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Modelling the fate and serogroup variability of persistent *Listeria monocytogenes* strains on grated cheese at different storage temperatures

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ABSTRACT

Processed cheese from cow's milk is one of the most consumed dairy products worldwide. Since this product is defined as ready-to-eat, foodborne pathogens such as *Listeria monocytogenes* can represent a health concern for susceptible populations. In this study, the individual and combined kinetic behaviour of four *L. monocytogenes* serogroups (namely, 1/2a, 1/2b, 1/2c and 4b) persistently isolated from a Spanish cheesemaking factory was modelled on grated cheese at different isothermal conditions (4 and 12 °C) during a 120-days period. The serogroup variability was characterized over the storage time by the isolation and identification of the different serogroups in the cocktail containing the four strains. This processed cheese did not support the growth of *L. monocytogenes* during storage. Survival patterns described by the log-linear type model indicated a high variability of *L. monocytogenes* serotypes at the tested temperatures: *L. monocytogenes* serogroup 4b showed a more rapid decrease rate at 4 °C than at 12 °C, while the opposite trend was found for the rest of serogroups and the *L. monocytogenes* cocktail containing all the strains. Survival rate of *L. monocytogenes* serogroup 1/2c at 4° C was 0.007 log CFU/d being the most resistant serotype while at 12° C, serogroup 1/2a showed the lowest survival rate (0.011 log CFU/d), thus showing a prolonged survival at this temperature. This study highlights the potential implications of *L. monocytogenes* contamination in processed cheese and shows that serogroup variability should be considered when evaluating survival patterns in contaminated products. Finally, the predictive models developed here could be useful to assist food operators to set specific storage conditions and formulations to avoid *L. monocytogenes* growth and survival in grated cheeses.

Keywords:

Foodborne pathogens, *Listeria monocytogenes*, persistence, cheese, survival rate, log-linear models, shelf-life

INTRODUCTION

Cheese consumption has grown in EU in recent years largely due to the improvement in the process quality and the increase of the number of European Quality Labels. For example, cheese is the second most consumed dairy product in Spain and represents more than 20% of the dairy products consumed by Spanish households in 2015, totalling 8 Kg per capita (MAPAMA, 2016), with an inter-annual increase of 1.4% in 2016 (MERCASA, 2017). Herd certification programs adapted Hazard Analysis Critical Control Point (HACCP) systems and systematic microbiological quality control throughout the supply chain (European Union, 2004) have been developed to guarantee the safety of cheese. However, cheese has been identified in risk assessment as a food of greater concern to public health due to listeriosis (Arrese and Arroyo-Izaga, 2012). *L. monocytogenes* can contaminate cheeses made from either raw or pasteurized milk, and listeriosis has become the emblematic example of severe illness transmitted by dairy products and particularly cheeses (Montel et al., 2014). Several *L. monocytogenes* outbreaks have been reported linked to cheese (Martinez-Ríos and Dalgaard, 2018), and recently, the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has warned about a case of meningitis in Madrid (Spain) implicated to the consumption of cheese in Spain (AECOSAN, 2018).

Predictive microbiology can help towards a better understanding of the behaviour of foodborne pathogens, by mathematically modelling reproducible patterns that can be used to predict microbial growth or survival over a range of conditions (Pérez-Rodríguez and Valero, 2013). Various mathematical models have been recently published for *L. monocytogenes* in dairy products, such as milk (Koutsoumanis et al., 2010), smear soft cheese (Ferrier et al., 2013), mold ripened cheese (Lobacz et al., 2013), mature raw sheep milk cheese (Valero et al., 2014), soft blue-white cheese

(Rosshaug et al., 2012) and in other cheese types or cheese model systems (Demers-Mathieu et al., 2013; Schwartzman et al., 2010; Spanu et al., 2015). Grated cheeses are widely commercialized in the market as side food ingredients for several composite dishes. These cheeses are subjected to different handling processes in dairy industries (slicing, packaging etc.), making them more prone to eventual bacterial contamination. Little scientific information is currently available on *L. monocytogenes* behaviour in such types of cheeses, and there is a lack of data on genotypic variation in *L. monocytogenes* isolated from the processing environment of dairy industries and how they can persist over long storage periods.

In this study, the kinetic behaviour of four cheese-borne, persistent *L. monocytogenes* strains belonging to the 4 major genetic groups of this species (1/2a – sequence type (ST) 204-, 1/2b –ST 5-, 1/2c –ST9- and 4b –ST6-) was modelled on grated cheese at different isothermal conditions (4 and 12 °C). Finally, *L. monocytogenes* variability was characterized over the storage time through the isolation and identification of the most persistent serotypes.

