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Survival of Shiga toxin-producing *Escherichia coli* O157:H7 in Minas frescal cheese

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 strains (isolated by cattle's faeces and a reference strain, EDL933), were inoculated into pasteurized milk (10^2 and 10^3 cells.mL^{-1}) to prepare the Minas frescal cheese. As control was used uninfected milk. Physicochemical and microbiological analyses were performed to milk and elaborated cheese. The O157:H7 strains were quantified in the stages of cheese processing and during 0, 2, 4, 5, 7, 10 and 15 storage days at 8 °C onto Sorbitol MacConkey Agar supplemented with potassium tellurite and cefixime (CT-SMAC). O157:H7 was not present in the pasteurised milk prior to the artificial inoculation. At the end of the processing the cheese had 10 to 100 times more STEC O157:H7 than the initial inoculum. During the storage, the Minas frescal cheese exhibited the largest population increase on the 4th and 5th day when inoculated with 10^2 and 10^3 cells.mL^{-1}, respectively. Additionally, viable cells were found up to the 10th and 15th day, according to the amount of initial inoculum. This number of cells is able to cause infection in humans, and therefore, Minas frescal cheese, even when stored under refrigeration, is a potential vehicle of disease caused by STEC O157:H7.

Keywords: cheese; psychrotrophic bacteria; foodborne disease.

Practical Application: This study demonstrates that the Minas frescal cheese may be an important vehicle for STEC O157:H7, since this microorganism remains viable in this food for a long period even under refrigeration. In this study we can observe the psychrotrophic behavior of STEC O157:H7 in this rich food which is Minas frescal cheese.

1 Introduction

O157:H7 is a particularly virulent serotype of Shiga toxin-producing *Escherichia coli* (STEC), recognised as a human pathogen in 1982 (Riley et al., 1983). STEC O157:H7 has been considered to be a threat to public health in many countries because it has been involved in many outbreaks and caused a variety of diseases, such as haemorrhagic colitis, haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura. Complications from these diseases can lead to death, especially in children, elderly patients and immunocompromised patients (Centers for Disease Control and Prevention, 2000). In Brazil, there is no systematic surveillance system for infection with STEC O157:H7 and HUS cases. However, research conducted in Brazil has demonstrated the occurrence of important STEC serotypes in human diseases, such as bloody diarrhoea, haemolytic anaemia and HUS (Guth et al., 2002; Souza et al., 2011).

STEC O157:H7 naturally colonises the gastrointestinal tract and recto-anal junction of cattle, which are the main reservoir of STEC, and these animals are generally asymptomatic (Lim et al., 2007). Many outbreaks caused by this pathogen are associated with the consumption of foods from bovine origin, as well as food and water that may have come into contact with the animal or its faeces (Centers for Disease Control and Prevention, 2000). Pasteurised milk and other dairy products, including cheese, have been associated with disease outbreaks in which the aetiological agent is STEC O157:H7 (Goh et al., 2002).

Due to the severity of the disease, the high potential for contamination, and the large-scale outbreaks caused by food contaminated by STEC O157:H7, studies have been undertaken to evaluate the behaviour and the viability of this pathogen in foods ready for consumption and during manufacturing (D'Amico et al., 2010; Breidt Junior & Caldwell, 2011).

In Brazil, cheese is one of the most important products in the dairy industry, particularly the MFC. MFC is characterised as a fresh cheese obtained by enzymatic coagulation of milk with rennet and/or other suitable coagulating enzymes, supplemented or not with specific lactic bacteria. This is semi-hard cheese, very high humidity, to be eaten fresh (Brasil, 1997, 2004). The physico-chemical characteristics of this cheese increase the potential risk of its contamination with certain pathogens because it constitutes a suitable medium for their growth. Therefore, it is of great importance to assess the behaviour and the viability of STEC O157:H7 during the processing and storage of MFC produced from artificially contaminated pasteurised milk. The objectives of this study were, to evaluate the behaviour and the viability of two strains of STEC belonging to serotype O157:H7 during the processing of MFC and its storage at 8 °C.

