Project Number: 1448

Project Acronym: ArtiSaneFood

Project title: Innovative Bio interventions and Risk Modelling Approaches for Ensuring Microbial Safety and Quality of Mediterranean Artisanal Fermented Foods

Deliverable 5.3: Report on the Survival of Foodborne Pathogens in Artisanal Fermented Products Elaborated using Enhanced Process Variables and Bio-Interventions (R, PU; March 2022)

Summary

1. Introduction

The selection of a product and its prototype elaboration process to be tested in fate studies of pathogens are defined within activities performed under Tasks 5.1, 5.2 and 5.3. For the sake of simplicity, the factors considered, and the different experimental designs performed by the partners may be described for a global understanding of the fate studies carried out with the different prototype products. This proposal is supported by the considerations defined in the Deliverable 5.2, summarising the adapted treatments carried out to assess the effects of the different factors on microbial responses in the fermented food products.

In the following sections, relevant information about the fate studies carried out by the different ArtiSaneFood partners with the different prototype fermented products are summarised, as well as the main results obtained so far.

2. Partner UCO

2.1. Summary of Material & Methods

A lab-scale fresh goat cheese was elaborated following the main production steps and orientations of a cheese producer located in Malaga (Spain) (Figure 1). The ingredients used for cheese elaboration were pasteurised goat milk, salt (NaCl, 10%v/v), calcium chloride ($CaCl_2$, 0.28%v/v) and rennet (0.28%v/v). Starter cultures were not used for cheese elaboration.

Cheese samples of 10 g placed in Falcon tubes were inoculated with a three-strain cocktail of *L. monocytogenes* at 10^2-10^3 cfu/g. Inoculation was carried out with the aid of a pipette and the inoculum was placed inside the samples (i.e., perforation in the central point). The inoculated strains were isolated from clinical cases and obtained from the Spanish Type Culture Collection (CECT 4032, CECT5366, CECT935). The inoculated samples were stored at different storage temperatures (4, 12, 18, 25°C) and withdrawn at proper intervals up to 20 days (product shelf-life) for microbial analysis. The temperature range selected reflects the logistic distribution chain conditions. *L. monocytogenes* and Lactic Acid Bacteria (LAB) were detected and quantified by ISO methods (ISO 11290 and ISO 15214, respectively). Samples were analysed in duplicate and the experiments were repeated at least three times.

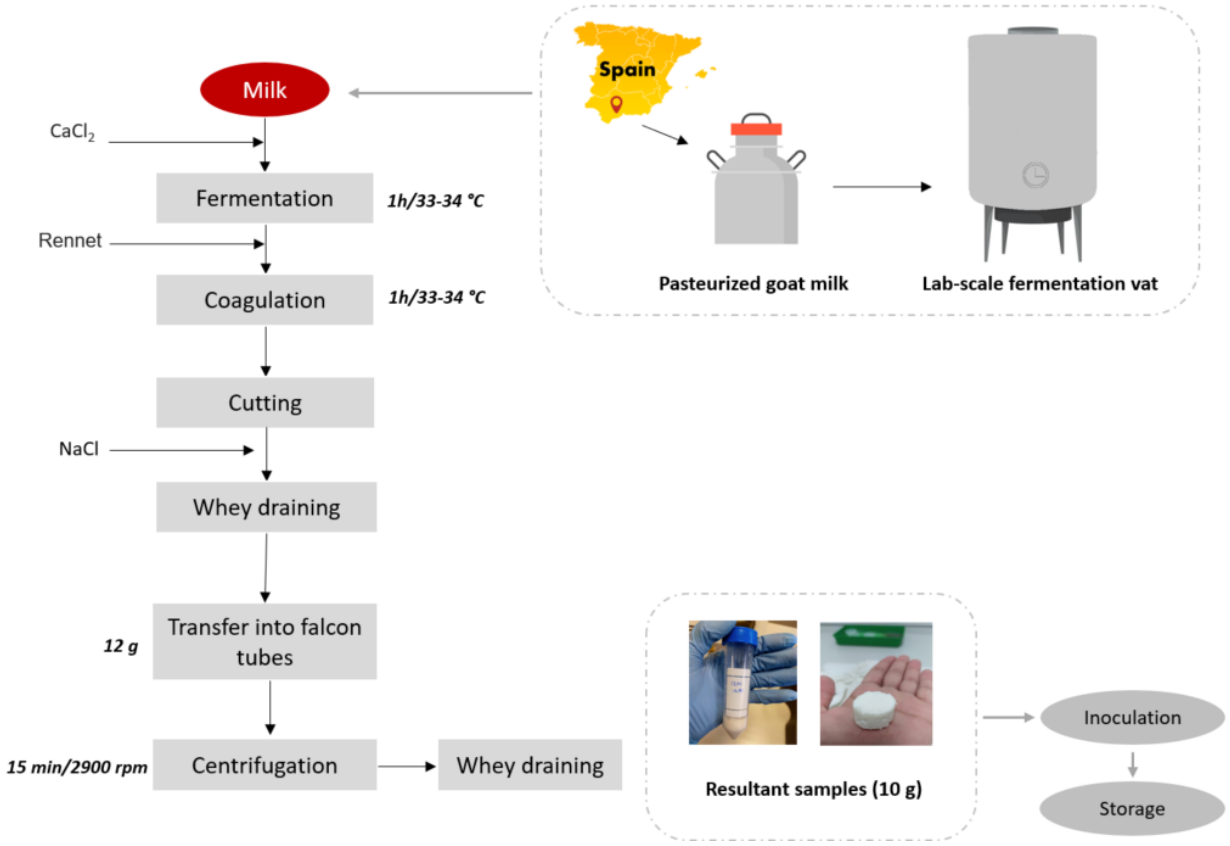

Figure 1. Scheme of the methodology applied to obtain the fresh goat cheese samples.

The pH and a_w of samples were also measured over storage. The initial pH of samples ranged within 6.50-6.60, while the a_w values were around 0.999.

2.2. Summary of Results

The main results of the fate studies are depicted in Tables 1, 2, 3 and 4 for each storage temperature. The duration of each study was adjusted to the product shelf-life at the different temperatures tested (20, 15, 10 and 5 days at 4, 12, 18 and 25°C, respectively). *L. monocytogenes* and LAB were able to grow in the product at all temperatures evaluated. Overall, the pH of samples dropped while the a_w of samples did not vary over storage. At 4°C (Table 1), the mean increase in *L. monocytogenes* concentrations during 20 days of storage was approximately 3.15 log cfu/g. LAB growth potential was lower, with a mean increase of 1.33 log cfu/g after 20 days.

Table 1. Results obtained from the analysis of fresh goat cheese stored at 4°C for 20 days.

Time (days)	0	2	5	8	12	20
LM* (log cfu/g)	2.97±0.01	3.38±0.02	3.73±0.61	4.60±1.26	5.44±1.67	6.12±1.39
LAB (log cfu/g)	4.21±1.23	5.02±0.41	4.85±0.54	4.85±0.81	5.15±0.91	5.54±0.99
pH	6.66±0.06	6.66±0.13	6.63±0.29	6.64±0.43	6.45±0.17	6.43±0.17

*LM= *Listeria monocytogenes*

Regarding the results obtained at 12°C, *L. monocytogenes* showed an increase of 3.04 log cfu/g with respect to the initial concentration after 15 days of storage. LAB growth was more evident than at 4°C, presenting a mean growth potential of 4.05 log cfu/g. Accordingly, the pH of samples decreased over time, ranging from average values of 6.66 to 5.85.

Table 2. Results obtained from the analysis of fresh goat cheese stored at 12°C for 15 days.

Time (days)	0	2	5	8	12	15
LM* (log cfu/g)	3.04±0.05	4.50±0.65	5.90±0.80	6.06±0.73	6.40±0.49	6.08±0.73
LAB (log cfu/g)	4.21±1.23	5.78±0.64	6.80±0.74	7.73±1.06	8.56±1.01	8.26±0.46
pH	6.66±0.06	6.52±0.14	6.40±0.31	6.18±0.30	6.04±0.27	5.85±0.24

*LM= *Listeria monocytogenes*

At 18°C, the average results for *L. monocytogenes* revealed a growth potential of 2.07 log cfu/g after 10 days of storage. The LAB reached a maximum average concentration of 8.60 log cfu/g at the end of storage. The drop in pH values during storage was similar to that observed at 12°C.

Table 3. Results obtained from the analysis of fresh goat cheese stored at 18°C for 10 days.

Time (days)	0	1	2	5	10
LM* (log cfu/g)	3.04±0.05	4.58±0.98	5.28±0.63	5.30±1.76	5.11±1.64
LAB (log cfu/g)	4.21±1.23	5.65±0.58	7.11±0.80	8.36±0.77	8.60±0.62
pH	6.66±0.06	6.62±0.14	6.36±0.05	5.89±0.14	5.81±0.29

*LM= *Listeria monocytogenes*

The results obtained from the analysis of samples stored at 25°C revealed a mean growth potential for *L. monocytogenes* of 2.40 log cfu/g. Among all the temperatures tested, the LAB

presented the highest growth potential at 25°C, with an increase of 4.82 log cfu/g over storage. The average pH value decreased from 6.66 to 5.72.

Table 4. Average results obtained from the analysis of fresh goat cheese samples stored at 25˚C for 5 days, for three experimental repetitions.

Time (days)	0	1	2	3	4	5
LM (log cfu/g)	3.01±0.06	5.25±0.76	5.59±0.36	5.23±0.66	5.13±1.00	5.41±0.60
LAB (log cfu/g)	4.21±1.23	7.27±0.99	8.16±0.52	8.32±0.49	9.11±0.19	9.02±0.18
pH	6.66±0.06	6.32±0.15	5.92±0.14	5.74±0.15	5.74±0.11	5.72±0.14

*LM= *Listeria monocytogenes*

In general, growth rates of *L. monocytogenes* and LAB increased with the increase in storage temperature. However, *L. monocytogenes* maximum population density decreased by increasing storage temperatures. This could be associated with the competition between the pathogen and LAB (i.e., Jameson effect). The decrease of pH was faster at higher temperatures due to the higher LAB growth rate and lactic acid production.

