Survival of *Escherichia coli* O157:H7 during the manufacture and ripening of *Cacioricotta* goat cheese.

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Abstract

The aim of this study was to assess the growth and survival of *Escherichia coli* O157:H7 during the manufacturing and ripening of *Cacioricotta* goat cheese. Goat milk was artificially contaminated with *E. coli* O157:H7 and the bacterial load was monitored from production up to 90 days of ripening. Goat milk was inoculated with 10^2cfu ml^{-1} of *E. coli* O157:H7 and the bacterial count of the curd at time zero was 2.31 log_{10} cfu g^{-1}. During the first day of ripening, the bacterial load has increased to 5.73 log_{10} cfu g^{-1} to more than 6.20 log_{10} cfu g^{-1} during the first week. The bacterial load remained constant up to 28 days and then slightly decreased until the end of ripening, with values of a_w and pH of 0.88 and 5.41 respectively.

The results of this study highlighted that *E. coli* O157:H7 is able to survive the manufacturing process and they suggest that the 90-day period of ripening alone is insufficient to remove *E. coli* O157:H7 in contaminated *Cacioricotta* goat cheese. Moreover, these results support the assumption that the presence of a low contamination of milk with *E. coli* O157:H7 could represent a potential source of infection and a threat to consumers.

Keywords

Challenge test, goat milk cheese, *Escherichia coli* O157:H7, *Cacioricotta* goat cheese.

1. Introduction

Escherichia coli O157:H7 is an important foodborne pathogen, which is able to cause severe disease in humans. In 1982, it was first recognized as a human pathogen thanks to the discovery of its ability to produce Shiga-like toxins and haemolysin, both important virulence factors that can lead to serious diseases such as haemorrhagic colitis, haemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (Karmali et al., 2010). *E. coli* O157:H7 can grow at temperatures ranging from 7°C to 50°C, with an optimum temperature at 37 °C, in acidic foods at pH of 4.4, and in foods with a minimum activity water (a_w) of 0.95 (World Health Organization, 2016).

52 The European Food Safety Authority reports that the number of confirmed cases of infection in
53 Europe for *E. coli* Verocytotoxic (VTEC) is 5955 with an increasing trend from 2008 to 2014
54 (EFSA-ECDC, 2015). Foodborne outbreaks of *E. coli* O157:H7 infection have been associated with
55 a wide range of food products, including raw and pasteurized milk and dairy products (Dorn, 1988;
56 Morgan et al., 1993; Upton and Coia, 1994; Bielaszewska et al., 1997; Keene et al., 1997;
57 Heuvelink et al., 1998; CDSC, 1999; Goh et al., 2002; McIntyre et al., 2002). Among them, cheese
58 made from raw milk is known to be the most frequently contaminated products (Bielaszewska et al.,
59 1997; EFSA, 2013) and it is documented that contaminated raw milk cheeses, with short ripening
60 time (less than 60 days), could generate severe outbreaks (Public Health Agency of Canada, 2013).
61 Many regional cheese specialities, throughout Europe are manufactured from unpasteurised milk in
62 small processing facilities that employing technological barriers on an empirical basis. For these
63 reasons, there is a growing concern that these products may pose a threat to consumer safety by
64 transmitting pathogens such as *E. coli* O157:H7 (Schoder et al., 2003; Vernozy-Rozand et al., 2005;
65 Jayarao et al., 2006; Latorre et al., 2009; Hospital et al., 2012; Meloni, 2015). Furthermore, Food
66 Business Operators (FBOs) have to check the hygienic quality of their products observing the
67 Commission Regulation (EC) n. 2073/2005 (European Commission, 2007), on which, however, is
68 not required the research for VTEC in dairy products.
69 Among Italian traditional cheeses made from unpasteurized milk, goat's milk and goat's milk
70 cheeses are associated by consumers with healthy foods due to their intrinsic properties, such as low
71 allergenic potential, high digestibility and nutritional value (Raynal-Ljutovac et al., 2008). The lipid
72 composition of goat milk determines its nutritional quality, particularly fatty acids (primarily
73 linoleic acid), are involved in the quality of dairy products and directly affect the taste aspects of
74 milk derivatives (Delacroix-Buchet and Lamberet, 2000; Ribeiro et al., 2011).
75 Several reports have shown that *E. coli* O157:H7 is able to survive and grow in different kind of
76 cheese and in unpasteurised goat milk cheese (Vernozy-Rozand et al., 2005; Schlesser et al., 2006;
77 D'Amico et al., 2010). In fact, it is able to survive at low temperatures (Massa et al., 1997; Massa et