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2 Materials and methods

2.1 Raw material

The pasteurized milk (3% of fat) was used as raw material for MFC production. The pasteurized milk was subjected to analyses for the verification of some of the minimum requirements for physicochemical and microbiological properties described in Brazilian law (Brasil, 2011). All analyses were performed in duplicate. The total acid concentration of the sample was measured by volumetric neutralisation. The results were expressed as the percentage of lactic acid (Brasil, 2006). The presence of antibiotic residues was investigated by Kit Eclipse 50 (Cap-lab®, São Paulo, Brazil).

Twenty-five mL of milk samples were homogenised in 225 mL of buffered peptone water to 1% (w/v) (BPW). Serial dilutions were performed for the enumeration of *E. coli* in Petrifilm™ EC (3M Company, St. Paul, EUA) according to the manufacturer's recommendations. *Salmonella* was investigated as described by APHA (American Public Health Association, 2001). The presence of *E. coli* O157:H7 was also investigated to ensure the absence of these microorganisms prior to the artificial contamination of the cheese. After enrichment for 3 h at 35 °C, 100 µL of the homogenate in BPW was seeded onto Sorbitol MacConkey Agar (SMAC; Himedia, Mumbai, India) supplemented with potassium tellurite (2.5 mg.L^{-1}) and cefixime (0.05 mg.L^{-1}) (CT-SMAC) (Zadik et al., 1993) and incubated at 35 °C for 24 to 48 h.

2.2 Bacterial strains

Were used two strains of STEC O157:H7 separately: RJ581, which has genetic virulence markers specific to the pathogenic group of STEC and was isolated by cattle's faeces from Rio de Janeiro, Brazil (data unpublished), and a reference strain, EDL933 (ATCC 43495), which was isolated from ground beef involved in an outbreak in a chain of fast food restaurants in Michigan, USA.

2.3 Milk artificial contamination

A loop (~10 µL) of each bacterial strain was transferred to 5 mL of Trypticase Soy Broth (Himedia) at 35 °C for 18 to 20 h. The concentration of the culture was adjusted to 10^8 cells.mL^{-1} based on the optical density (OD) measured by a spectrophotometer (UV-2600; Shimadzu, Tokyo, Japan) at a wavelength of 600 nm, and serial dilutions were performed to contaminate different batches of milk with 10^2 and 10^3 STEC O157:H7 cells.mL^{-1}. The different contaminated batches, as well as uninfected milk (negative control), were used for the preparation of cheese.

2.4 Minas frescal cheese manufacture

The cheeses were prepared from four group of milk samples (L1, L2, L3 and L4) of the same brand (Table 1). The milk was heated to 35 °C, and 200 ppm of $CaCl_2$ and 0.0625 g.L^{-1} of rennet Halamix were added (Chr. Hansen A/S, Horsholm, Denmark). The temperature was maintained at 35 °C, and the coagulation process lasted for approximately 40 min. After coagulation, the clot obtained was cut, manually agitated and maintained at 45 °C for 15 min until the flakes settled to the bottom of the container. Subsequently, the clot was separated from the whey and placed directly into Minas cheese moulds. The salt (2.1%; w/v) was added directly into the cheese mass (Furtado, 2005). The cheeses were stored at 8 °C for up to 15 days in closed plastic. Were prepared twelve MFC, six cheeses for each bacterial strain studied (Table 1).

Table 1. Samples of Minas frescal cheese (MFC) according to the bacterial strain used, artificial initial contamination amount and pasteurised milk samples used as raw material.

MFC samples	Level of artificial contamination	Milk sample	Bacterial strain
Q1	10^2	L1	RJ581
Q2	10^3	L1	RJ581
Q3	UN	L1	RJ581
Q4	10^2	L2	RJ581
Q5	10^3	L2	RJ581
Q6	UN	L2	RJ581
Q7	10^2	L3	EDL933
Q8	10^3	L3	EDL933
Q9	UN	L3	EDL933
Q10	10^2	L4	EDL933
Q11	10^3	L4	EDL933
Q12	UN	L4	EDL933

Q: manufactured cheese samples; UN: uncontaminated.

2.5 Physicochemical analysis of the cheese

A physicochemical analysis was performed to verify that the cheese met the minimum identity and quality standards established by Brazilian law (Brasil, 1996, 1997, 2004) with respect to fat and moisture. All analyses were performed in duplicate. The moisture content was determined by the gravimetric method (Brasil, 2006). The fat content was determined by the Gerber method (Brasil, 2006).