3. Partner UIZ

3.1. Summary of Material & Methods

The laboratory-scale preparation of fresh goat's cheese (*Jben*) was conducted as detailed in Figure 2. Ten L of goat's milk was distributed into five bottles with 2 L/bottle, pasteurised at 65 °C for 30 min, and rapidly cooled to 34 °C. For coagulation, calcium chloride ($CaCl_2$, 0.05%w/v) and rennet (0.03% v/v) were used, following the main production steps of a cheese producer located in Marrakech, without using a starter culture. The curds were cut into 8–15 mm cubes and transferred into small-perforated plastic moulds overnight at 4 °C to drain off the whey. The Jben was next salted (2%) and mixed for 5 min.

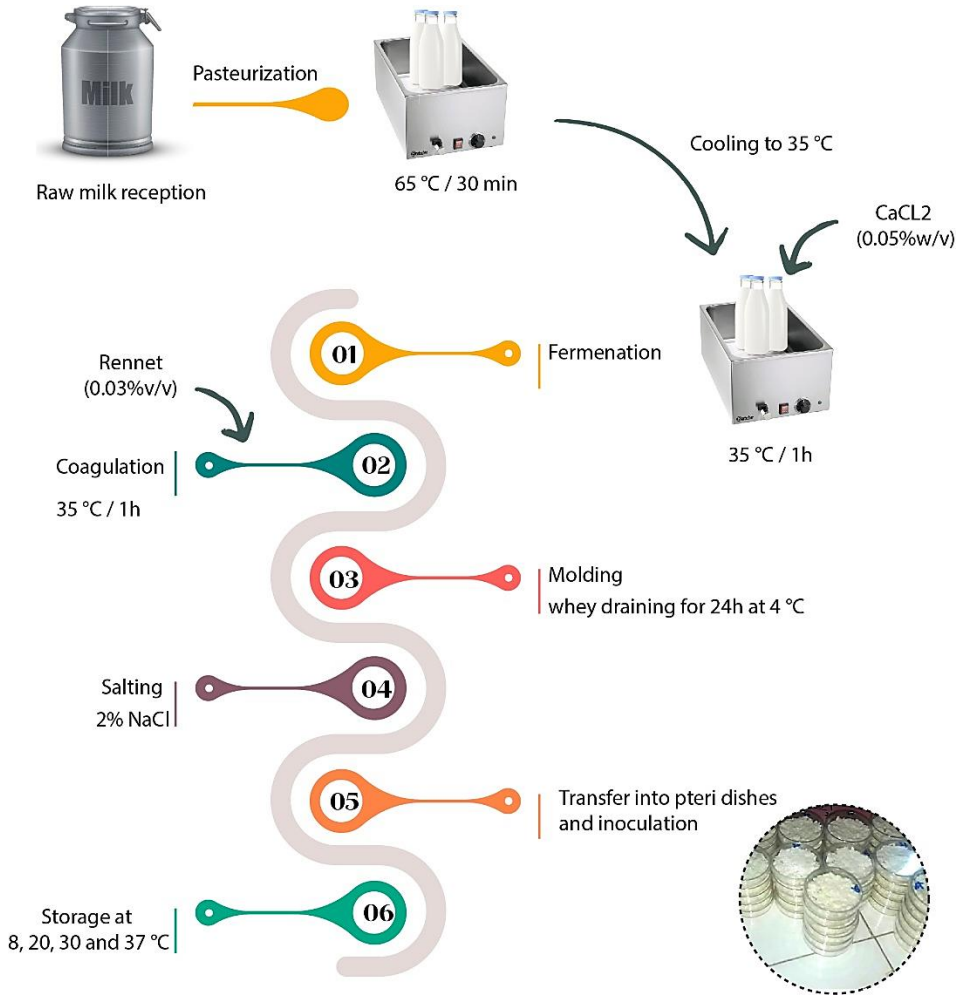

Figure 2. Flow chart of the methodology followed to produce the fresh goat cheese samples.

After salting, 10-g cheese samples were placed in sterilised Petri dishes (60 mm x 15 mm) and inoculated with a single strain of *S. aureus* (CECT 976) at 10^{2}-^{3} cfu/g. The parafilm was used to cover the Petri dishes and stored at different temperatures (8, 20, 30, and 37 °C) for 19 days (product shelf-life).

For bacteriological analysis, cheese samples were taken at different intervals depending on the temperature of storage and serially diluted to determine *S. aureus* and LAB populations by standard plate count methods. *S. aureus* was enumerated on Baird Parker agar after incubation at

37 °C for 48 h, and LAB were counted on MRS agar after 48 h of incubation at 30 °C. Samples were analysed in duplicate and the experiments were repeated three times.

The pH and a_w of cheese samples stored at different temperatures were also monitored during storage. The initial pH of samples ranged between 6.38 and 7.05, while the a_w values were around 0.906.

3.2. Summary of Results

Growth of the *S. aureus* population in fresh goat's cheese during different storage temperatures is reported in Tables 5, 6, 7, and 8. In general, fresh cheese supported significant growth of *S. aureus* at all temperatures evaluated.

In samples stored at 8 °C, the *S. aureus* population reached a maximum average concentration of 8.21 log cfu/g at the end of storage. Similarly, the same behavior was observed for the LAB population, reaching 9.25 log cfu/g after 19 days of storage.

Table 5. Results obtained from the analysis of fresh goat cheese stored at 8°C for 19 days.

Time (days)	**0**	**1**	**2**	**6**	**8**	**14**	**19**
***S. aureus* (log cfu/g)**	2.84±0.53	4.38±0.9	5.51±1.1	6.09±0.89	7.15±1.16	7.54±0.6	8.21±0.19
LAB (log cfu/g)	3.51±0.46	6.00±0.09	6.26±0.8	6.79±0.57	8.33±0.66	8.19±0.7	9.25±0.18
pH	6.14±0.24	6.53±0.61	6.48±0.30	6.19±0.10	6.31±0.02	6.38±0.48	6.74±0.24

At 20 °C, the *S. aureus* population was about 8.08 log cfu/g after 8 days of storage, while this population-level was observed after 19 days of storage at 8 °C. On the other hand, the LAB population was approximately 9 log cfu/g after 2 days of storage.

When samples were stored at 30 °C and 37 °C, the *S. aureus* population was approximately 5 log cfu/g after 6 h, reaching close to 7 log cfu/g after one day of storage. No difference in counts was observed between samples stored at 30 and 37 °C after 4 days of storage, except that samples

stored at 37 °C showed early spoilage after 4 days of storage, while the *S. aureus* population stored at 30 °C reached a maximum average concentration of 8.08 log cfu/g after 8 days of storage. LAB growth was similar at 30 and 37 °C, with growth accelerating in the first hours of storage and reaching a maximum average concentration of 9.52 and 9.31 log cfu/g after 4 days at 30 and 37 °C, respectively.

Table 6. Results obtained from the analysis of fresh goat cheese stored at 20°C for 19 days.

Time (days)	**0**	**1**	**2**	**6**	**8**	**14**	**19**
S. aureus (log cfu/g)	2.84±0.53	6.46±0.47	6.98±0.49	7.58±0.53	8.08±0.48	8.70±0.14	8.42±0.11
LAB (log cfu/g)	3.51±0.46	7.20±0.45	8.98±0.27	9.54±0.08	9.37±0.03	8.74±0.70	9.34±0.54
pH	6.14±0.24	6.08±0.31	5.79±0.28	5.47±0.14	5.44±0.12	6.15±0.01	7.60±0.40

Table 7. Results obtained from the analysis of fresh goat cheese stored at 30°C for 8 days.

Time (days)	**0**	**0.25**	**1**	**2**	**4**	**6**	**8**
***S. aureus* (log cfu/g)**	2.84±0.53	5.08±0.89	6.99±0.26	6.80±0.18	7.17±0.48	7.64±0.43	8.06±0.40
LAB (log cfu/g)	3.51±0.46	6.70±0.62	8.88±0.25	9.30±0.26	9.52±0.30	9.28±0.16	9.49±0.09
pH	6.14±0.24	6.080.28	6.10±0.32	5.46±0.16	5.29±0.19	5.79±0.14	6.55±0.25

The pH values of cheese decreased from 6.14 to 5.44 after 6 days of storage at 20 °C, while no significant changes were observed in cheese stored at 8 °C. After 4 days of storage at 30 and 37 °C, the pH values of the cheese decreased from an initial mean value of 6.14 to 5.29 and 5.57, respectively. Also, the pH increased over time until the end of the storage period for cheese stored at 20 and 30 °C. This increase in pH may be due to microbial proteases producing

peptides, amino acids, and nitrogenous substances that affect the organic acids generated by LAB. However, the a_w remained almost stable during the storage period.

Table 8. Results obtained from the analysis of fresh goat cheese stored at 37°C for 4 days.

Time (days)	0	0.25	0.42	1	2	4
***S. aureus* (log cfu/g)**	2.84±0.53	5.68±0.43	6.33±0.60	6.98±0.48	6.88±0.28	7.11±0.18
LAB (log cfu/g)	3.51±0.46	6.55±0.09	6.91±1.15	9.18±0.37	9.18±0.16	9.31±0.52
pH	6.14±0.24	6.11±0.31	5.90±0.30	5.77±0.20	5.47±0.05	5.57±0.21

Generally, Jben supported faster growth of *S. aureus* over storage time, and as the temperature increased, a shorter time was required to reach a stationary phase. However, no significant competition was found between the *S. aureus* and LAB populations.

4. Partner ISBST/UMA

4.1. Summary of Material & Methods

The laboratory-scale preparation of artisanal dry sausages was performed according to the traditional recipe and was conducted as detailed in Figure 3. After mincing the raw beef meat (69%) and fats (20%), salt (1.8%), hot paste Harissa, spices including coriander caraway, red pepper, fennel seeds powder, dried mint and garlic were added to the batter.