MATERIAL AND METHODS

2.1 Grated cheese samples

Processed grated cheese from cow's milk was used in this study. Cheese samples were provided by a local producer, packaged in modified atmosphere (50% CO₂ + 50% N₂). This type of cheese contains a moderate concentration of fat and proteins (approximately 21% each). The shelf-life of the commercial portions of this type of products usually is 3 months (90 days). The main ingredients are cow's milk cheese, butter, milk proteins, potato starch and modified potato starch, whey powder, melting salts, cream, salt, food preservative (E-202), and citric acid. Ten 25-g cheese samples were tested to confirm the absence of *L. monocytogenes* using the ISO 11290-1 detection method (ISO, 2017a). The pH and a_w values were measured on three non-inoculated samples every week along the timeframe of the study. The a_w was measured at 25 °C using an Aqualab water activity meter (Aqualab model Series 4 Decagon Devices Inc., Pullman, WA), whereas the pH was measured after blending 10 g of cheese in 90 mL of distilled and deionised water using a pH meter Crison Basic 20+ equipped with an electrode pH 0- 14 (Crison Instruments, S.A., Barcelona, Spain).

2.2 Bacterial strains and preparation of *L. monocytogenes* inocula

Four strains of *L. monocytogenes* were used in this study: LBMM1009 (serogroup 4b, sequence type ST6), LBMM 1111 (serogroup 1/2a, ST 204), LBMM 1104 (serogroup 1/2b, ST5) and LBMM 1109 (serogroup 1/2c, ST9) strains isolated from the dairy company providing the cheese samples. The four strains were chosen due to previous evidence that they can survive the stressful environment of cheesemaking better than other strains, as they were repetitively isolated in the facility of the cheese company. All strains were maintained at -80 °C in cryovial containing beads and cryopreservatives

(Oxoid TP15731 Maintenance Freeze medium, Oxoid, Hampshire, UK). Prior to the start of the experiment, a bead of each strain was surface-plated onto a Petri dish with Brain Heart Infusion (BHI) agar (Beckton, Dickinson and Co.) and incubated at 37 °C for 24h. Then, a loopful of one colony was transferred aseptically into 10 ml of BHI Broth (Beckton, Dickinson and Co.) and incubated at 37 °C overnight. To determine the initial concentration of each working inoculum, an aliquot was serially diluted, and surface plated onto BHI agar, incubated at 37 °C for 24h and colonies were counted. Each working inoculum was diluted and mixed to create a final cocktail of the four *L. monocytogenes* strains (approx. 2.5×10^6 CFU/ml at stationary phase). To ensure that the same concentration of each strain was present in the mixed cocktail, individual cultures were plated and microbial concentration was further checked.

2.3. Artificial contamination of cheese samples and storage

Grated cheese was aseptically removed from the commercial bags and transferred into sterile co-extruded polyamide/polyethylene packing bags (Industrias Pargón, Salamanca, Spain). Five different scenarios of artificial inoculation were tested: i) inoculation with the *L. monocytogenes* strain LBMM1009 (serogroup 4b), ii) inoculation with the *L. monocytogenes* strain LBMM 1111 (serogroup 1/2a), iii) inoculation with the *L. monocytogenes* strain LBMM 1104 (serogroup 1/2b), iv) inoculation with the *L. monocytogenes* strain LBMM 1109 (serogroup 1/2c), and v) inoculation with a cocktail containing the four *L. monocytogenes* strains. Samples of 25 g of grated cheese were inoculated with 100 µl of PBS containing *L. monocytogenes*. To facilitate a homogeneous distribution of the inoculum, the microbial culture was evenly distributed in ten different surface areas of 10 µl each (for a final concentration of $\sim 1 \times 10^4$ CFU/g). Then, inoculated samples were air-dried in a biosafety cabinet, and

subsequently packaged at the same atmosphere above described and stored at two different temperatures (4 and 12 °C).

2.4 Microbiological analyses

Microbiological studies on the presence and enumeration of *L. monocytogenes* at each sampling point were conducted using three independent inoculated cheese samples according to ISO 11290-1 (ISO, 2017a) and ISO 11290-2 (ISO, 2017b), respectively. The analysis period covered 109 and 118 days at 4° C and at 12°C, respectively in relation to the established shelf-life by the manufacturer (Beaufort et al., 2014). The quantification limit was 10 CFU/g and the counts from the triplicate samples were expressed as log CFU/g. It was assumed that in case that cheeses samples yielding three successive negative results using the standard detection method were not further analyzed. In parallel enumeration of lactic acid bacteria (LAB) was conducted in each sampling time according to ISO 15214 (ISO, 1998).