2.6 Microbiological analysis of the cheese

A microbiological analysis was performed to verify the compliance of the cheese with the standards established by Brazilian law (Brasil, 1996). The assay for *Salmonella* sp, *E. coli* and total coliform count were performed as described above for milk samples. For *Listeria monocytogenes*, 25 g of the sample was homogenised in 225 mL of Listeria enrichment broth (Oxoid, United Kingdom) in a homogenizer sample (MK1204, Cap-Lab®, São Paulo, Brazil) for 120 sec and incubated at 30 °C for 24 h. Was transferred 0.1 mL of the culture to 10 mL of Fraser broth and incubated at 30 °C for 24 to 48 h, after than, was transferred to agar Palcam (Oxoid) supplemented with Listeria selective supplement (Oxoid) and incubated at 30 °C for 24 to 48 h. *Staphylococcus aureus* in the BPW culture was enumerated by plating 1 mL of serial dilutions on Petrifilm™ EC Staph Express (STX) (3M, St. Paul, USA) as recommended by the manufacturer.

The survival of the O157:H7 strains was evaluated during the MFC processing steps and after 0, 2, 4, 5, 7, 10 and 15 days of storage at 8 °C (Figure 1).

One hundred microliters of serial dilutions of each sample were plated, in duplicate, on CT-SMAC and incubated for 24 to 48 h at 35 °C. Colonies typical of *E. coli* O157, which are non-sorbitol fermenting (clear) with a black point in the centre (tellurite reduction), were counted. After counting, five typical colonies of *E. coli* O157 were selected and subjected to PCR (polymerase chain reaction) for *rfb*O157 gene research, this gene encodes for the O157 antigen (Paton & Paton, 1998). Two replicates of each test were performed.

2.7 Statistical analyses

Count data were log transformed for all analyses. To evaluate the behaviour of STEC O157:H7 during the storage of MFC, a linear mixed-effects model was applied to the data. Time (in days) and the combination of strain and level of artificial contamination were used as fixed effects and milk sample as random effect. Contrasts of interests were tested with the use of lme package in R software.

3 Results and discussion

3.1 Milk quality

Milk quality is defined by determining parameters for chemical, physicochemical and microbiological composition. Acidity is one of the minimum parameters required to demonstrate the quality and identity of pasteurised milk, and this value should be 0.14 to 0.18 g% (w/v) of lactic acid (Brasil, 2011). The values determined by this study ranged from 0.17 to 0.18 g of lactic acid, which is in accordance with the permitted limits. Another important factor in evaluating the milk quality is the presence of antibiotic residues. This type of substance, when present in milk, can confound the results of quality analyses and interfere with the production of milk derivatives (Nascimento et al., 2001). All milk samples used for cheese manufacture had a negative result regarding the presence of antibiotics (Table 2).

To evaluate the microbiological quality of pasteurised milk, the samples were qualitatively assessed for the presence of *Salmonella* sp and *E. coli* O157:H7 and quantitatively assessed for *E. coli*. *E. coli* O157:H7 was not present in the pasteurised milk prior to the artificial inoculation. The pasteurised milk samples did not show *Salmonella* sp in 25 mL, which is the threshold established by Brazilian law (Brasil, 1996). However, the number of *E. coli* found as faecal contamination indicator were notably high, with values ranging from 3.5 x 10^4 to > 10^5 CFU.mL^{-1} of milk. Brazilian law does not provide standards for *E. coli*. However, the maximum allowable count of thermotolerant coliforms at 45 °C is 4 MPN (most probable number).mL^{-1} of pasteurised milk. One should consider that among the faecal indicator bacteria, *E. coli* is the true indicator of this contamination type because it is the only member of the group of coliform bacteria that is exclusively of faecal origin and can provide information on the sanitary conditions of production, processing and storage (International Commission