The homogenised paste was divided in five lots: lot 1 and lot 2 were used respectively positive and negative controls while the studied mint extract was added to the other three lots at 0.156% (T1), 0.468. % (T2) and 0.936% (T3).

Dried cattle natural casings (20-25 cm of length and about 4-5 cm of diameter) were used. Prior to embedding, the casings were soaked in potable water and vinegar for approximately two days. The stuffing of the sausages was done after resting period while avoiding the air bubbles in the mass that can cause unwanted oxidation.

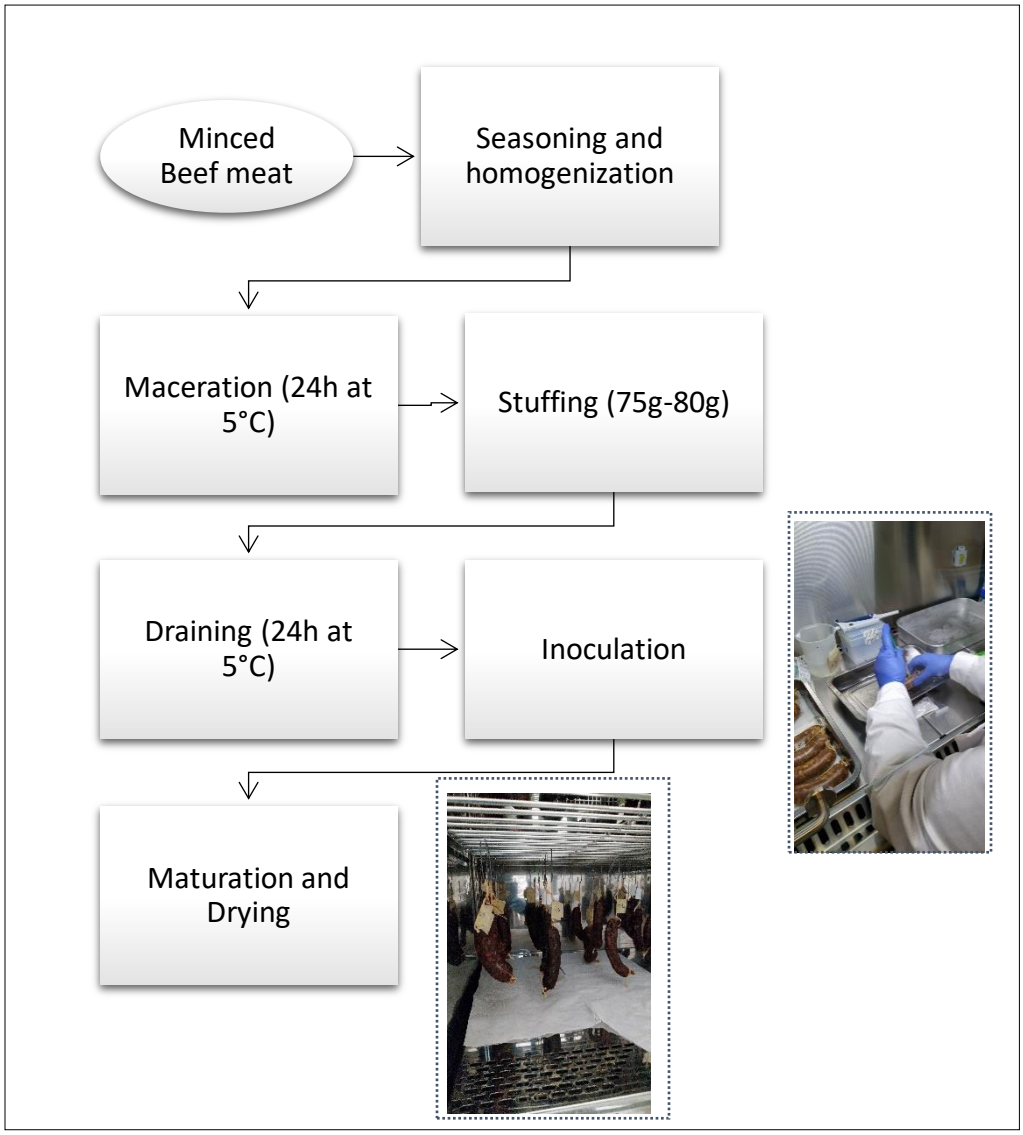

Figure 3. Scheme of the methodology applied to prepare the sausages (collaboration between Polytechnic Institute of Bragança and University of Manouba, internship of Ben Hmidène I)

The sausages were then hung in the controlled humid chamber to dry until reaching the desired dehydration. Relative humidity and temperature ranges selected consider the traditional processing conditions during the wintertime as indicted in the Table 9.

Table 9. Relative humidity and temperature during the maturation and drying steps

Conditions	Maturation	Drying
RH	97%	50-63%
Temp	10°C	27°C
Duration	24 hours	15 days

The inoculation procedure was performed in such a way that product formulation is not changed. Therefore, the inoculum volume did not exceed 1% of the product weight or volume and was injected into the sausages (25cm/ 75g each) prepared manually by piercing with a syringe needle at different points of the sausage.

Then the inoculated sausage was directly stomached when analysing and the level of contamination was confirmed by testing control positive unit after the inoculation. *Listeria monocytogenes* WDCM 00019, obtained from the Polytechnic Institute of Bragança stock collection, was used for all experiments. A loop of culture kept on Nutrient Agar slants was inoculated separately in 10 mL of TSB broth. Broth tubes were incubated at 37 ◦C for 16 h, following two successive inoculations, to achieve a concentration of approximately 108 CFU/mL. *L. monocytogenes* and Lactic Acid Bacteria (LAB) were detected and quantified by ISO methods (ISO 11290 and ISO 15214, respectively). Samples were analysed in duplicate.
The pH and a_w of samples were also measured during the drying step.

Lben is a spontaneously fermented milk produced in North of Africa, always produced from raw cow milk. The traditional manufacture process of Lben involves a fermentation of raw milk until a weak coagulum is formed. The formed coagulum is churned to separate sour milk and butter. The obtained acidic milk called “Lben”. The material used in the fermentation process are: cow milk, starter culture and as supplemented ingredient; citrus extract and/or lactic bacteria isolated and identified from fermented milk used as potential bio-preservative. The study will be carried against *Staphylococcus aureus* at 10^3cfu/mL level and will be conducted in two stages: stage I during fermentation process and stage II during storage process. In the first group (control samples), raw milk was fermented flavored and unflavored with citrus and inoculated or not with

lactic bacteria. In the second group (Test unit), *S. aureus* will be added separately with citrus extract, with LAB and with mixture and left to incubate until coagulation. During fermentation, samples will be analyzed at 0, 2, 4, 6, 8, 10 and 24 h. For stage II, the amount of 10^3cfu/mL of *S. aureus* added after butter and lben separation. Test unit samples will be kept at 5°C for 5 days. Sampling will be done on a daily basis.

At each sampling time, lactic acid bacteria, *S. aureus*, total bacteria count were determined and for physicochemical parameters pH, aw and lactic acid production performed in three triplicate. The summary of the adopted diagram represented in Figure 4.

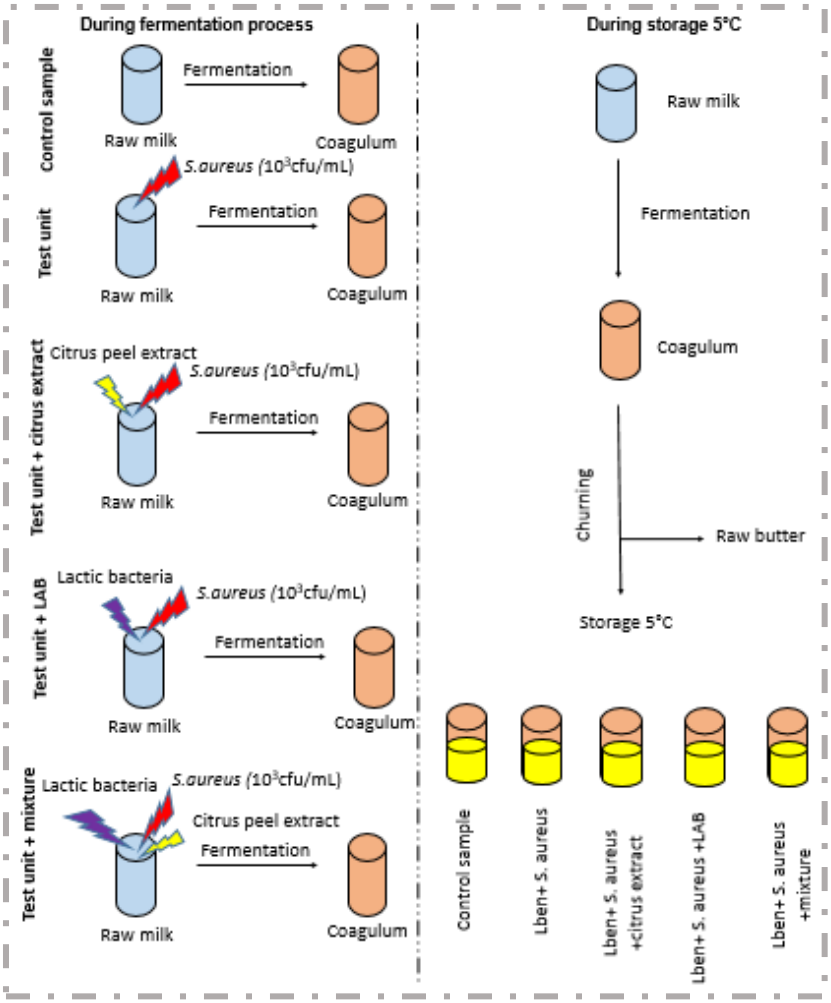

Figure 4. Proposed experimental methodology for fate study in Lben (collaboration between University of Bologna and University of Manouba, internship of Mkadem W)

4.2. Summary of Results

The main results of the fate studies are illustrated in Tables 10 and 11 for control batch and the batch with 0.936% of dried mint extract. The duration of each study was adjusted to the artisanal product desired final characterisation and water activity.