2.5 PCR-Serogrouping of *L. monocytogenes*

From the experiment of the *L. monocytogenes* cocktail, twenty colonies with *L. monocytogenes* characteristics grown in the ALOA agar plates were assayed in each sampling event for each grated cheese sample to determine the percentage of each *L. monocytogenes* serogroup. *L. monocytogenes* serogrouping was determined using a multiplex PCR targeting the specific target genes lmo0737, lmo1118, ORF2819, ORF2110 and *Listeria* spp. specific prs published by Doumith et al. (2004) and amended by Leclercq et al. (2011) for PCR IVb-VI.

2.6 Data modelling

The survival patterns of the different *L. monocytogenes* serotypes in the grated cheese samples were constructed by plotting the logarithm of the number of colony-forming

units per g of sample (log CFU/g) against the storage time (d). The log-linear model (Equation 1) was fitted to survival curves using the GInaFiT add-in for Excel® (Geeraerd et al., 2005).

$$\log N(t) = \log N_0 - k_{max} \cdot t \quad \text{Equation 1}$$

where $N(t)$ is the number of survival cells (log CFU/g) at time t (d); N_0 corresponds to the initial inoculum level (log CFU/g); and k_{max} is defined as the maximum survival rate (log CFU/d).

On the other hand, growth of LAB was also monitored, and log count data were processed to MS Excel. The Baranyi model (Baranyi and Roberts, 1994) was fitted using DMFit v 3.5 (Institute of Food Research, Norwich, England). Kinetic growth parameters; namely maximum growth rate (μ_{max} , log CFU/d) and maximum population density (MPD , log CFU/g) were estimated from the observed data at each condition studied.

For both survival and growth models, Root Mean Squared Errors (RMSE) and adjusted coefficient of determination ($R^2_{adj.}$) were determined to assess the goodness-of-fit of the estimated models.

2.7 Statistical analysis

All the experiments were carried out in triplicate to capture biological variability. The univariate Analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$), was achieved to evaluate significant differences in the estimated kinetic parameters between storage temperatures and *L. monocytogenes* serotypes. The Statistica® v12.0 software was used (Statsoft, Portugal).

RESULTS AND DISCUSSION

3.1 Survival ability of *L. monocytogenes* serogroups in grated cow's milk cheese

Survival patterns of *L. monocytogenes* individual serotypes as well as for the cocktail during the storage period are shown in Figure 1A-E. Although this monitoring period exceeded the shelf-life period recommended by the manufacturer (90 days), it was intended to study the survival ability of *L. monocytogenes* to better know its potential persistence in abusive storage conditions. The results obtained in the present study corroborated that processed cheese does not support the growth of *L. monocytogenes* regardless different combinations of strains, inoculum levels and storage temperatures, as already shown in other studies (Angelidis et al., 2010; Valero et al. 2014; Wemmenhove et al., 2013; Yousef and Marth, 1990). Interestingly, the four strains selected for this study were persistent in the facility of the company providing the grated cheese samples (i.e. an identical PFGE type was detected in a lapse of time bigger than 6 months).

Final concentrations of all *L. monocytogenes* serogroups remained above 2 log CFU/g at the end of the storage period, which indicates that, although this cheese matrix cannot support the growth of *L. monocytogenes*, it can survive during prolonged storage at refrigeration temperatures. The results obtained showed that *Listeria* survival on grated cow's milk cheese was temperature and strain dependent. The survival patterns of *L. monocytogenes* serogroup 4b, 1/2c as well as the cocktail showed significant differences ($p < 0.05$) in the obtained survival rates at the tested temperatures (Table 1). *L. monocytogenes* strain LBMM 1009 (serogroup 4b, ST6) was the only one showing a more rapid decrease rate at 4 °C than at 12 °C (Figure 1A), thus confirming the sensitivity of this serogroup to refrigeration temperatures. The opposite trend was found

for the serogroup 1/2c and the *L. monocytogenes* cocktail containing the four strains (Figure 1B-E). This could indicate that survival behavior of the cocktail was dominated by the serogroup 1/2c.

On the other hand, the serogroups 1/2a and 1/2b did not show significant differences in k_{max} values at the tested temperatures ($p \geq 0.05$). This result is interesting, as *L. monocytogenes* strains belonging to ST6 are reported to be hypervirulent and often involved in listeriosis with severe outcome (Kremer et al., 2017; Severi et al., 2017; Smith et al., 2016).