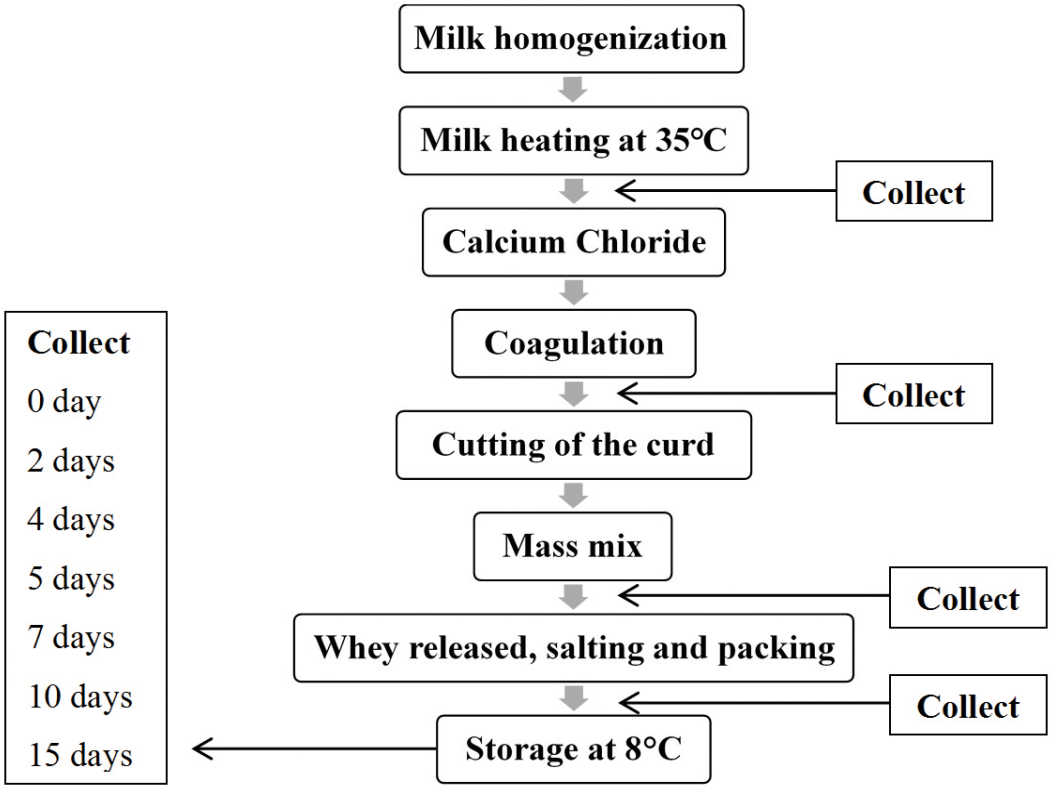

Figure 1. Sample collection points during the process and storage steps of artificially contaminated Minas frescal cheese.

on Microbiological Specifications for Foods, 1978). *E. coli*, including pathogenic *E. coli*, are bacteria commonly found in raw and pasteurised milk produced in Brazil (Ataíde et al., 2008). These results indicate a need for the revision or implementation of hygiene practices and evaluation of the effectiveness of processes adopted by producers in the pasteurisation of milk and more careful selection of suppliers. Despite displaying *E. coli* above the standards established by Brazilian law, the milk samples were used as raw material for the preparation of MFC, because they were not contaminated with serotype O157:H7.

3.2 Minas frescal cheese identity and quality

To verify the identity and quality standards of the MFC, the percentage of fat in dry matter (FDM) and moisture in the control cheese for each test were measured (Table 3). The results for the parameters determined for FDM and moisture in the cheeses allowed for samples Q6, Q9 and Q12 to be characterised as MFC based on the standard of identity and quality for this cheese (Brasil, 1997). Sample Q3, because of a higher fat content than the standard of identity and quality (Brasil, 1997), could not be characterised as MFC. Although the Q3 sample did not fall within the standards according to fat content, this parameter was not considered to be an exclusion criterion for this sample because only a relatively small number of microorganisms are able to utilise fat as an energy source, while the moisture content is a crucial parameter for microbial growth and could contribute to a change in the behaviour of the strains studied. Therefore, all samples of cheese were used to evaluate the behaviour of the strains studied.

The microbiological quality of the MFC prepared with pasteurized milk that was artificially contaminated with 10^2 and 10^3 CFU.mL^{-1} of O157:H7 strains EDL 933 and RJ581 was evaluated by comparing the results of the samples with the values established by Brazilian law (Brasil, 1996) (Table 4).

All of the MFC samples produced were in accordance with the standards established by legislation (Brasil, 1996) for *Salmonella* and *L. monocytogenes*. The Brazilian legislation (Brasil, 1996) established the standard for *Staphylococcus* as

Table 2. Physicochemical and microbiological parameters of the pasteurised milk samples used as raw material.