The results shows that all samples from different batches supported the growth of L. *monocytogenes* and spontaneously LAB. Overall, the pH and the water activity of samples dropped over the drying in 27°C simultaneously with the increase of the LAB suggesting the start of the natural fermentation process. For the control batch (Table 10), the mean increase in *L. monocytogenes* concentrations during 7 days of storage was approximately 2.47 log cfu/g. LAB growth potential was higher, with a mean increase of 6.94 log cfu/g after 9 days. Until the 7th day, the inactivation of *L. monocytogenes* is observed in all samples. A decrease of 4.72 log cfu/g was measured at 15th day of drying.

Table 10. Results obtained from the analysis of artisanal dry sausages: Control.

Time (Days)	0	3	5	7	9	11	15
LM (log cfu/g)	5.21±0.016	7.4±0.14	7.26±0.008	7.68±0.085	6.89±0.014	4.34±0.053	2.97±0.1
LAB (log cfu/g)	4.42±0.2	6.36±0.14	7.03±0.04	8.61±0.06	11.36±0.07	10.17±0.09	7.8±0.1
pH	5.785±0.015	5.625±0.005	5.57±0.02	5.45±0.04	5.42±0.03	5.5±0.02	5.64±0.03
a_w	0.9672± 0.001	0.8717± 0.008	0.7736± 0.007	0.6437± 0.057	0.6715± 0.014	0.6086± 0.002	0.5972± 0.006

*LM= *Listeria monocytogenes*

Concerning the results obtained for the treated batch T1 (with 0.156% of mint extract), *L. monocytogenes* showed an increase of 2.29 log cfu/g. LAB growth was less evident than the control, presenting a mean growth potential of 5.41 log cfu/g. Accordingly, the pH and aw of samples decreased over time, ranging from average values of 5.79 to 5.49 and 0.961 to 0.615, respectively. The results obtained from the analysis of samples treated with 0.47% of mint extract (0.468% of mint extract) revealed a mean growth potential for *L. monocytogenes* of 2.24 log cfu/g similar to samples treated with lower extract concentration.

An increase of LAB growth was recorded by mean of 6.66 log cfu/g during 9 days of drying and 3.58 log cfu/g at the final day of the drying process. The LAB presented the highest growth potential at this concentration The average pH value decreased from 5.81 to 5.58. Likewise, the value of water activity reached 0.611 at 15th day of drying from 0.96 at the beginning of the experiment.

As for the final batch T3 (with 0.936% of mint extract) incorporated with the highest amount of extract, the average results for *L. monocytogenes* revealed a growth potential of 2.25 log cfu/g after 7 days of drying and a significant drop of 4.37 log cfu/g at 15 days of drying in the inactivation phase. The LAB reached a maximum average concentration of 6.33 log cfu/g at the 9th day and 3.45 log cfu/g at end of process. The drop in pH and water activity values during the drying was similar to that observed in different batches.

Table 11. Results obtained from the analysis of artisanal dry sausages treated with 0.936% of mint extract for 15 days.

Time (Days)	0	3	5	7	9	11	15
LM (log cfu/g)	4.76±0.03	7.02±0.12	7.2±0.43	7.01±0.14	6.49±0.16	3.63±0	2.64±0
LAB (log cfu/g)	4.41±0.04	6.96±0.12	7.96±0.2	8.57±0.02	10.73±0.01	9.14±0.11	7.85±0.36
pH	5.83±0.03	5.79±0.16	5.71±0.04	5.46±0.1	5.32±0.04	5.56±0.07	5.56±0.06
a_w	0.965±0.001	0.842±0.015	0.763±0.016	0.723±0.042	0.684±0.024	0.636±0.017	0.594±0.012

*LM= *Listeria monocytogenes*

Overall, there is two distinguished phases in all four production; gowth (log phase) and inactivation phase for the selected pathogen. The growth of *L. monocytogenes* increased with change of the temperature in 24 h reaching its maximum after 7 days of drying at 27C. However, the growth rates were significantly affected by the incorporation of the extract (**result not shown)** of the mint extract on *L. monocytogenes* growth was demonstrated in the final batch where the least growth potential was recorded. *L. monocytogenes* maximum population density decreased was followed by an increase in the LAB population suggesting a competition between the pathogen and LAB.

The addition of plant extract as demonstrate in this fate studies showed a positive effect on the safety of naturally fermented dry sausage. However, further investigations are currently undergoing to improve and refine these results.

5. Partner ANSES/CNIEL

5.1. Summary of Materials & Methods CONFIDENTIAL

Soft Camembert raw milk cheeses were produced according to the technological diagram provided by the Camembert de Normandie PDO (Table 12).

Table 12. Manufacturing condition of Camembert de Normandie.

Stage	Characteristics	Values
Skimming	Standardization in MG to 28 g/L	
Primary maturation	Temperature	13°C
	Mesophilic ferments	
	Ripening micro-organisms	
	CaCl2	25mL/100L
	Duration	<24h
	Target pH	6,50-6,60
Secondary Maturation	Temperature	35°C
	Duration	60 - 90 min
	targeted pH	6,40-6,45
Coagulation	Temperature	35°C
	Rennet dose	20mL/100L
	setting	7-8 min
	hardening	
Slicing	Blade spacing	2.5 x 2.5 cm
	pH	6,15 - 6,25
Moulding	Ladle	5 steps at 40-minute intervals
	Room temperature	30-32°C
	Curd temperature	> 28°C
	pH	6,15-6,25
	Duration	200 to 300 minutes
Acidification / draining / turning	Turning	3: end of moulding + 30 min / +1h / +2h
	Duration	18h min from the first ladle
	Room temperature	> 22°C
	Target pH	< 6.10 at 1h and < 5.60 at 2h
	Planing	M+30min; M+1h; M+2h
Demoulding	Temperature	20°C
	pH	4,70 [4,65-4,75]
Dry salting		
Drying	Temperature	12 to 18°C

	Hygrometry	85-90%
	Duration	4h
Ripening	Temperature	12-13°C
	Hygrometry	95-98%
	Duration	13 days min - re-drying 24h - storage at 4°C

Two microbial species were studied: *Listeria monocytogenes* and *Salmonella enterica* spp. enterica. Two strains of each genus were selected to perform challenge-tests. All strains were isolated from dairy products:

- *L. monocytogenes* AER101 isolated from milk, serotype 4b CC6
- *L. monocytogenes* UNIR100 isolated from cheese, serotype 4b (CC under identification)
- *Salmonella* Mbandaka 274-079 isolated from soft raw milk cheese
- *Salmonella* Dublin isolated from uncooked pressed cheese

The inoculums were prepared for each strain according to the following steps : the strains were first isolated of selective media (Compass Listeria or XLD/IRIS Salmonella specific agar) and incubated for 24-48h at 37°C. A first preculture (C1) was then prepared from an isolated colony in 10mL of BHI broth and incubation for 18 hours at 37°C. The inoculum culture (C2) was then prepared from precultures in 1% BHI and incubated for 18h at 37°C. The inoculum cultures were then adapted to cold conditions by storing them at 2°C. Dilution of the adapted cultures were then carried out with the aim of obtaining a target inoculation of around 100 CFU/mL in raw milk.

Fabrications were conducted in triplicate for each microbial species. The milk used was collected each morning. For each production run, two tanks were prepared in order to inoculate one strain of each microbial species per tank (e.g., FAB 1 tank 1-Listeria strain 1 / tank 2-Listeria strain 2; etc). In order to produce a sufficient number of cheeses, 38L of milk were used per vat, allowing the production of 14 cheeses. These 38 L of milk were inoculated with the Lm or Salmonella spp. strains before the primary ripening step.

The cheeses produced were kept for 13 days in the cellar under the conditions specified in the Camembert de Normandie production diagram, i.e., at a temperature of 13°C and a humidity of 95-98%. One cellar was provided for each type of contamination, i.e., one cellar for maturing

cheeses contaminated with Lm and one cellar for maturing cheeses contaminated with Salmonella spp. Following the ripening period, the cheeses were packaged and then stored at 4°C to start the 60-day DVM phase (the DVM normally applied being 50-70 days). The thermal scenario applied during this MVD was as follows: 1/3 of the MVD at 4°C and then 2/3 of the MVD at 8°C with a 2h break in the cold chain at room temperature between these two periods (see diagram below). The temperature was recorded by thermal chips from the time of entry into the cellar until the end of the DVM.

The microbiological analytical methods used were the following:
- *L. monocytogenes*: detection (ALOA ONE DAY), enumeration (ALOA medium)
- *Salmonella* spp.: detection (VIDAS EASY), enumeration (XLD medium)
- Enumeration of Enterobacteriaceae: 30°C (NF V08-054)
- Total flora enumeration: 30°C (ISO 4833-1)

For physico-chemical characterization the following parameters were measured: lactates (enzymatic determination), lactose and galactose (HPLC - adapted from AOAC 980.13 and 982.14), acetates (enzymatic determination), dry extract (Oven 102 +/-2°C), fat content (HEISS method NF V 04-287), salt content (chlorides/potentiometric), NH3 (enzymatic kit), lipolysis, aw (Aqualab) and pH.

5.2. Summary of results

Figures 5 and 6 below show the results of pathogen counts inoculated into milk for the manufacture of Camembert at each key stage from inoculation at the start of prematurity to the end of the shelf-life. Each point corresponds to the average of 3 test analysis and are given for each of the three productions (1 production corresponding to 1 colour). The diamond-shaped points represent the results obtained for the analyses carried out on the milk and then the cheese paste from the ripening period onwards. The dots in crosses represent the results obtained for the analyses carried out on the rind of the cheeses, only from the ripening period until the end of the DVM.