al., 1999; Giacometti et al., 2012) and low pH values (Jordan et al., 1999). However, there are few data on viability of *E. coli* O157:H7 during the manufacture and ripening of goat cheeses, in particular, of *Cacioricotta* cheese. It is a cheese produced from whole goat milk according to a traditional Italian technology that involves the use of unpasteurized milk that is only heat treated before the added of liquid veal rennet, as stated in the product specification. This typical Apulian cheese is recognized as "*Prodotto agroalimentare tradizionale*" (PAT), officially approve on proposals from Basilicata, Calabria, Campania, Lazio and Apulia Italian regions and included in the Sixteenth Revised Regional and National list of PAT (Gazzetta Ufficiale della Repubblica Italiana, 2016). It is traditionally produced according to each regional production specification and it can be eaten fresh (1-30 days) or after storage like grating cheese (2-3 months).

Therefore, the aim of this study is to investigate the growth and survival of *E. coli* O157:H7 during the manufacture and ripening period of *Cacioricotta* goat cheese by using artificially contaminated milk during the cheese making process.

2. Materials and methods

2.1. Bacterial strains

The strain used in this study was a non-toxigenic *E. coli* O157:H7 (NCTC 12900) kindly provided by Institute Zooprofilattico of Apulia and Basilicata (Foggia, Italy). The strain was cultured on Brain Heart Infusion Agar (BIOKAR Diagnostic, Beauvais Cedex, France) and incubated at 30°C for 24 h. The suspension of *E. coli* O157:H7 in sterile saline solution (NaCl 0,85%) was compared with the turbidity standard McFarland 2.0 Barium Sulphate (Liofilchem, Teramo, Italy) in order to obtain approximately the homogeneous suspension of 600 x 10^6 cfu ml^{-1}. The culture was diluted to obtain a concentration of 10^5 cfu ml^{-1} and 50 ml of this culture were added to 50 L of milk so that the final concentration of *E. coli* O157:H7 was approximately 10^2 cfu ml^{-1}.

2.2. Raw goat milk samples

Raw goat milk was purchased and delivered to the laboratory scale plant, in a bulk tank at 4±0.5°C within 6 h from the production, and it was artificially contaminated during cheese making.

The experimental test consists of two replicates of inoculated batches with non-toxigenic *E. coli* O157:H7 and two non-contaminated batches (control samples).

The milk used in the assessment was subjected to microbiological analysis for the detection and isolation of *E. coli* O157:H7 (UNI EN ISO 16654:2001).

2.3. Cheese making

A goat milk cheese, namely *Cacioricotta,* was produced in laboratory scale plant, according to production specification (Ars Alimentaria, 2016).

Briefly: 50 L of raw goat milk was heated to 90°C, left to cool under stirring until reaching 37°C, and supplemented with 650 g (1.3%) of sea salt and 15 ml of liquid veal rennet. It was left to rest for about 30 minutes.

Afterwards, the curd was cut twice into cubes of 4 cm and then into smaller cubes of about 0.5 cm.

