A., Todaro, M., Cannas, A., Atzori, A. S., Francesconi, A. H. D., & Trabalza-Marinucci, M. (2015). Feeding and management techniques to favour summer sheep milk and cheese production in the Mediterranean environment. Small Ruminant Research, 126, 43-58.
25. Slaghuis, B. A., TeGiffel, M. C., Beumer, R. R., André, G. (1997). Effect of pasturing on the incidence of *Bacillus* spores in raw milk. International Dairy Journal, 7, 201-205.

26. Spanu, C., Scarano, C., Spanu, V., Penna, C., Viridis, S., De Santis, E. P. L. (2012). *Listeria monocytogenes* growth potential in Ricotta salata cheese. International Dairy Journal, 120, 122-24.
27. Spanu, C., Spanu, V., Pala, C., Viridis, S., Scarano, C., De Santis, E. P. L. (2013). Evaluation of a post-lethality treatment against *Listeria monocytogenes* on Ricotta salata cheese. Food Control, 30, 200-205.
28. Spanu, C., Scarano, C., Spanu, V., Pala, C., Di Salvo, R., Piga, C., Buschettu, L., Casti, D., Lamon, S., Cossu, F., Ibba, M., De Santis, E. P. L. (2015). Comparison of post-lethality thermal treatment conditions on the reduction of *Listeria monocytogenes* and sensory properties of vacuum packed ricotta salata cheese. Food Control, 50, 740-747.
29. Svensson, B., Monthan, A., Shaheen, R., Andersson, M. A., Salkinoja-Salonen, M., Christiansson, A. (2006). Occurrence of emetic toxin producing *Bacillus cereus* in the dairy production chain. International Dairy Journal, 16(7), 740-749.
30. TeGiffel, M. C., Wagendorp, A., Herrewegh, A., Driehuis, F. (2002). Bacterial spores in silage and raw milk. Antonie van Leeuwenhoek, 81(1-4), 625-630.
31. Vissers, M. M. M., TeGiffel, M. C., Driehuis, F., De Jong, P., Lankveld, J. M. G. (2007). Predictive modeling of *Bacillus cereus* spores in farm tank milk during grazing and housing periods. Journal of Dairy Science, 90(1), 281-292.

Table 1. Evolution of *B. cereus* ( $\log_{10}$  cfu g<sup>-1</sup>; mean $\pm$ SD) in nine batches of vacuum-packed Ricotta salata stored refrigerated until 90 days after production.

| Batch | T <sub>0</sub>                           | T <sub>30</sub>                          | T <sub>60</sub>                          | T <sub>90</sub>                         |
|-------|--|--|--|---|
| h     |  |  |  |   |
| 1     | 4.51 $\pm$ 0.00 <sup>a</sup> (n = 1/2)   | 2.79 $\pm$ 0.79 <sup>a</sup> (n = 2/2)   | N.D. (n = 2/2)                           | N.D. (n = 2/2)                          |
| 2     | 4.37 $\pm$ 0.37 <sup>a</sup> (n = 2/2)   | 5.04 $\pm$ 0.00 <sup>a</sup> (n = 1/2)   | 2.94 $\pm$ 1.00 <sup>a</sup> (n = 2/2)   | N.D. (n = 2/2)                          |
| 3     | 4.68 $\pm$ 0.24 <sup>a</sup> (n = 2/2)   | 4.49 $\pm$ 0.20 <sup>a</sup> (n = 2/2)   | 3.26 $\pm$ 0.93 <sup>ab</sup> (n = 2/2)  | 1.30 $\pm$ 0.00 <sup>b</sup> (n = 1/2)  |
| 4     | 4.50 $\pm$ 0.33 <sup>a</sup> (n = 2/2)   | 4.68 $\pm$ 0.20 <sup>a</sup> (n = 2/2)   | 2.15 $\pm$ 0.00 <sup>b</sup> (n = 1/2)   | 1.78 $\pm$ 0.68 <sup>b</sup> (n = 2/2)  |
| 5     | 4.66 $\pm$ 0.35 <sup>a</sup> (n = 2/2)   | 3.85 $\pm$ 0.21 <sup>a</sup> (n = 2/2)   | N.D. (n = 2/2)                           | N.D. (n = 2/2)                          |
| 6     | 4.88 $\pm$ 0.00 <sup>a</sup> (n = 1/2)   | 3.81 $\pm$ 0.74 <sup>a</sup> (n = 2/2)   | 3.45 $\pm$ 0.16 <sup>a</sup> (n = 2/2)   | N.D. (n = 2/2)                          |
| 7     | 4.0 $\pm$ 0.00 <sup>a</sup> (n = 1/2)    | 3.62 $\pm$ 0.00 <sup>b</sup> (n = 1/2)   | 2.38 $\pm$ 0.00 <sup>a</sup> (n = 2/2)   | N.D. (n = 2/2)                          |
| 8     | 6.19 $\pm$ 0.14 <sup>a</sup> (n = 2/2)   | 4.83 $\pm$ 0.43 <sup>b</sup> (n = 2/2)   | 3.78 $\pm$ 0.00 <sup>bc</sup> (n = 1/2)  | 2.56 $\pm$ 0.00 <sup>c</sup> (n = 1/2)  |
| 9     | 3.77 $\pm$ 0.46 <sup>ab</sup> (n = 2/2)  | 5.43 $\pm$ 0.89 <sup>b</sup> (n = 2/2)   | 2.46 $\pm$ 1.67 <sup>a</sup> (n = 2/2)   | 2.26 $\pm$ 0.00 <sup>a</sup> (n = 2/2)  |
| total | 4.65 $\pm$ 0.74 <sup>a</sup> (n = 15/18) | 4.27 $\pm$ 0.90 <sup>a</sup> (n = 16/18) | 2.91 $\pm$ 0.84 <sup>b</sup> (n = 12/18) | 1.99 $\pm$ 0.55 <sup>c</sup> (n = 6/18) |

