Study of the bacterial flora and the growth of *Listeria monocytogenes* in raw milk butter

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Abstract

Listeria monocytogenes is a foodborne pathogen for which growth in food depends on the latter's physico-chemical characteristics and the presence of inhibitors such as lactic acid bacteria. To study the growth of *L. monocytogenes* in raw milk butter, challenge test was conducted. Two approaches of butter production were simulated. The first one used cream matured at 4° C while the second one used cream matured at 14° C. Samples of cream and butter were taken at several days for pH and microbiological analyses. Metagenetic analyses were also conducted on all samples. The two batches of butter showed different bacterial profile. The first batch made from refrigerated cream presented a big diversity and was characterized by an abundance of psychotropic bacteria. This batch showed an increase in the levels of *L. monocytogenes* during butter storage. The growth potential of *L. monocytogenes*, defined as the difference between the median of the counts at the end and the median of the counts at the beginning of storage, was 1.81 log cfu/g. The second batch made from cream matured at 14° C was characterized by a dominance of *Lactococcus*. The latter was even more abundant in inoculated samples than in control samples. This batch presented a decrease of *L. monocytogenes* during butter storage with a growth potential of -1.72 log cfu/g. These data suggest that cream maturation is an important factor to the growth of *L. monocytogenes* and lactic acid bacteria especially *Lactococcus*.

Introduction

Listeria monocytogenes is the causative agent of listeriosis, a severe foodborne disease with high mortality rate. The ingestion of contaminated food, especially ready-to-eat (RTE) including butter, is the principal route of transmission of *L. monocytogenes*. However, the survival or growth of the pathogen depends on the food's formulation and characteristics. Many intrinsic and extrinsic factors such as temperature, pH and the presence of antimicrobials or competitive microbiota, like lactic acid bacteria (LAB). were shown to affect the growth of *L. monocytogenes* in food.

The objective of this study was to use next-generation sequencing to analyze bacterial content of raw milk butter, processed during a challenge test, depending on cream maturation temperature and on the presence or not of artificially inoculated *L. monocytogenes.*

Methodology

Challenge test was conducted on raw milk butter made from artificially contaminated cream with a mix of two strains of *L. monocytogenes.* To simulate a contamination during fabrication, the inoculation was done in the cream before maturation at a level of 50 cfu/ml. Two batches were produced. The creams were kept for three days at 4 °C for the first batch and at 14 °C for the second one. Samples were taken at each day for pH and microbiological analysis (total flora, LAB and levels of *L. monocytogenes*). After three days, the matured creams were transformed into butter by churning. Butter was then stored at 9 °C for 30 days. Samples of butter were taken at several days for analysis (**Figure 6**).

All samples (cream and butter) were also submitted to DNA extraction and sequencing. The bacterial **V1-V3** region of the 16S rRNA gene were amplified by PCR for library preparation. Sequencing was conducted on an Illumina.

Results of challenge test

pH and LAB evolution in cream: At the beginning, the pH values of the two batches of cream were 6.77 ± 0.01 and 6.75 ± 0.01, respectively. During maturation, the pH of B2 cream (maturation at 14 °C) decreased significantly compared to B1 (maturation at 4 °C). The values obtained at the end of maturation were 4.58 ± 0.01 and 6.76 ± 0.01, respectively (**Figure 1**, line graph).

Levels of LAB were higher in B2 samples than in B1 samples with maximum values of 8.03 ± 0.47 and 5.13 ± 0.11 log cfu/g, respectively (**Figure 1**, histogram).