The curd was stirring heated at 44-45°C for about 5 min, left to rest for 10 min up to deposition; finally, it was moulded by hand with light pressure into the traditional cylindrical wooden moulds, which have an internal diameter of 102 mm with a height of 50 mm, to facilitate the draining of whey. Forty-eight shapes of cheese were produced totally (12 contaminated for 2 replicates, and 12 as control samples for 2 replicates), so each shape of cheese was used only once for laboratory tests and then discarded. Each shape of cheese weighed about 400 to 450 g.

The moulds were left to drain at room temperature for 24 h during which they were twisted three times. Ripening of *Cacioricotta* goat cheese was made at 11°C with relative humidity of 70% for 90 days with turning movements. After the first three days, the cheese was removed from the moulds.

The bacterial inoculum of *E. coli* O157:H7 was added to goat milk after cooling to 37°C, before the addition of the sea salt and liquid veal rennet.

2.4. E. coli O157:H7 *count*

For each inoculated batch and for non-contaminated control samples, count of *E. coli* O157:H7 was performed in duplicate at time 0, immediately after the extraction of cheese, and during ripening (1, 3, 7, 9, 14, 21, 28, 35, 42, 49, 60 and 90 days). For non-contaminated control samples the count of *E. coli* O157:H7 was performed at 0, 7, 21, 35, 49, 60 and 90 days of ripening.

The count was performed by 10-fold dilution and direct plating (0.1 mL in duplicate) on sorbitol MacConkey Agar plates containing cefixime (0.05 mg L^{-1}) and potassium tellurite (2.5 mg L^{-1}) (Conda, Madrid, Spain), and incubated at 37°C for 24 h.

Five colonies, showing morphological characters of *E. coli* O157:H7 (sorbitol negative, translucid, 1-3 mm in diameter, opaque centre), were replated on Tryptone Soya Agar (Oxoid, Hampshire, United Kingdom) and subjected to indole testing.

All the strains which tested indole positive were confirmed biochemically as *E. coli* by API 20E (bioMérieux, Marcy l'Etoile, France) and, the strains identified as *E. coli,* were examined by latex-agglutination test with the *E. coli* O157 latex kit (Oxoid). Presumptive *E. coli* O157:H7 colonies were counted.

2.5. Microbiological analysis

The samples of curd and cheese contaminated and non-contaminated (control sample), aseptically collected, were subjected to the following analysis during the ripening period to the same times listed in the previous section.

25 g of each samples, were decimally diluted with sterile saline solution (NaCl 0,85%), separately, and subjected to Total Lactic Acid Bacteria Count (LAB), in de Man Rogosa Sharpe (ISO) Agar (Conda), and Total Thermophilic Lactococci Count, in M17 Agar (Conda), using pour plate method (1ml of each dilution). These inoculated plates were incubated at 37°C and 44°C respectively, for 48-72 h in microaerophilic condition.

155 For the Total Bacterial Count (TBC), 30 g of each sample, were added to 270 ml of Buffered
156 Pepton Water (BPW) (Liofilchem), homogenized in stomacher (Lab-Blender 400, PBI, Milan,
157 Italy), decimally diluted and pour plate on Plate Count Agar (Liofilchem). The bacterial culture was
158 incubated at 30°C±1°C for 72±3 h.
159 For the Total Enterobacteria and Total Coliforms, 30 g of each samples were added to 270 ml of
160 BPW (Liofilchem), homogenized in stomacher (PBI) and decimally diluted. 1 ml of each dilution
161 was added to Violet Red Bile Glucose Agar (Conda) and on Violet Red Bile Lactose Agar (Conda)
162 respectively, and incubated at 37±1°C for 24±2 h. After incubation, the count of typical colonies
163 was carried on.
164 For the Coagulase Positive Staphylococci Count (CPS) 25 g of each sample was diluted with 225
165 ml of BPW (Liofilchem), homogenized in a stomacher (PBI), seeded onto Baird-Parker RPF agar
166 (Biolife, Milan, Italy) and incubated aerobically at 35°C for 24-48 h; after incubation, the count of
167 typical colonies was carried on.
168 Furthermore, the search of the following pathogens has been carried out in each sample:
169 *Staphylococcus aureus*, according to UNI EN ISO 6888-1:2004 protocol, *Salmonella* spp.,
170 according to UNI EN ISO 6579:2008 protocol and *Listeria monocytogenes,* according to UNI EN
171 ISO 11290-1:2005 protocol; checks have been carried out until six successive analyses were
172 negative.
173
174 ***2.6. pH and a_w determination***
175 The water activity (Dew Point Water Activity Meter 4TE, AquaLab, USA) and pH (Lab pH meter,
176 © XS Instruments, Italy) were measured for each sample of contaminated and non-contaminated
177 milk. All analyses were performed in duplicate.
178
179 ***2.7. Statistical analysis***