The *L. monocytogenes* LBMM 1009 (serogroup 4b, ST6) decreased from 3.84 log CFU/g to 2.13 log CFU/g in 109 days at 4 °C, while the final concentration remained above 2.7 log CFU/g at 12° C (Figure 1A). A similar behavior was observed at 4 and 12 °C for *L. monocytogenes* LBMM 1111 (serogroup 1/2a, ST204), with a 2.5 log reduction in approx. 110 days storage (Figure 1B). Noteworthy, this serogroup was persistently isolated in the grated cheese area of the facility providing the cheese samples for this study. *L. monocytogenes* strains belonging to ST204 have been isolated from food and food environments (Allnut et al., 2016; Haase et al., 2014; Ruckerl et al., 2014), and the persistence of the *L. monocytogenes* ST204 has been also described in dairy processing environment in Czech Republic (Stessl et al., 2014). Lower decrease than the serogroup 4b was observed for the *L. monocytogenes* LBMM1104 (serogroup 1/2b, ST5) since final values were slightly above than 1-log reduction at the end of the storage period (Figure 1C). This prolonged survival is relevant for cheesemaking industries as *L. monocytogenes* serotypes belonging to ST5 are globally distributed and highly prevalent in cheese processing environments, and recently have been also

associated with foodborne outbreaks (Chen, et al., 2017; Lomonaco et al., 2013, Maury et al., 2016; Muhterem-Uyar et al., 2018).

L. monocytogenes strain LBMM 1109 (serogroup 1/2c, ST9) presented the highest survival ability at both storage temperature scenarios tested (Figure 1D). Serotypes of *L. monocytogenes* belonging to ST9 have been associated with food processing plants, foods and listeriosis cases (Henri et al., 2016; Martin et al., 2014; Parisi et al., 2010; R uckerl et al., 2014; Wang et al., 2012). There were significant differences for this serogroup in survival rates ($p < 0.05$) at 4 °C (~0.5 log CFU/g decrease in 109 days of storage) in comparison with 12 °C (~ 1-log CFU/g decrease in 118 days of storage). This result could demonstrate that that *L. monocytogenes* strain LBMM 1109 (serogroup 1/2c, ST9) was cold adapted in this cheese matrix, thus indicating its high persistence. Finally, the inoculated cocktail containing the four *L. monocytogenes* serogroups showed a similar trend to that obtained for *L. monocytogenes* strain LBMM 1109 (serogroup 1/2c, ST9) (Figure 1E), highlighting that this serotype could have imposed over the others throughout the whole-time span of the study regardless of the storage temperature.

Differences between strains at various temperatures cannot be easily retrieved from literature since most published studies refer to an inoculation with a cocktail of *L. monocytogenes*. Kagkli et al. (2009) observed differences in monitoring single-strain inocula between low (i.e. 5 °C) and high temperatures (i.e. 20 °C) in Greek soft cheese (*Katiki*). Though similar survival was observed at different temperatures in some cases, longer survival of *L. monocytogenes* was observed when cheese was stored at low temperature, in agreement with the findings in the present study, apart from the *L. monocytogenes* strain 1009 (serogroup 4b) with longer survival at 12 °C. The results

shown in this study contrast with those obtained by Angelidis et al. (2010), where *L. monocytogenes* strains belonging to serogroup 4b were able to survive for a longer time at low temperatures. Other studies also demonstrated that some *Listeria* isolates showed less resistance to ripening and storage conditions of semi-hard or hard cheeses, such as Gouda where *L. monocytogenes* strain Scott A was reduced by more than 1.0 log after 28 weeks and longer (Wemmenhove et al., 2013).

Microbial responses of *L. monocytogenes* to stressful conditions according to their serogroup and origin were studied by Lianou et al. (2006), showing variability in heat and acidic resistance patterns between serotype 4b and Scott A. The lesser survival at 4 °C of *L. monocytogenes* serogroup 4b could be attributed to the lack of adaptation to the cheese environment at low temperatures. Comparisons in survival patterns should be made with caution, since the physicochemical parameters of cheeses as well as indigenous microbiota could influence the survival ability of *L. monocytogenes*. The log-linear model parameters and goodness-of-fit indices for describing survival of *L. monocytogenes* are presented in Table 1. A common linear survival pattern was obtained. All microbial counts were included for model fitting because there were within the limit of quantification of the analytical technique (10 CFU/g). The log-linear model showed a good adjustment to observed data, being able to describe survival patterns of *L. monocytogenes* individual serotypes as well as for the cocktail containing the four strains ($R^2_{adj.} > 0.758$; RMSE < 0.349).

3.2 Physicochemical parameters and LAB growth over storage of grated cheese

There were not significant differences in pH and a_w during the storage at both temperatures ($p > 0.05$). Average pH values were 6.33 ± 0.16 at 4° C, and 6.38 ± 0.04 at 12° C while a_w values were between 0.939 and 0.955 (mean 0.949 ± 0.001) at 4° C and

between 0.942 and 0.955 (mean 0.949 ± 0.001). No significant variations were observed in both parameters during storage.