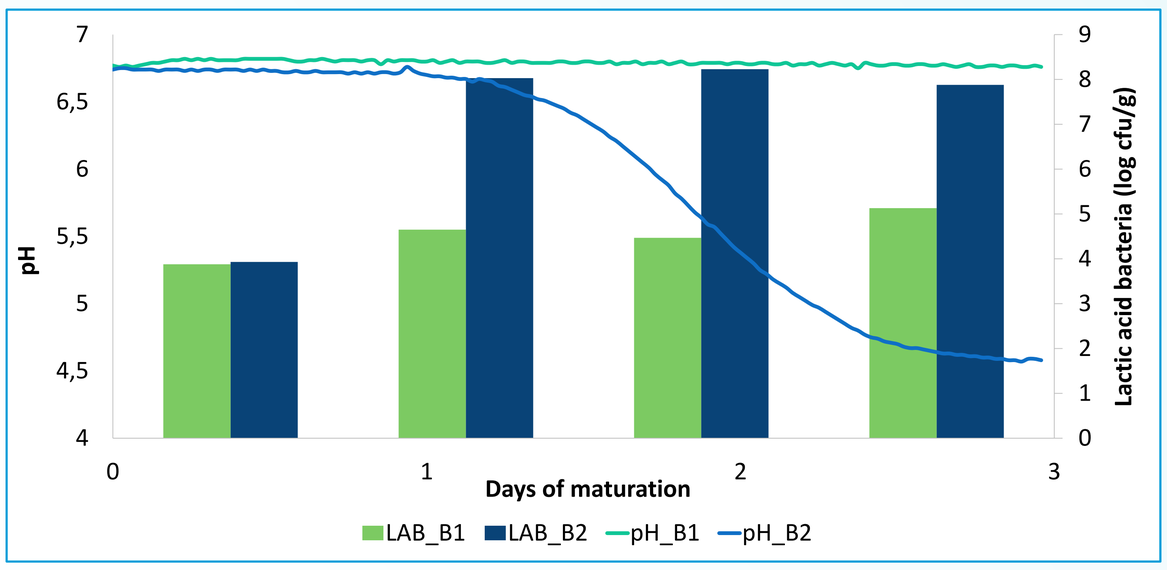

Figure 1: Evolution of pH (line graph) and lactic acid bacteria (histogram) in the two batches of raw cream during maturation.
B1: First batch with cream matured at 4 °C, B2: Second batch with cream matured at 14 °C .

***L. monocytogenes* evolution in cream**: As shown in **Figure 2**, the level of *L. monocytogenes* increased by 1.56 log cfu/g after maturation for cream stored at 4 °C (B1) and by 3.43 log cfu/g for cream stored at 14 °C (B2). The levels obtained for the two batches were 3.75 ± 0.06 and 5.18 ± 0.04 log cfu/g, respectively.

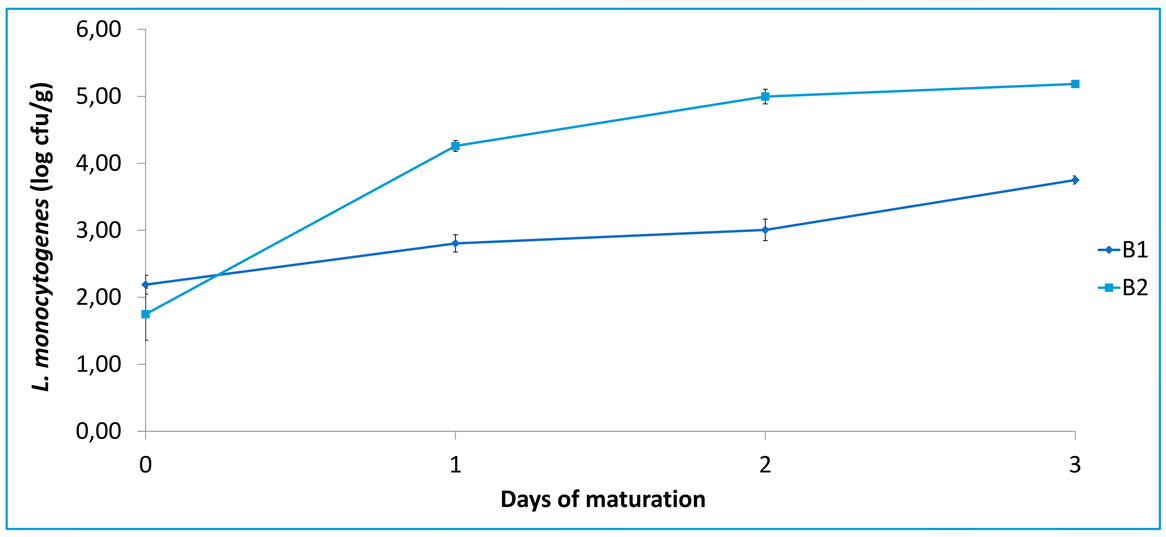

Figure 2: Evolution of levels of *L. monocytogenes* in the two batches of raw cream during maturation.
B1: First batch with cream matured at 4 °C, B2: Second batch with cream matured at 14 °C .