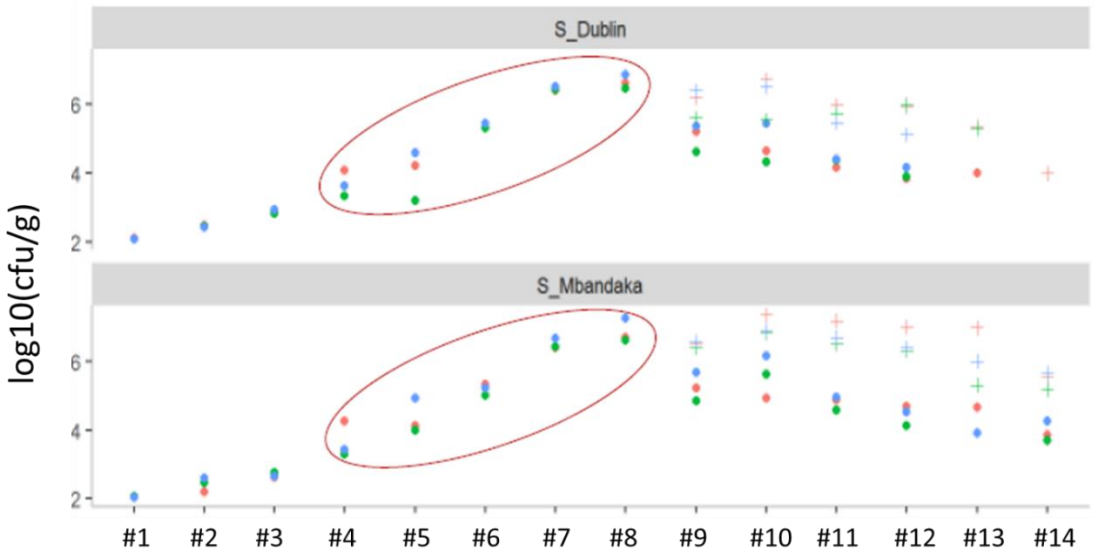

Figure 5. Evolution of the two *Salmonella* strains (log10 CFU/g) during the manufacture and storage of Camembert (in orange assay 1, green assay 2 and blue assay 3). Legend: #1 Start of prematuration step (inoculation of strains), #2 End of prematuration, #3 End of secondary maturation, #4 Moulding 1st layer, #5 3rd layer, #6 5th layer, #7 4h after moulding, #8 24 h after moulding, #9 After 10 days of ripening, #10 End of ripening #11 20th day of storage, #12 35th day of storage, #13 45th day of storage , #14 60th day of storage.

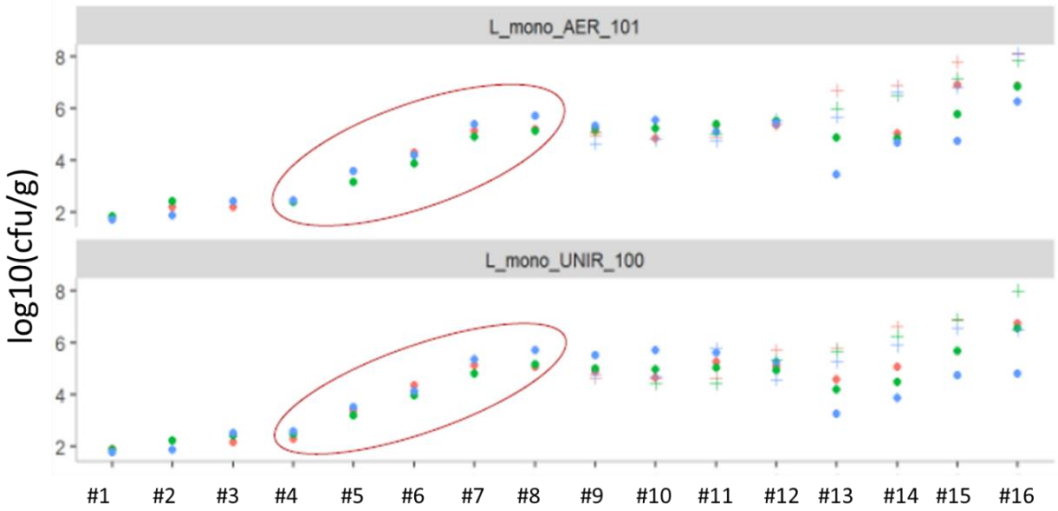

Figure 6. Evolution of the two *L. monocytogenes* strains (log10 CFU/g) during the manufacture and storage of Camembert (in orange assay 1, green assay 2 and blue assay 3). Legend: #1 Start of prematuration step (inoculation of strains), #2 End of prematuration, #3 End of secondary maturation, #4 Moulding 1st layer, #5 3rd layer, #6 5th layer, #7 4h after moulding, #8 24 h after moulding, #9 After 3 days of ripening, #10 6 days of ripening, #11 9 days of ripening, #12 End of ripening #13 20th day of storage, #14 35th day of storage, #15 45th day of storage , #16 60th day of storage.

The results showed that the growth of the two pathogenic micro-organisms was relatively similar. Their exponential growth phase occurred during the first 50 hours of production, with microbial populations reaching values between 6 and 8 log10 CFU/g. The population of *L. monocytogenes* remained then stable during the maturation process and consumer stage storage, while the *Salmonella* population tended to decline. These observations and the values obtained were repeatable for the three productions carried out for each of the two strains studied per pathogen.

With regard to the physicochemical parameters of the cheeses, the pH and lactate curves in the cheeses were consistent with this type of technology. The pH of the cheeses on removal from the mould was also in line with the expected target values (between 4.60 and 4.70). Furthermore, the values obtained were repeatable for each production run. The aw values were between 0.940 and 0.960 during the shelf-life of the products.

6. Partner AUA

6.1. Summary of Material & Methods

The fate of *L. monocytogenes* and/or *Salmonella* spp. on katiki cheese and/or on slices of nouboulo sausage during the storage was evaluated. More specifically, commercially available Katiki cheese in 200-g packs were purchased from the retail market and immediately transferred to the laboratory for analysis. Cheese samples of 100 g placed in sterile plastic tubes were inoculated with a three-strain cocktail of either *L. monocytogenes* or *Salmonella* spp. to a final population of 3 Log CFU/g and 6 Log CFU/g, respectively (Table 13). The inoculum was homogeneously applied to the cheese samples by mixing with a sterile spatula before the packs were resealed. The inoculated cheese samples were stored at 7°C (to mimic temperatures at retail and consumer storage) and analyzed throughout storage at regular time intervals up to 20 days (product shelf-life).

Moreover, commercially available nouboulo sausage was obtained from the retail market. The nouboulo was processed on a meat slicer preset to yield 4- to 5-g slices and transferred to the laboratory for surface inoculation. The upper surface of each slice was inoculated with 0.020 ml of the *Salmonella* spp. culture (three-strain cocktail, Table 13) by spreading the inoculum over

the entire surface with a sterile bent glass rod. Following a 10-min resting period for bacterial attachment, the slices were flipped over, and the other side was inoculated. Three slices (~10 g) were placed into clear polyethylene bags, packaged under vacuum, and stored at 10^{o}C. Samples were analyzed throughout storage at regular time intervals up to 20 days.

Table 13. Bacterial strains used to inoculation of katiki cheese and/or nouboulo sausage.

Microorganism	Strain ID	Origin
Listeria monocytogenes	C5	Surface isolate- dairy farm environment- Serotype 4b
Listeria monocytogenes	6179	Food isolate from farmhouse cheese- Serotype 1/2a
Listeria monocytogenes	Scott A	Human isolate- epidemic strain- Serotype 4b
Salmonella enterica subsp. enterica Le Minor and Poppof serovar Typhimurium	4/74	Calf bowel
Salmonella enterica subsp. enterica serovar Agona	23	Feeds
Salmonella enterica subsp. enterica serovar Infantis	167	Feeds

*Bacterial strains used in this study maintained in the Laboratory of Food Quality Control and Hygiene Culture Collection

Microbiological analyses of both products included the enumeration of *L. monocytogenes* on Agar *Listeria* acc. to Ottaviani & Agosti (ALOA) (ISO 11290-2:2017), incubated at 37 ^{o}C for 48 h , *Salmonella* spp. on Xylose Lysine Deoxycholate (XLD) agar, incubated at 37 ^{o}C for 24 h , lactic acid bacteria (LAB) on the de Man, Rogosa, Sharpe agar (MRS), incubated at 30 ^{o}C for 48–72 h and yeasts and moulds on Rose Bengal Chloramphenicol agar (RBC), incubated at 25 ^{o}C for 3–5 days. The pH and a_w of samples were also measured over storage.

The above procedures were performed twice, and the samples were analysed in duplicate.

6.2. Summary of Results

The survival of *L. monocytogenes* on katiki cheese during storage is shown in Figure 7. The microorganism was found to be able to survive (< 1-log reduction) during the storage of katiki cheese at 7^{o}C.

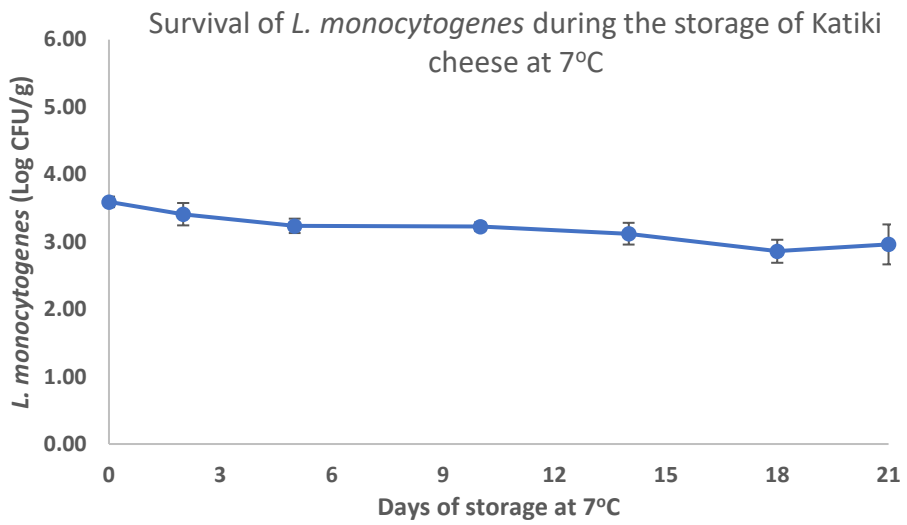

Figure 7. Survival of *L. monocytogenes* during the storage of Katiki cheese at 7°C.