Microbiological data were transformed into logarithms of the number of colony forming units (cfu g^{-1}), the average and standard deviations of microbial counts and physical-chemical values were determined from the average of two replicates of inoculated batches and two not contaminated control batches at each sampling time. Two Way Analysis of Variance (ANOVA) was carried out to evaluate the difference of microbial counts during production and ripening using Statview (ver. 5.0, SAS Institute Inc. Cary, NC) with statistical significance settled at $P < 0.05$.

3. Results and discussion

The results of *E. coli* O157:H7 count, TBC, Total Enterobacteria, Total Coliforms, LAB and Thermophilic Lactococci, pH and a_w values, in samples of *Cacioricotta* goat cheese experimentally contaminated during the manufacture and ripening, are described in Table 1 and Figure 1. The results of control samples are described in Table 2.

In detail, the strain of *E. coli* O157:H7, used in this study, survived during the entire ripening period and its load was increased from 2.31 $\log_{10}$ cfu g^{-1} on day 0, up to 5.73 $\log_{10}$ cfu g^{-1} on day 1 of the whey drainage at room temperature.

Afterwards, during the first week of ripening, the load of *E. coli* O157:H7 has further increased to 6.35 $\log_{10}$ cfu g^{-1}, while values remained essentially unchanged over the next 28 days. Subsequently, there has been a decrease in the load up to values of 4.28 $\log_{10}$ cfu g^{-1} (Table 1). In control cheese samples the results of *E. coli* O157:H7 have been always negative (Table 2).

The TBC has increased during the ripening period going from average values of 4.31 $\log_{10}$ cfu g^{-1} on the first day up to 8.11 $\log_{10}$ cfu g^{-1} at the end of the maturation process.

In the same way, the Total Enterobacteria and the Total Coliforms load were increased from 2.31 $\log_{10}$ cfu g^{-1} (day 0) to 4.68 $\log_{10}$ cfu g^{-1} (day 90) and from 2.31 $\log_{10}$ cfu g^{-1} (day 0) up to 4.54 $\log_{10}$ cfu g^{-1}, respectively, at the end of the ripening period (Table 1).

The comparison between results of control samples and those of contaminated samples showed significant differences ($P = 0.003$) of the values of Total Enterobacteria and Total Coliforms.