LAB growth was modelled using the Baranyi primary model, which showed a reasonably good fit to the observed concentrations ($R^2 > 0.82$; standard error of fit < 0.334). The fitted curves are shown in Figure 2. It is observed that LAB were able to grow over storage, however, as expected, temperature influenced on the maximum growth rate 0.143 ± 0.013 log CFU/day at 12 °C and 0.025 ± 0.005 log CFU/day at 4 °C) and that storage at low temperatures significantly affected the maximum LAB population density as well (4 °C: 3.84 ± 0.093 log CFU/g; 12 °C: 6.93 ± 0.06 log CFU/g). Interestingly, LAB grew immediately over the storage period since there was not lag phase, thus indicating that most of the LAB groups could correspond to microbial species adapted at refrigeration conditions. A sharp increase of LAB population was observed at 12 °C; from 2 to 7.28 log CFU/g in 97 days, while only an increase of 2.5 log CFU/g was observed at 4 °C during the same period. A lower growth of LAB was observed in cured raw sheep milk cheese at the same temperatures, these differences being attributed to the lower pH of the cheese samples in that study (5.46) by Valero et al. (2014). In the present study, pH variations over the storage period were not significant despite the increased concentration of LAB at 12 °C. It could be hypothesized that there was a high proteolytic activity producing ammonia compounds together with the added ingredients which could have a buffering capacity able to neutralize lactic acid production by LAB.

*3.3 Persistence of *L. monocytogenes* serogroups over storage at different temperatures*

A representative amount of *L. monocytogenes* colonies of the cocktail containing the four *L. monocytogenes* serogroups (20 per cheese sample in each sampling event over

the storage period) were further identified to examine the evolution and potential differences of the survival of the different serotypes in the cheese. A graphical representation of the percentage of *L. monocytogenes* serotypes at 4 °C and at 12 °C is shown in Figure 3. *L. monocytogenes* serogroup 1/2a (ST204) was the most prevalent during the first two days of storage at 4° C (Figure 3A). However, as the storage time increased (>7 days), *L. monocytogenes* serogroup 1/2c (ST9) was the most prevalent, being present in more than 80% of isolates after the study period at both storage temperatures (Figure 3A-B). These results are in line with those obtained in the survival curves (Figure 1A-E), which showed that *L. monocytogenes* serogroup 1/2c presented the highest concentrations. The *L. monocytogenes* LBMM 1111 and 1104 belonging to the serogroups 1/2a and 1/2b were recovered mostly in the initial sampling periods, however, their presence was becoming lower as long as storage time increased. Interestingly, although the four strains were persistent in the cheese facility providing the cheese samples (i.e. isolated several times with a lapse of time of more than 6 months), *L. monocytogenes* LBMM 1111 (serogroup 1/2a, ST204) and *L. monocytogenes* LBMM 1109 (serogroup 1/2c, ST9) were persistently isolated in the grated cheese area during a sampling study of one year (Melero et al., unpublished).

In conclusion, a high variability in the survival behavior of *L. monocytogenes* was observed, directly related to the *L. monocytogenes* serotypes present in the grated cheese. While the *L. monocytogenes* strain belonging to the serogroup 4b (ST6) was the most sensitive to refrigeration temperatures, the *L. monocytogenes* serogroup 1/2c (ST9) exhibited the most resistant survival pattern at both temperatures tested, yielding the highest concentration values and being detected up to the end of the storage period. Selection of the most appropriate *L. monocytogenes* strains (considering both the

genetic background and the capacity for persistence in the specific food processing environments) should be taking account prior to conducting a challenge test, particularly when defining best- and worst-case scenarios for microbial risk assessment (Lianou et al., 2006). This study highlights the potential implications of *L. monocytogenes* contamination in cheese and shows that *L. monocytogenes* serogroup variability should be considered when evaluating survival patterns in contaminated food products. Finally, the predictive models developed here could be useful to assist food operators to set specific storage conditions and formulations to avoid *L. monocytogenes* growth and survival in dairy products, particularly in grated cheese.

CONFLICT OF INTEREST

None

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Table 1. Log linear model parameters; $\log N_0$ (initial inoculum level, \log CFU/g); k_{max} (maximum survival rate, \log CFU/d), and goodness-of-fit indices (Root Mean Squared Error, (RMSE) and adjusted determination coefficient, (R^2 adj.)) describing survival of *L. monocytogenes* serotypes in grated cow's milk cheese stored at 4 and at 12 °C. Standard errors are represented between brackets.