The results from the microbiological and physicochemical analysis of katiki cheese (Table 14) showed that population of Yeasts/Moulds increased by 2 log CFU/g (from 4.88 to 7.07 log CFU/ g) while the population of lactic acid bacteria remained constant at high levels (>8.0 log CFU/g) during the storage of katiki cheese at 7°C. The pH and water activity of the product slightly increased by 0.2 U (from 4.3 to 4.5) and 0.010 U, respectively.

Table 14: Results obtained from the analysis of katiki cheese stored at 7°C.

Time (days)	0	5	14	21
Yeasts/Moulds (Log CFU/g)	4.88 ±0.16	6.61 ±0.16	7.53 ±0.31	7.07 ±0.45
Lactic Acid Bacteria (Log CFU/g)	8.14 ±0.26	8.16 ±0.10	8.41±0.32	8.32 ±0.24
pH	4.31±0.03	4.34±0.06	4.40±0.09	4.53±0.12
a_w	0.974±0.002	0.975±0.002	0.975±0.003	0.983±0.001

The survival of *Salmonella* spp. on katiki cheese during storage is shown in Figure 8**.** The population of *Salmonella* spp. gradually decreased (> 3 log reduction) but still survived at significant levels (3 log CFU/g) during the storage of katiki cheese at 7°C.

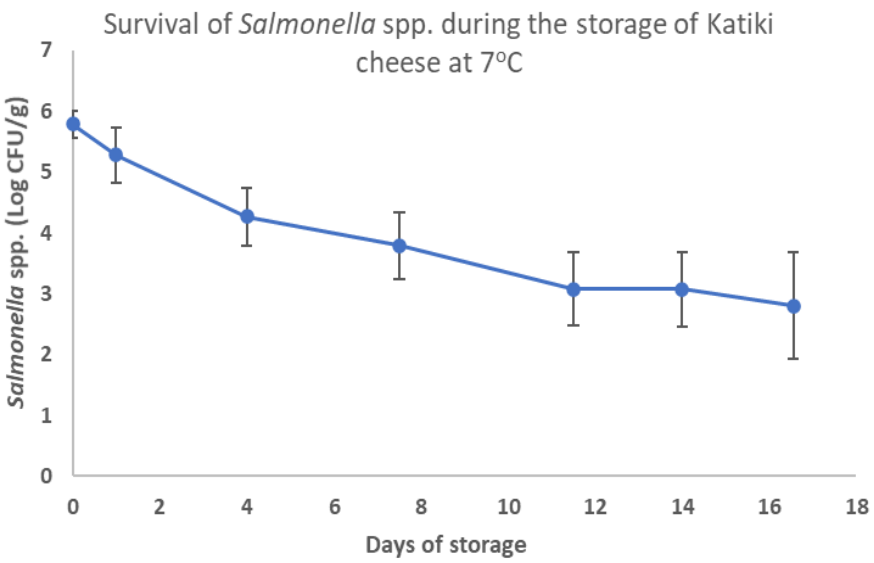

Figure 8. Survival of *Salmonella* spp. during the storage of Katiki cheese at 7°C.

The above results confirm that both *L. monocytogenes* and *Salmonella* spp. can survive during refrigeration storage in chesses such as katiki cheese. The adaptation of cells to the acid and osmotic conditions such as those of katiki cheese may result in increased microbial resistance to harsh conditions, which could lead to an increased risk of listeriosis to consumers.

The fate of *Salmonella* spp. on slices of nouboulo during the storage is shown in Figure 9.

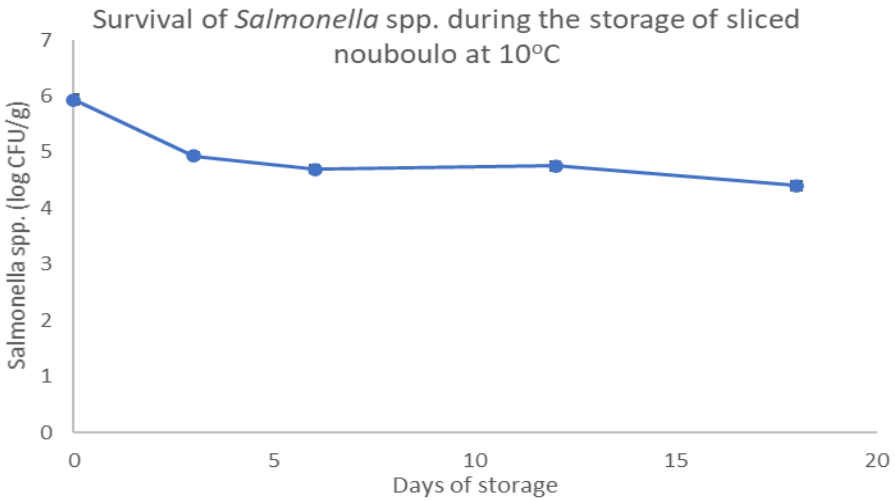

Figure 9. Survival of *Salmonella* spp. during the storage of sliced nouboulo at 10°C.

The inactivation curve of *Salmonella* spp. was biphasic with an initial rapid inactivation phase within the first days of storage, followed by a slower inactivation phase or a profound ‘‘tailing’’ (Figure 9), indicating a possible adaptation or selection of the pathogen to the stressful environment (i.e., low a_w, Table 15).

Table 15. Results obtained from the analysis of nouboulo slices stored at 10°C.

Time (days)	0	6	18
Yeasts/ Moulds (Log CFU/g)	3.45 ±0.15	4.18 ±0.25	4.57 ±0.25
Lactic Acid Bacteria (Log CFU/g)	7.76 ±0.16	7.65 ±0.11	7.70±0.11
pH	5.09±0.06	-	5.03±0.08
a_w	0.922±0.002	-	0.918±0.006

The results from the microbiological and physicochemical analysis of nouboulo slices (Table 15) showed that population of Yeasts/Moulds increased from 3.45 to 4.57 log CFU/ g while the population of lactic acid bacteria remained constant at high levels (7.70 log CFU/g) during the storage of nouboulo slices at 10^{o}C. The pH and water activity of the product did not vary over storage.

7. Partner IPB

7.1. Summary of Material & Methods

Fate studies were conducted to evaluate the antimicrobial effect against *S. aureus* of (i) sage and black cumin extracts in alheira sausages during maturation; and (i) lemon balm and spearmint extracts in goat’s raw milk cheeses during ripening. Lyophilised extracts were obtained using ethanol 70% as solvent in a shaking water bath (60 °C for 90 min at 150 rpm; sample/solvent ratio of 1 g/20 mL). *Staphylococcus aureus* subsp. *aureus* Rosenbach (ATCC 11632) kept on a fresh slant was cultivated overnight in brain heart infusion broth (BHI) at 37 °C. On the day of inoculation, the inoculum was prepared from a second subculture in early stationary phase (~9.0 log CFU/mL) diluted in physiological water to reach 7.0 log CFU/mL.

Mini-cheeses were prepared in a similar manner as explained in Figure 1, yet using goat's raw milk, without adding $CaCl_2$, and salting by immersion in brine at the end of the process. Milk was inoculated with *S. aureus* to reach ~5 log CFU/g and 0% or 1% (w/w) of each extract was added to the drained curd, while a non-inoculated control was kept. Cheeses were kept in a chamber at 10 °C/98% RH for 15 days.

Mini-alheiras were prepared by soaking sliced wheat white bread (22%) in hot boiled water (60%) for 20 min. After breaking down, garlic powder (1%), red pepper powder (1%), table salt (1%), and finely shredded cooked chicken meat (10%) were added and mixed to form a well-integrated batter. Separately, virgin olive oil (5%) was heated to ~50 °C and added with 0.0, 0.5, 1.0 or 1.5% (w/w) of the lyophilised sage extract and incorporated still warm into the batter. The batter was mixed throughout and stuffed in pre-washed natural pig casings to produce mini-alheiras of 80-90 g approximately. The weight in g of each alheira was annotated (W). Mini-alheiras were then inoculated by individually syringing a volume of 10W µL of the inoculum into the test units. Through this standardised procedure, each mini-alheira reached a *S. aureus* target concentration of ~5.0 log CFU/g. Mini-alheiras were hung in a climate-controlled chamber (10 °C, 85% RH) for fermentation/maturation to take place during 10 days.

On every sampling day, two units of cheeses and alheiras were separately analysed per treatment run; and physicochemical (pH, water activity, moisture content and weight loss) and microbiological determinations (*S. aureus* and lactic acid bacteria [LAB] counts) were carried out in triplicate. LAB was counted on MRS agar (Liofilchem, Italy), overlaid with 1.2% bacteriological agar (Liofilchem, Italy), and incubated at 30 °C for 48 h. *S. aureus* colonies were enumerated on Baird-Parker agar (Liofilchem, Italy), supplemented with Egg Yolk Tellurite (Liofilchem, Italy). Typical colonies were counted after 48 h following incubation at 37 °C and confirmed.

7.2. Summary of Results

When the alheiras were not formulated with sage extract (i.e., control; Figure 10), the inoculated concentration of *S. aureus* (5.0 log CFU/g) persisted throughout maturation despite acidification and dehydration, whereas the indigenous LAB proliferated more rapidly until reaching ~10.5 log CFU/g at the 10th day of maturation. From the alheiras added with sage extract, it became evident that the higher the extract concentration, the greater the effect on the populations of *S.*

aureus and LAB (Figure 10). While sage extract produced a (desired) inactivation effect of *S. aureus*, it produced a delay in the development of LAB due to an increase in the lag phase and a decrease in the maximum concentration. As can be seen in Table 16, the pH of alheiras formulated with sage extract only dropped from 5.95 to 5.90, reinforcing that that the course of fermentation was affected by the extract. On the other hand, the aw did not change significantly from the fourth day of maturation onwards; whereas, even towards the end of maturation, alheiras continued to dehydrate significantly (see moisture content; Table 16). By the end of maturation, the addition of 0.5 – 1.5% sage extract inactivated *S. aureus* population in 0.476 – 0.672 log CFU/g, respectively, in relation to the control treatment.