206 Whereas, the LAB and Thermophilic Lactococci increased from 3.49 log_{10} cfu g^{-1} to 8.85 log_{10} cfu
207 g^{-1} and from 3.58 log_{10} cfu g^{-1} to 8.84 log_{10} cfu g^{-1}, respectively, in the first week of ripening; and
208 then decreased until the end of ripening to reach 7.43 and 7.84 log cfu g-1 respectively (Table 1).
209 Statistical analysis showed that not significant difference existed between LAB and Thermophilic
210 Lactococci loads on contaminated samples and control samples ($P > 0.05$).
211 Values of CPS and *Staphylococcus aureus* were negative and the search of *Salmonella* spp. and
212 *Listeria monocytogenes* was negative for six consecutive analyses in all samples tested.
213 The value of pH decreased during all the ripening days, from 6.36 (day 0) up to 5.41 (day 90), as
214 well as the a_w value is reduced from 0.99 (day 0), up to 0.88 (day 90) in contaminated and
215 uncontaminated samples (Figure 1).
216 The aim of this study was to assess the viability of a strain of *E. coli* O157:H7, during the
217 production of a typical Italian cheese, made from goat's milk, named *Cacioricotta*. The
218 contamination of goat milk with *E. coli* O157:H7 occurred after the production step, which provides
219 for the heat treatment and the addition of rennet. The results show that *E. coli* O157:H7 is able to
220 survive during the manufacturing process and that its concentration increases during the first day of
221 ripening, remains substantially stable up to 35 days and then decreases slowly until the end of the
222 curing period.
223 The results obtained in our work, point out that in *Cacioricotta* goat cheese, experimentally
224 contaminated, the long period of ripening (90 days), is not sufficient to eliminate *E. coli* O157:H7.
225 The survival and replication of the pathogen in cheeses with long ripening periods could be due to
226 several factors such as the cheese processing temperature, the decrease in pH, the addition of salt
227 and starter cultures that do not reach values able to ensure guarantee the elimination of the pathogen
228 (Govaris et al., 2002).
229 In fact, the production of traditional cheeses must faithfully follow the production specification that
230 often use temperature for the milk cooking not always able to devitalize the pathogen.

231 During the production of *Cacioricotta* goat cheese, for example, making cheese initially involves a
232 step of heating the milk to high temperatures (90°C), able to devitalize *E. coli* O157:H7. However,
233 in case of post-treatment contamination, to work of tools or food operators, the required
234 temperatures in the production specification (45°C to 11°C), do not induce the death of the
235 pathogen and they can even promote its replication (Table 1). The risk of post-treatment
236 contamination is more prevalent in traditional dairy products. In fact the combination of artisan
237 practices employed in traditional food manufacturing, and the potential for poor hygienic
238 conditions, prevailing especially in small-scale family-owned processing installations often attached
239 to the farms, may result in the contamination and survival of foodborne pathogens that may be
240 present throughout the distribution chain until the time of consumption (Kousta et al., 2010;
241 Panagou, et al., 2013).
242 This does not occurs at the time when the temperatures during the production of cheeses are high.
243 For example, during the production of *Cottage cheese* or *Mozzarella cheese* experimentally
244 contaminated, to an initial increase of the loads, follows the death of the pathogen due to the
245 application of a temperature of 80°C for the cooking of the curds and whey in the case of the
246 *Cottage cheese,* and for spinning and forming in the case of the *Mozzarella cheese* (Arocha et al.
247 1992; Spano et al., 2003).
248 In addition to the application of high temperatures, another factor limiting the survival of *E. coli*
249 O157:H7 is the inhibitory effect played by lactic acid bacteria on the pathogen, due to the products
250 of their final metabolism such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins. The
251 starter cultures are used in the food industry for the production of many fermented foods, to ensure
252 their hygienic, nutritional and sensory quality (Cerri et al., 2006; Dellaglio et al., 1995).
253 Lactobacilli and Lactococci have an important role during the acidification of the curd, as they
254 cause a decrease of the pH value, the demineralization of the casein and the proteolytic action,
255 which helps to give flavor to the cheese during ripening (Cerri et al., 2006; Dellaglio et al., 1995).