T(°C)	Model parameters	<i>L. monocytogenes</i> serotype				
		<i>4b</i>	<i>1/2 a</i>	<i>1/2 b</i>	<i>1/2 c</i>	<i>Cocktail</i>
4	$\log N_0$	3.808* ^{Aa}	3.909 ^{Aa}	4.008 ^{Aa}	4.096 ^{Aa}	3.936 ^{Aa}
		(0.049)	(0.059)	(0.023)	(0.025)	(0.031)
	k_{max}	0.034 ^{Aa}	0.011 ^{Ac}	0.023 ^{Ab}	0.007 ^{Bd}	0.013 ^{Bc}
		(0.002)	(0.001)	(0.001)	(0.001)	(0.001)
		<i>RMSE</i>	0.112	0.349	0.052	0.057
R^2 adj.	0.955	0.895	0.979	0.758	0.888	
12	$\log N_0$	3.928 ^{Aa}	3.865 ^{Aa}	3.950 ^{Aa}	4.076 ^{Aa}	3.801 ^{Aa}
		(0.055)	(0.025)	(0.049)	(0.083)	(0.051)
	k_{max}	0.022 ^{Ba}	0.011 ^{Ac}	0.024 ^{Aa}	0.017 ^{Aab}	0.022 ^{Aa}
		(0.002)	(0.001)	(0.002)	(0.003)	(0.002)
		<i>RMSE</i>	0.099	0.249	0.089	0.150
R^2 adj.	0.947	0.978	0.961	0.808	0.951	

* Different lower-case letters indicate significant differences in the same row, while different upper-case letters denote significant differences in the same column ($p < 0.05$)

FIGURE LEGENDS

Figure 1. Fitted log-linear models describing the survival of *L. monocytogenes* (Lm) serotypes in grated cow's milk cheese stored at 4 and at 12 °C. **1A.** Lm strain LBMM 1009 (serogroup 4b); **1B.** Lm strain LBMM1111 (serogroup 1/2a); **1C.** Lm strain LBMM1104 (serogroup 1/2b); **1D.** Lm strain LBMM1109 (serogroup 1/2c); **1E.** Cocktail of all Lm serogroups. Limit of quantification was set at 10 CFU/g.

Figure 2. Observed growth and fitted Baranyi models for lactic acid bacteria populations in grated cow's milk cheese stored at 4 and at 12 °C.

Figure 3. Graphical representation of the percentage of isolated *L. monocytogenes* serotypes in the grated cow's milk cheese over storage time (d) at 4 (**3A**) and at 12 °C (**3B**).