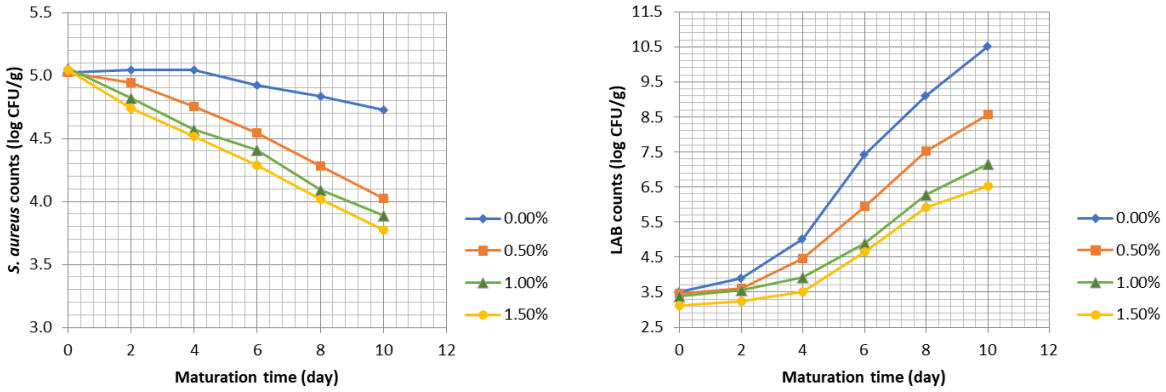

Figure 10. Kinetics of inoculated *S. aureus* and indigenous lactic acid bacteria (LAB) in mini-alheiras formulated with 0.0, 0.50, 1.00 and 1.50% (w/w) lyophilised sage extract during maturation at 10 ºC and 85% RH.

Table 16: Evolution of physicochemical properties of alheira sausages formulated with 1.50% sage extract during maturation

Time (days)	0	2	4	6	8
pH	5.952±0.011	5.918±0.002	5.911±0.005	5.908±0.018	5.907±0.001
a_w	0.9902±0.0012	0.9877±0.0010	0.9778±0.0011	0.9741±0.0041	0.9728±0.0004
Moisture (%)	59.6±0.50	58.4±0.20	50.9±1.45	49.7±2.80	41.1±2.00

Black cumin inactivated *S. aureus* in alheira sausages to a lesser extent than sage extract; and it did not seemingly affect the growth of LAB (Figure 11). In this sense, alheira sausages presented a normal fermentation, evidenced by the decrease in pH from 6.03 until 4.77 (Table 17). By the

end of maturation, the addition of 0.5 – 1.5% black cumin extract inactivated *S. aureus* population in 0.18 – 0.34 log CFU/g, respectively, in relation to the control treatment. In this way, black cumin extract was not considered as a sufficiently good bio-preservative for alheira sausages. By the end of maturation, alheira sausages formulated with black cumin presented a lower a_w (0.9444; Table 17) than the ones formulated with sage extract (0.9728; Table 16).

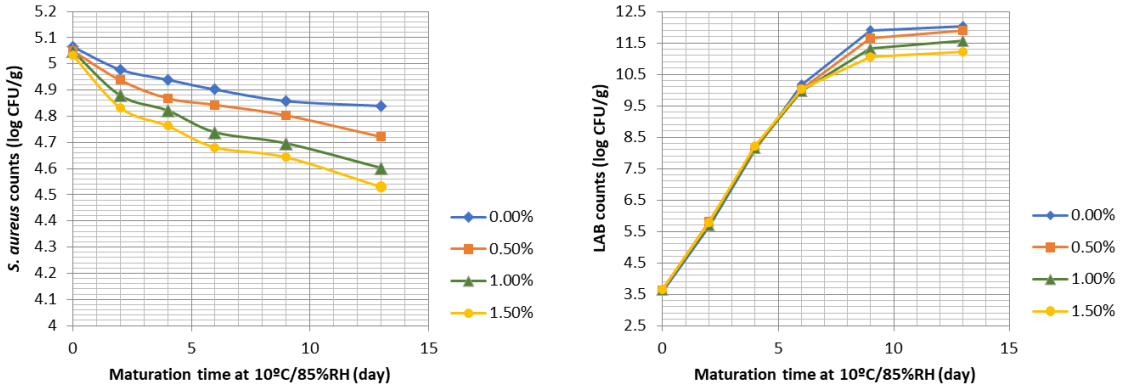

Figure 11. Kinetics of inoculated *S. aureus* and indigenous lactic acid bacteria (LAB) in mini-alheiras formulated with 0.0, 0.50, 1.00 and 1.50% (w/w) lyophilised black cumin extract during maturation at 10 ºC and 85% RH.

Table 17. Evolution of physicochemical properties of alheira sausages formulated with black cumin extract during maturation

Time (days)	0	2	4	6	9
pH	6.033±0.003	5.993±0.007	5.817±0.013	5.292±0.015	4.768±0.002
a_w	0.9929±0.0001	0.9833±0.0020	0.9742±0.0027	0.9648±0.0033	0.9440±0.0020
Moisture (%)	72.8±0.006	64.9±0.241	56.9±0.227	50.3±1.742	42.9±0.406

In the case of the cheese fate studies, three runs were carried out per extract: cheeses produced with extracts but not inoculated with the pathogen (Control 0), cheeses produced without extract but inoculated with the pathogen (Control 1), and cheeses produced with extracts and inoculated with the pathogen (Treatment). *S. aureus* and LAB kinetics in cheeses are presented in Figures 12 and 13.

According to Figure 12, cheeses inoculated with *S. aureus* (C1 and Treatment) presented lower LAB populations than the ones without *S. aureus* despite containing lemon balm extract. Seemingly, this extract did not affect fermentation, as can be also deduced from the normal pH drop of cheeses formulated with lemon balm extract, from 6.49 until 5.38 in 15 days of ripening (Table 18). It appears that 1.0% lemon balm in curd caused some inactivation of *S. aureus*; yet, as it was minimal, this extract will not be further studied as a potential bio-preservative (Figure 12).

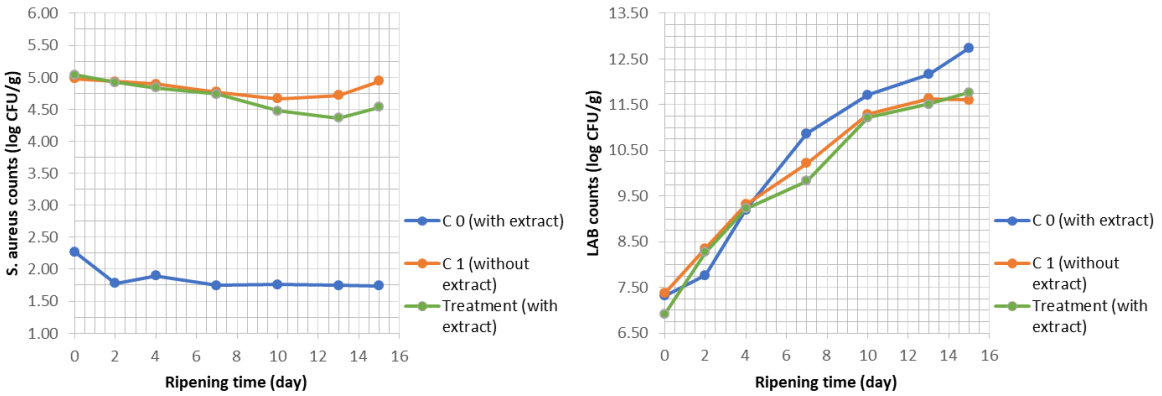

Figure 12. Kinetics of inoculated *S. aureus* and indigenous lactic acid bacteria (LAB) in mini-cheeses formulated with 0.0% (C0) and 1.0% lyophilised lemon balm extract (Treatment) during ripening at 10 ºC and 98% RH. C1 is an inoculated control formulated without extract.

Table 18. Evolution of physicochemical properties of mini-cheeses formulated with lemon balm extract during ripening

Time (days)	0	2	4	7	10	13	15
pH	6.49±0.01	6.07±0.02	5.83±0.02	5.52±0.13	5.34±0.055	5.37±0.01	5.38±0.025
a_w	0.966±0.003	0.974±0.008	0.958±0.001	0.956±0.001	0.958±0.003	0.956±0.001	0.961±0.001

The capability of spearmint extract of inhibiting *S. aureus* in goat's raw milk cheese during ripening was greater than that of lemon balm extract. Figure 13 shows that, on the 14th day of ripening, the decrease in *S. aureus* attained in the cheeses with extract was about 1.0 log CFU/g lower that of cheeses without extract (C1). The inhibitory activity of lemon balm against *S.*

aureus can be also seen in the non-inoculated control C0, where the enumerated pathogen was that originally present in the raw milk. In comparison with the cheeses without spearmint extract (C1), LAB counts in the cheeses formulated with extract were overall lower (Figure 13). This may explain why pH values of cheeses with spearmint extract (Table 19) were higher than those of cheeses with lemon balm extract (Table 18).