Parameters	Milk samples			
	L1	L2	L3	L4
Total acidity[a]	0.18	0.17	0.17	0.17
Antibiotics residue	negative	negative	negative	negative
E. coli (CFU.mL^{-1})	10^5	10^5	1.7×10^5	3.5×10^4
Salmonella sp.25 mL^{-1}	absent	absent	absent	absent
E. coli O157:H7.mL^{-1}	absent	absent	absent	absent

L: pasteurized milk; L1: milk used in the preparation of the Q1, Q2 and Q3 samples; L2: milk used in the preparation of the Q4, Q5 and Q6 samples; L3: milk used in the preparation of the Q7, Q8 and Q9 samples; L4: milk used in the preparation of the Q10, Q11 and Q12 samples. [a]g% of lactic acid.mL^{-1}.

Table 3. Physicochemical quality assessment of the Minas frescal cheese (MFC) uninoculated studied.

Parameters (g%)	MFC Samples				Standard[b]
	Q3	Q6	Q9	Q12	
FDM[a]	53.70	41.84	44.53	44.13	25 - 44.9%
Moisture	60.65	59.36	66.32	69.86	>55%

Q: manufactured cheese samples; [a]fat in dry matter; [b]Brazilian law (Brasil, 1996).

Table 4. Microbiological quality of Minas frescal cheese (MFC).

MFC samples	Parameters			
	Salmonella sp[b]	*L. monocytogenes*[b]	*S. aureus*[c]	*E. coli*[c]
Q1	absent	absent	< 10	1.29×10^7
Q2	absent	absent	< 10	1.4×10^6
Q3	absent	absent	< 10	9.2×10^6
Q4	absent	absent	< 10	1.2×10^7
Q5	absent	absent	< 10	1.4×10^7
Q6	absent	absent	< 10	1.9×10^7
Q7	absent	absent	< 10	3.0×10^6
Q8	absent	absent	< 10	5.2×10^6
Q9	absent	absent	< 10	9.5×10^5
Q10	absent	absent	< 10	1.3×10^5
Q11	absent	absent	< 10	4.5×10^5
Q12	absent	absent	< 10	5.0×10^5
Standard[a]	absent	absent	1.0×10^3	5.0×10^2

Q: manufactured cheese samples; [a]Brasilian law (Brasil, 1996); [b]Absent in 25 g; [c]CFU.g^{-1}.

coagulase-positive. However, the research was performed in *S. aureus* (a representative of the category), and there were < 10 CFU of this microorganism per g of cheese. The *E. coli* faecal contamination indicator showed counts of up to 5.2 x 10^6 CFU.g^{-1}. These high values for *E. coli* may be related to the quality of the raw material used, because a large number of bacteria in all of the samples of pasteurised milk were observed.

3.3 Search of STEC O157:H7

In the samples collected during the processing of cheese that was artificially contaminated with 10^2 cells of *E. coli* O157:H7. mL^{-1} of milk, it was not possible to count the bacteria through this methodology with the exception of the sample artificially contaminated with the EDL933 strain that was collected after heating at 35 °C (Table 5). However, after enrichment, *E. coli* O157:H7 cells were recovered from the samples, indicating the presence of viable cells. The high values for *E. coli* faecal contamination indicator may have affected the isolation of *E. coli* O157:H7 from the artificially contaminated cheeses with an initial contamination of 10^2 cells.mL^{-1} of milk was used.

During the manufacturing of MFC with milk artificially contaminated with 10^3 CFU of *E. coli* O157:H7.mL^{-1}, it was possible to perform bacterial counts on all of the samples, and an increase in the bacterial population of 1 log in the early stages and 2 log in the cheese-ready stage was observed (Table 5). All typical colonies of *E. coli* O157 in CT-SMAC, subjected to PCR, showed the sequence of 259 bp, confirming the presence of the *rfb*O157 gene. These results demonstrate that the processing steps do not interfere significantly with the growth of either *E. coli* O157:H7 strain.