256 The results we have obtained about the replication and survival of *E. coli* O157:H7 in *Cacioricotta*
257 goat cheese (90 days; Table 1), may be due to the different lactic strains naturally present in this
258 cheese and obtained from the fermentation of raw goat milk, as required by specification product.
259 A possible hypothesis is that the latter factor, has led, together with the low concentration of salt
260 used during the maturing stage, a slow lowering of the pH values and a_w, allowing the survival of
261 the pathogen.
262 As opposed, Osaili et al. (2014) observed a higher reduction of *E. coli* O157:H7 load, in samples of
263 white brined cheese experimentally contaminated and added with LAB starter, compared to cheese
264 samples without LAB starter addition. Hence, the Authors have suggested that the addition of
265 starter cultures is an important factor responsible for the reduction of contamination of cheese with
266 *E. coli* O157: H7 (Osaili et al., 2014).
267 The pH value, both in *Cacioricotta* goat cheese short curing (30 days) and in the more long
268 maturation cheese (from 60 days to 90 days), is never dropped to values below 5.41, compatible
269 with the survival of the pathogen.
270 In fact, *E. coli* O157:H7, when it is in a moderately acidic environment, has the ability to develop
271 an Adaptive Tolerance Response (ATR) which gives it a high resistance when it is exposed to
272 environmental conditions of strong acidity (Jordan et al., 1999; Maher et al., 2001; Vernozy-Rozand
273 et al., 2005).
274 In agreement with our research, several challenge studies have reported that *E. coli* O157:H7 is able
275 to survive, in low or high load, during the phases of production in various kind of cheese produced
276 from cow, sheep and goat milk, even for long periods of ripening.
277 Particularly, Vernozy-Rozand et al. (2005) had evaluated the survival of *E. coli* O157:H7 in cheeses
278 of raw goat's milk with the addition of starter cultures and experimentally inoculated at a final
279 concentration of 10, 100 and 1000 cfu ml^{-1}. The results obtained, showed an initial decrease in load
280 of *E. coli* O157:H7 by 1 log cfu g^{-1} in the curd just prior to molding. However, at 42 days of

281 ripening, *E. coli* O157:H7 was counted and isolated in all contaminated cheeses (Vernozy-Rozand
282 et al., 2005).
283 In the same way, the results obtained in another study from Cosciani-Cunico et al. (2014), showed
284 that higth loads (4.78 log cfu/ml) of *E. coli* O157:H7 increases to more than 1.5 log cfu g^{-1} during
285 the production of an Italian raw goat cheese named *Formaggelle*, and remained constant until the
286 end of ripening (30 days).
287 Furthermore, it was shown that the count of *E. coli* O157:H7 undergoes a significant reduction
288 (more than 6 log cfu g^{-1}), only after a long period of maturation equal to 90-120 days (Cosciani-
289 Cunico et al., 2015; D'Amico et al. 2010; Gill and Oudit, 2015).
290

291 **4. Conclusion**

292 *Cacioricotta* goat cheese is a traditional Italian cheese with a short or long ripening period; it is
293 characterized by a low allergenic potential, by high nutritional value and by good digestibility (Ars
294 Alimentaria, 2016). Contamination of cheese with low load of *E. coli* O157:H7 in the processing
295 phase may pose a risk to the consumer because *E. coli* O157:H7 survives in *Cacioricotta* goat
296 cheese experimentally contaminated, up to 90 days.
297 According to the results that we have obtained, we can conclude that the dairy industry and the
298 Food Business Operators of small processing plants, who use the production specification, should
299 employ strict sanitary control measures to prevent the contamination of raw milk and of the cheese
300 during all the processing steps.
301 The strict application of Good Manufacture Practice (GMP), and the implementation of the HACCP
302 system can help to improve the hygienic quality of milk during the milking process and storage.
303 Furthermore, it can help to prevent contamination during the production and handling of cheese, in
304 order to ensure a high bacteriological quality of traditional cheeses and reduce the risk to the
305 consumer who appreciates these typical products.
306

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Table 1. Results of *E. coli* O157:H7 count, Total Bacterial Count (TBC), Total Enterobacteria, Total Coliforms, Lactic Acid Bacteria (LAB) and Thermophilic Lactococci, in samples of *Cacioricotta* goat cheese experimentally contaminated during the manufacture and ripening period.