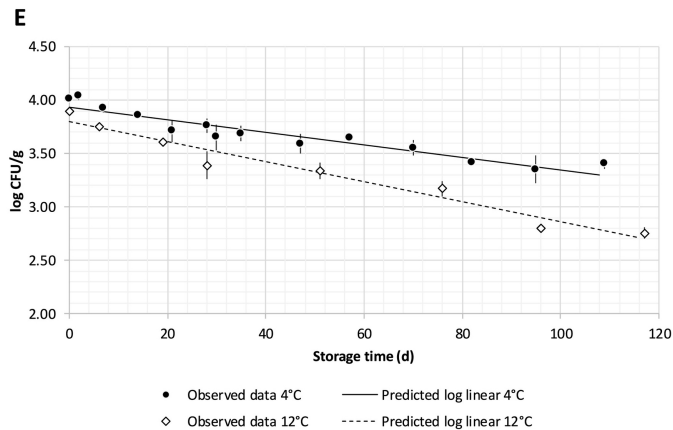
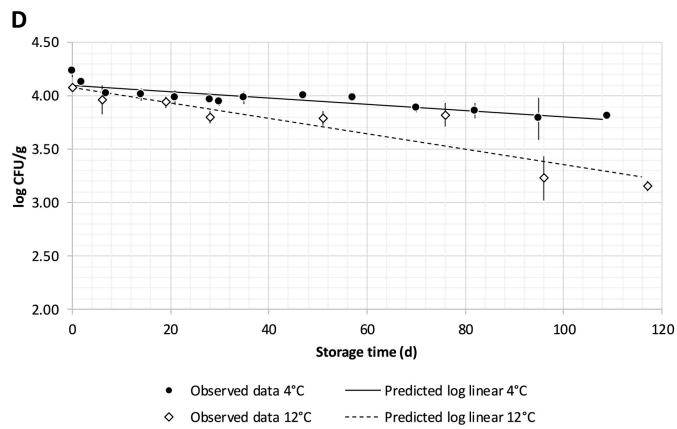
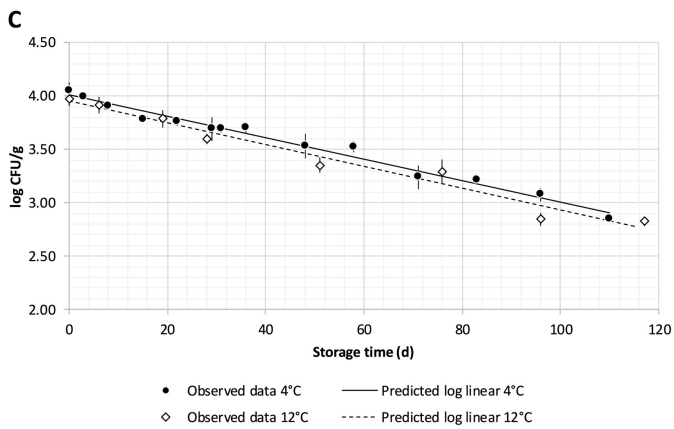
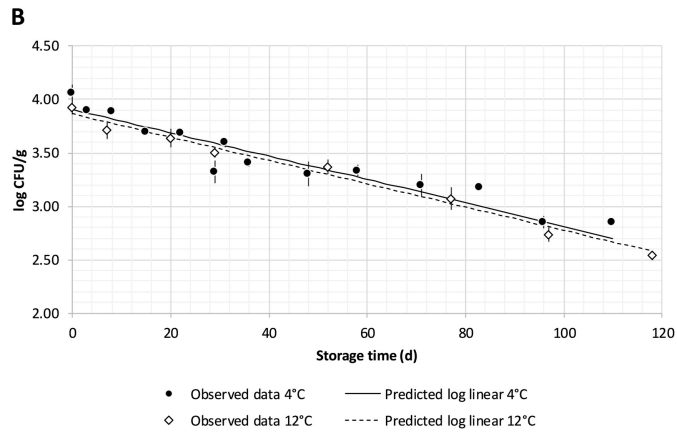
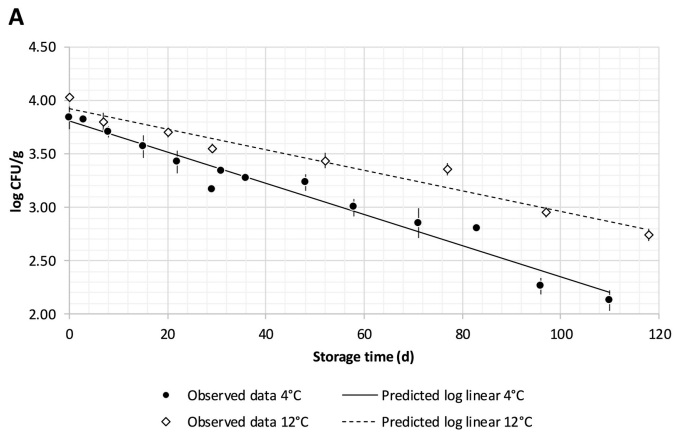