Sata et al. (1999) reported that the direct detection of *E. coli* O157:H7 using the conventional procedure of direct plating has a sensitivity of 10^2 to 10^4 CFU.g^{-1} or mL^{-1}, depending on the food. Simple plating on CT-SMAC has low sensitivity because this medium is not suitable for the detection of stressed cells (McCarthy et al., 1998). In cheese, the bacterial cells are commonly stressed, mainly due to the presence of lactic acid (Jordan & Maher, 2006). Jordan & Maher (2006) reported that the detection limit of *E. coli* O157:H7 from food using CT-SMAC is approximately 10^3 CFU.g^{-1}. This finding could explain the lack of detection of STEC O157:H7 in certain evaluations in this study. To increase the detection sensitivity for *E. coli* O157:H7 in food by CT-SMAC, an enrichment step is required (Zadik et al., 1993). However, this step increases the bacterial population and does not portray the real number of bacteria in the original product. Saad & Franco (1999) assessed the influence of the natural microbiota of raw meat on the growth of *E. coli* O157:H7 in ground beef, which was maintained at 8.5 °C for 96 h. When intermediate levels of 10^3 to 10^4 CFU.g^{-1} of *E. coli* O157:H7 were incubated with 10^6 to 10^7 CFU.g^{-1} of non-pathogenic *E. coli*, it was only possible to count *E. coli* O157:H7 up to 24 h of storage at 8.5 °C, and this count was equal to the initial contamination level, which most likely was observed due to the difficulty of enumerating the pathogen in the midst of high counts of non-pathogenic *E. coli*. When the contamination level was smaller, i.e., from 10^1 to 10^2 CFU.g^{-1}, the difficulties increased, and it was not possible count the pathogenic *E. coli*. A higher contamination level of *E. coli* O157:H7, 10^6 to 10^7 CFU.g^{-1}, mixed with non-pathogenic *E. coli* did not affect the growth and counting of the pathogen. After artificially contaminating cheddar and Gouda cheese with 20 CFU.g^{-1}, D'Amico et al. (2010) did not observe *E. coli* O157:H7 colonies by direct plating in approximately half of the analyses because there was an overgrowth of the natural microbiota of the cheese.

Thus, the results confirm that lower levels of initial contamination with *E. coli* O157:H7, such as 10^2 CFU.mL^{-1}, influence the detection of the pathogen. It is possible that the high numbers of *E. coli* found by the faecal contamination indicator may have interfered with the development and/or detection of the pathogen. There was no significant difference in the behaviour of the different *E. coli* O157:H7 strains evaluated during the processing steps for MFC. The process did not affect the growth and/or viability of the pathogen.

Although this work did not aim to evaluate the sensitivity of the detection method, the results show that direct counting on CT-SMAC exhibited a low level of sensitivity.

The results for each group were compared to identify differences with respect to the time of storage at 8 °C, artificial initial contamination level and bacterial strains. Results obtained after adjusting a linear mixed-effects model indicated no significant difference between the contamination levels (10^2 and 10^3) across time. After evaluating differences from baseline (day 0) it was detected a significant increase in the *E. coli* O157:H7 population on days 4 and 5 and, after that the population declined ($p<0.05$). A significant decrease, compared to baseline, occured on days 10 and 15 ($p<0.05$). There were no significant differences between strains EDL933 and RJ581 when considering the whole time course .

The storage temperature of 8 °C allowed for the growth of *E. coli* O157:H7 in MFC until the 4th or 5th day of the storage period, after which there was a reduction in the bacterial

Table 5. Counts of *E. coli* O157:H7 (CFU.g^{-1}) during the manufacturing steps of Minas frescal cheese.

Collection points	Artificial contamination level of bacterial strains			
	RJ581		EDL933	
	10^2	10^3	10^2	10^3
After heating to 35 °C	< 10^2	5.5 x 10^4	9.0 x 10^2	5.8 x 10^4
After curd formation	< 10^2	2.7 x 10^4	< 10^2	5.5 x 10^4
After salting and stirring at 42 °C	< 10^2	1.1 x 10^4	< 10^2	2.3 x 10^4
Cheese	< 10^2	4.7 x 10^4	< 10^2	1.2 x 10^5