Samples	Parameters					
	E. coli O157:H7*	TBC*	Total Enterobacteria*	Total Coliforms*	LAB*	Thermophilic Lactococci*
Day 0 (curd)	2.31 ± 0.01	3.31 ± 0.01	2.31 ± 0.01	2.31 ± 0.01	3.49 ± 0.69	3.58 ± 0.57
Day 1 (cheese)	5.73 ± 0.01	6.23 ± 0.71	5.76 ± 0.01	5.75 ± 0.01	5.55 ± 3.31	5.64 ± 3.12
Day 3	6.2 ± 0.01	8.74 ± 0.17	6.46 ± 0.19	6.44 ± 0.02	7.97 ± 1.27	8.4 ± 0.67
Day 7	6.35 ± 0.02	9 ± 0.15	6.49 ± 0.08	6.32 ± 0.09	8.85 ± 0.00	8.84 ± 0.00
Day 9	5.99 ± 0.03	8.91 ± 0.15	6.34 ± 0.16	6.16± 0.02	8.8 ± 0.03	8.33 ± 0.40
Day 14	6.18 ± 0.02	9.12 ± 0.15	6.39 ± 0.07	6.22 ± 0.09	8.61 ± 0.00	8.81 ± 0.02
Day 21	5.91 ± 0.02	8.95 ± 0.03	6.12 ± 0.20	6.06 ± 0.03	8.85 ± 0.31	8.72 ± 0.02
Day 28	6.68 ± 0.66	8.71 ± 0.18	6.13 ± 0.03	6.43 ± 0.66	8.44 ± 0.11	8.21 ± 0.19
Day 35	5.94 ± 0.06	8.9 ± 0.04	6.12 ± 0.00	6.58 ± 0.54	8.34 ± 0.15	8.29 ± 0.08
Day 42	5.66 ± 0.07	8.61 ± 0.06	6.15 ± 0.00	5.7 ± 0.00	8.22 ± 0.37	8.5 ± 0.01
Day 49	5.56 ± 0.03	8.46 ± 0.04	5.84 ± 0.08	5.72 ± 0.12	8.01 ± 0.80	8.31 ± 0.43
Day 60	5.15 ± 0.04	8.15 ± 0.21	5.6 ± 0.20	5.59 ± 0.00	7.75 ± 0.00	7.67 ± 0.00
Day 90	4.28 ± 0.17	8.11 ± 0.00	4.68 ± 0.06	4.54 ± 0.00	7.43 ± 0.37	7.84 ± 0.00

*Average values of two repetition expressed as Log cfu/g ± standard deviation.

493 **Table 2.** Results of *E. coli* O157:H7 count, Total Bacteria Count (TBC), Total Enterobacteria, Total
494 Coliforms, Lactic Acid Bacteria (LAB) and Thermophilic Lactococci, in control samples during the
495 manufacture and ripening period.

Samples	Parameters					
	E. coli O157:H7*	TBC*	Total Enterobacteria*	Total Coliforms*	LAB*	Thermophilic Lactococci*
Day 0 (curd)	0	3.31 ± 0,01	2 ± 0,14	1.91 ± 0,08	3.37 ± 0,30	3.61 ± 0,24
Day 7 (cheese)	0	9.04 ± 0,07	4.62 ± 0,05	4.36 ± 0,07	8.71 ± 0,08	8.89 ± 0,01
Day 21	0	8.97 ± 0	4.16 ± 0,11	4.15 ± 0,09	8.8 ± 0,3	8.76 ± 0,02
Day 35	0	8.37 ± 0,06	2.57 ± 0,16	2.36 ± 0,07	8.43 ± 0,01	8.33 ± 0,17
Day 49	0	8.25 ± 0,31	0	1 ± 0,07	8.01 ± 0,80	8.39 ± 0
Day 60	0	8.16 ± 0,19	0	0	7.66 ± 0,06	7.64 ± 0,02
Day 90	0	8.11 ± 0,14	0	0	7.79 ± 0,24	7.79 ± 0,05

496 *Average values of two repetition expressed as Log cfu/g ± standard deviation.

497 **Figure 1.** Performance of a_w and pH in *Cacioricotta* goat cheese experimentally contaminated
498 during the manufacture and ripening period.