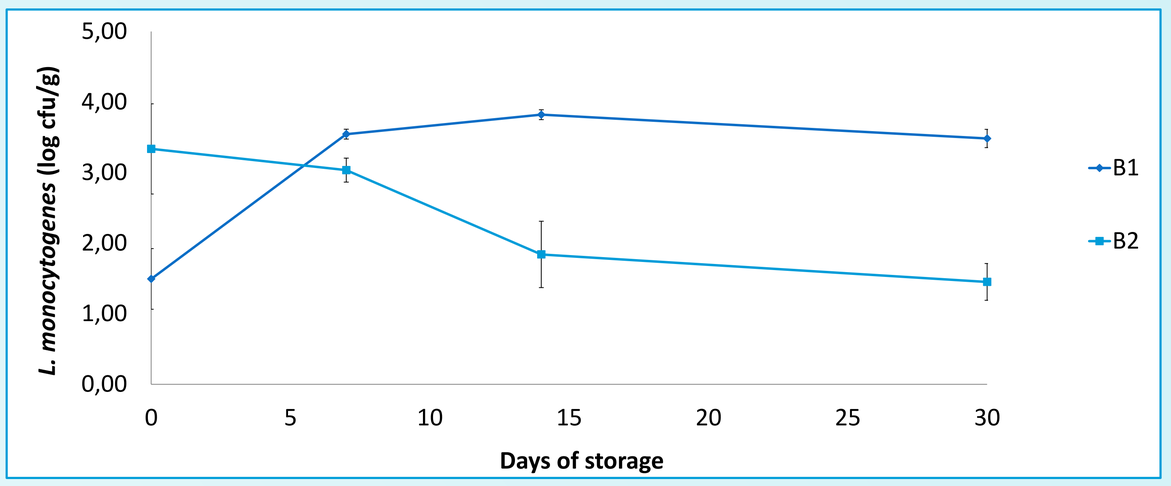

Figure 3: Evolution of levels of *L. monocytogenes* in the two batches of raw milk butter during storage.
B1: First batch with cream matured at 4 °C, B2: Second batch with cream matured at 14 °C .

***L. monocytogenes* evolution in butter and growth potential**: After production, a decrease in contamination levels was observed. The levels of *L. monocytogenes* in butter samples at D0 were respectively 1.49 ± 0.43 log cfu/g and 3.34 ± 0.64 log cfu/g for B1 and B2 (**Figure 3**).

After 30 days of storage, growth potential of 1.81 log cfu/g was obtained for B1 butter. This product therefore allowed the growth of *L. monocytogenes* unlike B2 butter. The second batch presented growth potential of - 1.72 cfu/g (**Table 1**).

Table 1: Results of growth potential (δ) in the two batches of raw milk butter.

	pH	a_w	*L. monocytogenes* (log cfu/g)				δ (med D30 – med D0)
			D0	D7	D14	D30	
B1	6.81 ± 0.01	0.98 ± 0.01	1.00 1.78 **1.70**	3.62 3.51 3.51	3.81 3.89 3.76	3.34 3.60 **3.51**	**1.81**
B2	4.75 ± 0.04	0.98 ± 0.01	2.95 4.08 **2.98**	2.85 3.15 3.11	2.18 1.30 2.05	**1.26** 1.48 0.95	**- 1.72**

Results of metagenetic

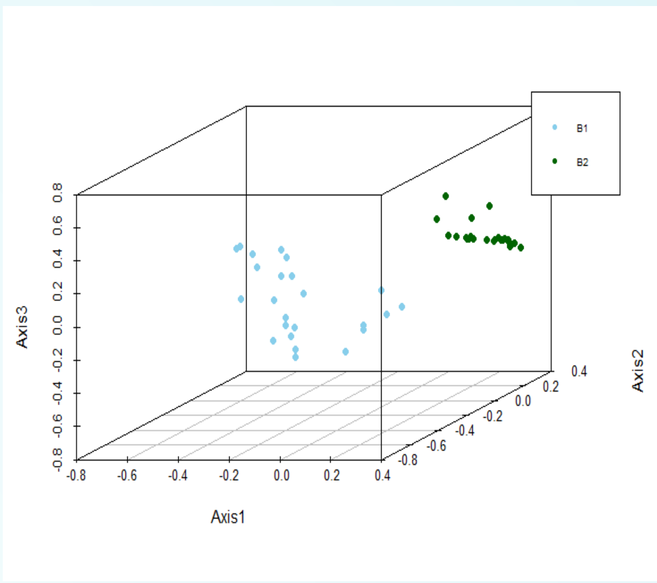

Figure 4: NMDS plot of butter samples generated via Yue & Clayton distance matrix, depending on cream maturation
B1: cream maturation at 4 °C, B2: cream maturation at 14 °C.

Comparison of bacterial community of butter samples: As shown in **Figure 4**, a significant difference was found between butter samples of the two batches. Analyzed results revealed that this significant difference could be owed to the abundance of *Lactococcus* in B2 samples. In contrast, psychrotrophic bacteria were more abundant in B1 samples (**Figure 7**).

Conclusion

- Good cream acidification at 14 °C compared to at 4 °C.
- No growth of *Listeria monocytogenes* in butter made from cream matured at 14 °C.
- Abundance of *Pseudomonas* in high pH butter, made from refrigerated cream.
- Dominance of *Lactococcus* in acidic butter, made from cream matured at 14 °C.
- Higher abundance of *Lactococcus* in inoculated samples compared to control samples.

Acknowledgements

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