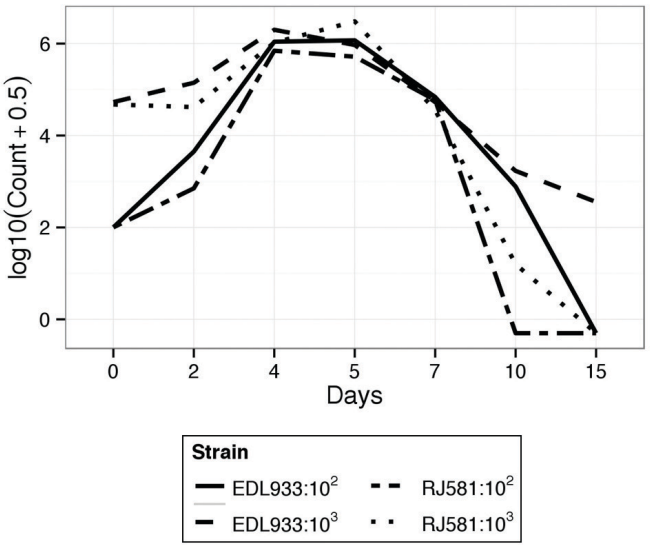

Figure 2. Behaviour of *E. coli* O157:H7 during storage at 8 °C.

population. In addition, viable cells were found until at the least the 7th and 10th storage day in the MFC manufactured with artificial initial contaminations level of 10^2 and 10^3 CFU.mL^{-1} of milk, respectively (Figure 2). This suggests a psychrotrophic behavior of the O157: H7 strains studied.

Marek et al. (2004), who evaluated the behaviour of mixed *E. coli* O157:H7 strains (initial concentration of 10^2 and 10^5 cells. mL^{-1}) in pasteurised and unpasteurised Cheddar cheese whey stored at temperatures of 4, 10 and 15 °C for 28 days, obtained similar results to those of this study. At the storage temperatures of 10 and 15 °C, regardless of the artificial initial contamination level, the numbers of *E. coli* O157:H7 had a significant increase on days 7 and 4 of storage, respectively, and a decline in their population was observed soon after. Other authors have also described the multiplication and survival of *E. coli* O157:H7 at low temperatures (Ramsaran et al., 1998).

The interest of STEC in the food safety from animal origin, appeared with increasing number of human infections caused by this pathogen (Hussein & Sakuma, 2005). The survival of STEC O157:H7 in different types of cheese have already been demonstrated (Marek et al., 2004; D'Amico et al., 2010), but there are no reports on MFC, a not matured cheese with great commercial importance in Brazil.

The low infectious dose showed by STEC O157:H7, together with the feasibility of survival in MFC, allows us to include this food as a potential public health problem, since the Brazilian cattle is an important reservoir of STEC. And studies in the literature show the high contamination of the MFC by *E. coli* (Araújo et al., 2002). However, the expression of virulence within the cheese matrix may be altered over time by prolonged exposure to salt, acid, low temperatures, low moisture, and the presence of starter culture (D'Amico et al., 2010). Perhaps, this can be included with other explanations for the low incidence of the STEC diseases, including serotype O157:H7, in Brazil.

Due the high frequency of STEC in cattle from different regions of Brazil (Cerqueira et al., 1999; Irino et al., 2005), was considered the MFC as a potential vehicle for transmission of STEC, because the conditions as the raw material, processing, transport and storage may allow contamination of this type of cheese by these bacteria, which has been demonstrated even in countries with better sanitary standards (Vernozy-Rozand et al., 2005).

Because of the variation in host susceptibility, a number of individuals exposed to cheeses contaminated with STEC O157:H7 may develop mild forms of illness that go unreported (D'Amico et al., 2010).

4 Conclusions

The processing of MFC does not eliminate STEC O157:H7 and the detection of viable cells at all stages of processing are possible.

The different *E. coli* O157:H7 strains studied showed the same behavioural profile under various conditions. Due his psychrotrophic behaviour, the temperature of 8 °C cannot be used to control the growth of *E. coli* O157:H7. *E. coli* O157:H7 requires a low infectious dose of fewer than 100 cells.g^{-1} or mL^{-1} of food. As a result, the capacity of this bacterium to remain viable during processing and storage of MFC is high, and special attention should be directed to this food. MFC is a potential vehicle for STEC O157:H7, which makes this food a problem, given the poor hygiene conditions of these products in the domestic market and in food processing. More studies should be conducted, including an assessment of the microflora present in the viability of the STEC O157:H7.

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