The sampling time (T<sub>0</sub>, T<sub>30</sub>, T<sub>60</sub> and T<sub>90</sub>) were respectively the day of packaging and 30, 60 and 90 days after the production. Means in the same row with different superscript letters are significantly different (P < 0.05); values within brackets indicate the prevalence of positive samples. N.D = data not definable, below the detection limit of the method.

Table 2. Intrinsic properties (mean  $\pm$  SD) and composition (%  $\pm$  SD) evolution during storage of ricotta salata wheels.

| parameter      | sampling times                  |                                |                                |                                |
|----------------|---------------------------------|--------------------------------|--------------------------------|--------------------------------|
|                | T <sub>0</sub>                  | T <sub>30</sub>                | T <sub>60</sub>                | T <sub>90</sub>                |
| pH             | 6.49 $\pm$ 0.10 <sup>a</sup>    | 6.18 $\pm$ 0.10 <sup>b</sup>   | 5.73 $\pm$ 0.14 <sup>c</sup>   | 5.63 $\pm$ 0.28 <sup>c</sup>   |
| a <sub>w</sub> | 0.978 $\pm$ 0.001 <sup>ab</sup> | 0.976 $\pm$ 0.002 <sup>a</sup> | 0.976 $\pm$ 0.002 <sup>a</sup> | 0.980 $\pm$ 0.001 <sup>b</sup> |
| % moisture     | 58.28 $\pm$ 2.91 <sup>a</sup>   | 58.56 $\pm$ 3.29 <sup>a</sup>  | 57.41 $\pm$ 2.99 <sup>ab</sup> | 56.23 $\pm$ 2.55 <sup>b</sup>  |
| % total solids | 41.72 $\pm$ 2.93 <sup>a</sup>   | 41.44 $\pm$ 3.26 <sup>a</sup>  | 42.59 $\pm$ 2.99 <sup>ab</sup> | 43.77 $\pm$ 2.49 <sup>b</sup>  |
| % fat          | 23.74 $\pm$ 3.92 <sup>a</sup>   | 23.13 $\pm$ 4.28 <sup>a</sup>  | 23.32 $\pm$ 3.73 <sup>a</sup>  | 23.39 $\pm$ 3.86 <sup>a</sup>  |
| % protein      | 12.65 $\pm$ 1.12 <sup>a</sup>   | 12.83 $\pm$ 1.16 <sup>a</sup>  | 13.01 $\pm$ 0.84 <sup>a</sup>  | 13.36 $\pm$ 1.14 <sup>a</sup>  |
| % salt         | 2.60 $\pm$ 0.30 <sup>ab</sup>   | 2.73 $\pm$ 0.24 <sup>b</sup>   | 2.49 $\pm$ 0.28 <sup>c</sup>   | 2.01 $\pm$ 0.49 <sup>d</sup>   |

The sampling time (T<sub>0</sub>, T<sub>30</sub>, T<sub>60</sub> and T<sub>90</sub>) were respectively the day of packaging and 30, 60 and 90 days after the production. Means in the same row with different superscript letters are significantly different (P < 0.05).