Figure 1

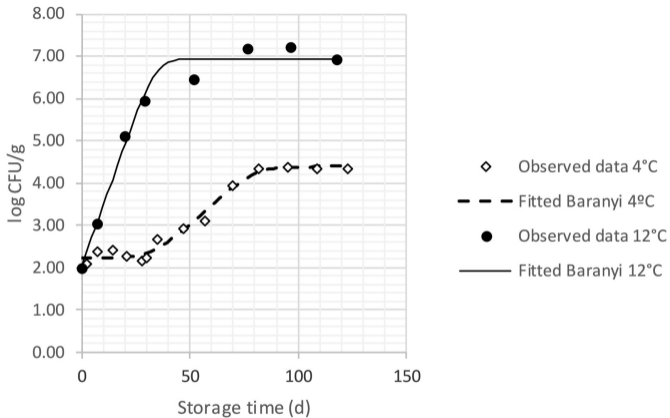


Figure 2

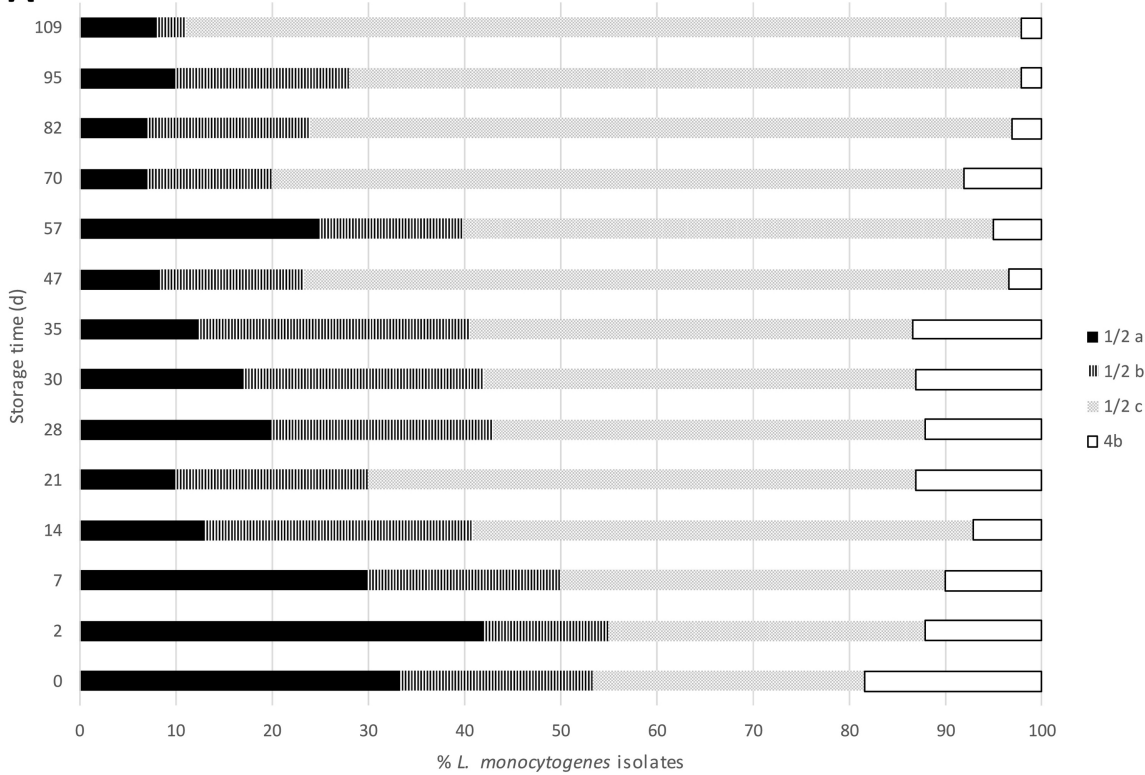
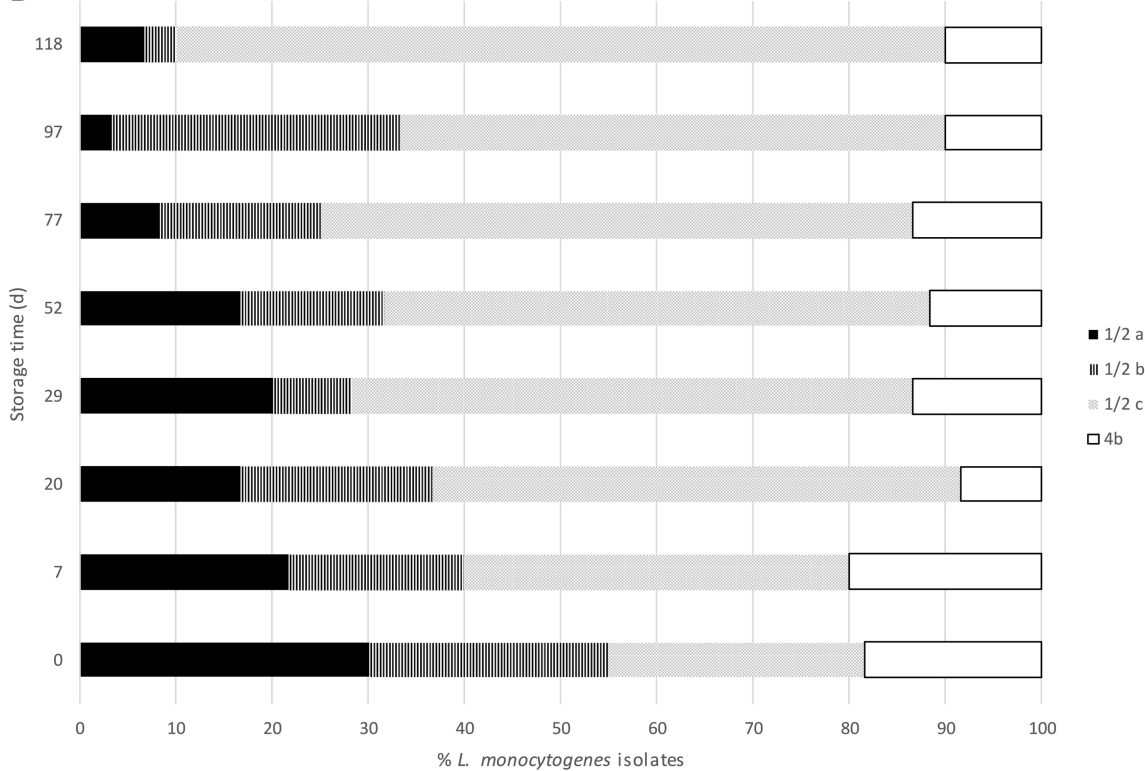
A**B**

Figure 3