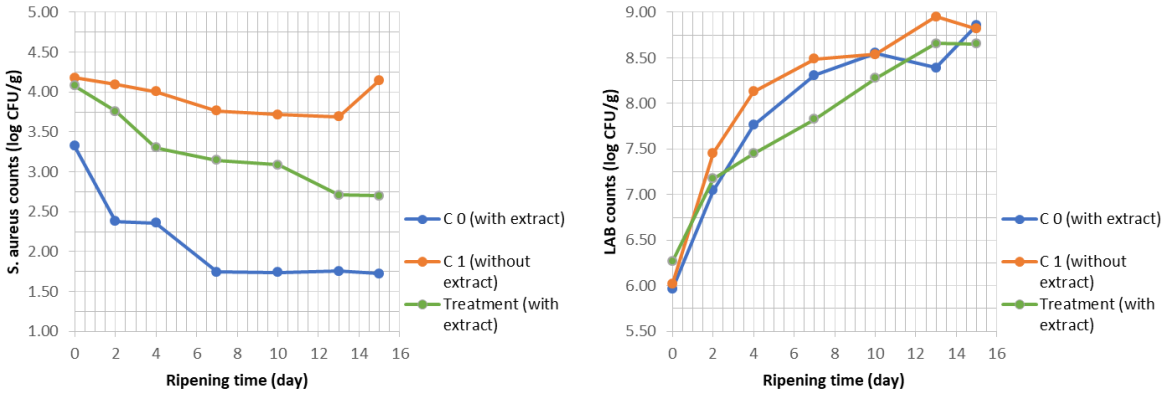

Figure 13. Kinetics of inoculated *S. aureus* and indigenous lactic acid bacteria (LAB) in mini-cheeses formulated with 0.0% (C0) and 1.0% lyophilised spearmint extract (Treatment) during ripening at 10 ºC and 98% RH. C1 is an inoculated control formulated without extract.

Table 19. Evolution of physicochemical properties of mini-cheeses formulated with spearmint extract during ripening

Time (days)	0	2	4	7	10	13	15
pH	6.56±0.055	6.15±0.005	6.09±0.02	5.82±0.05	5.73±0.005	5.65±0.14	5.79±0.03
aw	0.959±0.001	0.964±0.003	0.967±0.001	0.973±0.011	0.962±0.008	0.962±0.008	0.968±0.011

8. Partner UNIBO

8.1. Summary of Material & Methods

Italian fresh soft cheese with no rind "Squaquerone di Romagna" was provided by a cheese producer located in Cesena (Italy). In Figure 14 the flow diagram of the cheese production. The

ingredients were pasteurized cow milk, salt (NaCl, 0.4-0.8%v/v), rennet (30-50ml/100 l of milk), and starter culture of *Streptococcus thermophilus*.

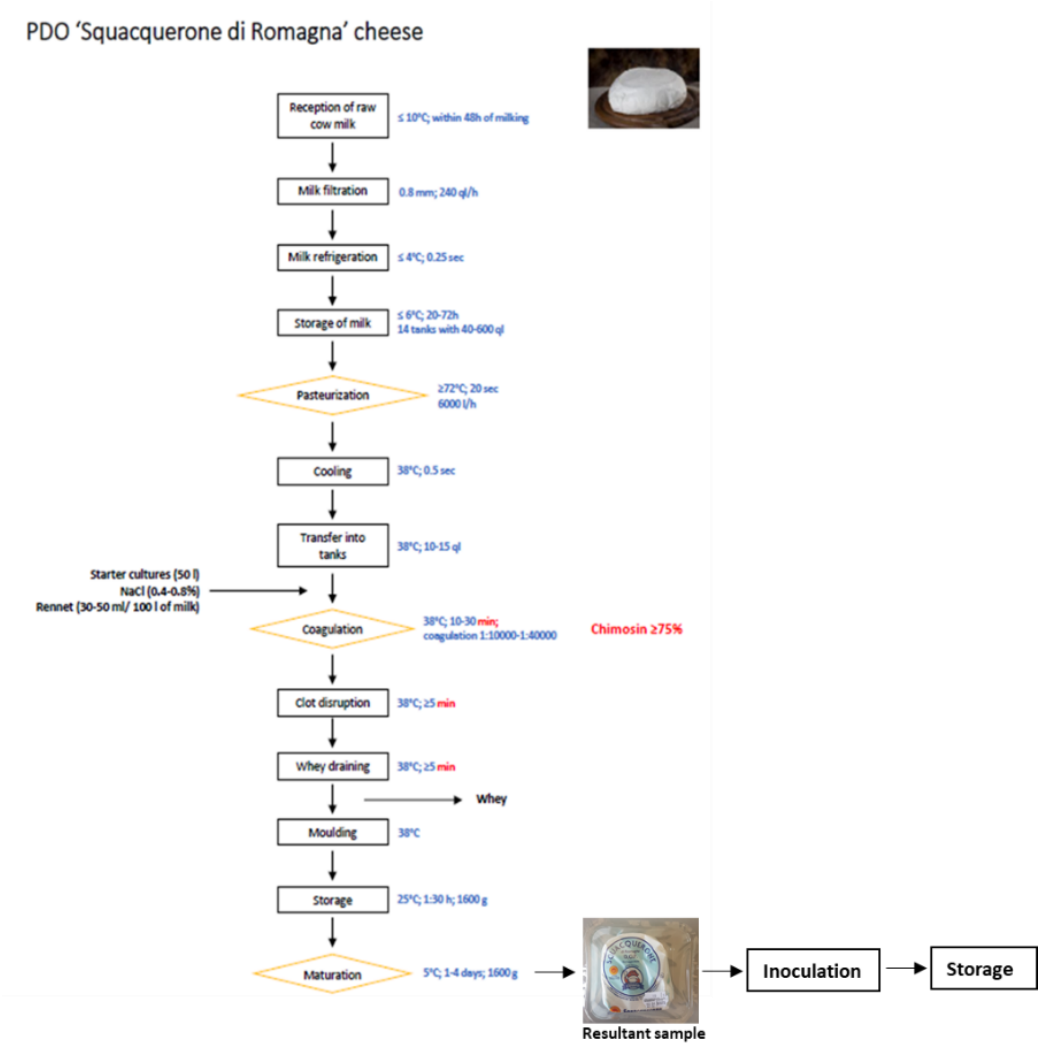

Figure 14. Scheme of the methodology applied to obtain the Italian fresh soft cheese samples.

Cheese samples of 50 g each in thermo-sealed plastic packaging were inoculated with a five-strain cocktail of *L. monocytogenes* at 10^2-10^3 cfu/g. Inoculation was carried out with the aid of a syringe and the inoculum was placed inside the samples (i.e., perforation in the central point). The inoculated strains were: two isolates from Italian RTE food production (LM26, LM27), two isolates from Italian cheese production (LMPC2 and LMPCDM8) and one reference strain (12 MONO13045). The inoculated samples were stored at different storage temperatures (3 and

10°C) and withdrawn at proper intervals up to 20 days (product shelf-life of 15 days + 33%) for microbial analysis. The temperature range selected reflect the logistic distribution chain conditions. *L. monocytogenes*, Lactic Acid Bacteria (LAB) and Total Colony Count (TCC) were detected and quantified by ISO methods (ISO 11290-2, ISO 15214 and ISO 4833, respectively). Inoculated and not inoculated (control) samples were analysed in triplicate. Control samples were tested for *L. monocytogenes* detection at the beginning, half, and the end of the storage period by ISO 11290-1. The pH and a_w of control samples were also measured over storage.

8.2. Summary of Results

The main results of the fate studies are depicted in Tables 20 to 23, for each storage temperature. The duration of each study was adjusted to the product self-life at 3°C (16 days) and to the product shelf-life + 33% (20 days) at 10°C. Control samples tested a day 0, 9 and 16 days were negative for *L. monocytogenes* detection. At 3°C *L. monocytogenes* showed a slight increase of 0.57 $\log_{10}$ CFU/ml along the storage period of 16 days in contaminated samples (Table 21).

Interestingly LAB were able to grow at 3°C in both contaminated and control samples with a higher increase in contaminated ones (2.5 vs 0.9 $\log_{10}$ CFU/g) (Tables 20 and 21). A concentration of approx. 6 $\log_{10}$ CFU/g of TCC was maintained in both contaminated and control (Tables 20 and 21). Overall, in control samples the pH slightly increased from 5.23 to 5.63 whereas the a_w value fluctuated within the range of 0.989-0.996 (Tables 20 and 21).

Table 20. Results (log cfu/g) obtained from the analysis of control samples of fresh soft cheese stored at 3°C for 16 days.

Time (days)	0	1	3	6	9	13	16
TCC (log cfu/g)	5.95±0.09	5.81±0.19	6.01±0.13	6.09±0.09	6.26±0.11	6.04±0.01	6.07±0.12
LAB (log cfu/g)	2.55±1.00	2.44±0.15	2.43±0.11	2.81±0.08	2.71±0.20	2.52±0.05	3.34±0.35
pH	5.23±0.02	5.51±0.15	5.25±0.17	5.68±0.08	5.43±0.19	5.51±0.06	5.63±0.12
a_w	0.993±0.001	0.994±0.002	0.989±0.005	0.992±0.002	0.993±0.003	0.996±0.004	0.991±0.002

*LM= *Listeria monocytogenes*

Table 21. Results obtained from the analysis of contaminated samples of fresh soft cheese stored at 3°C for 16 days.

Time (days)	0	1	3	6	9	13	16
LM* (log cfu/g)	2.97±0.25	3.12±0.43	3.47±0.05	3.32±0.18	3.60±0.08	3.55±0.09	3.54±0.06
TCC (log cfu/g)	6.08±0.39	6.01±0.05	6.03±0.07	6.06±0.14	6.22±0.05	6.26±0.33	6.19±0.12
LAB (log cfu/g)	2.36±0.35	2.39±0.08	2.55±0.06	3.1±0.38	3.544±0.52	4.00±0.00	4.93±0.20

*LM= *Listeria monocytogenes*

At 10°C (Table 23), the initial concentration of *L. monocytogenes* in contaminated fresh soft cheese was maintained along the 20 days of storage. On the contrary LAB and TCC grew in both control and contaminated samples with a sharp increase within the first 9 and 6 days of storage reaching values of approx. 7-8 $\log_{10}$ CFU/ml and 8-9 $\log_{10}$ CFU/ml respectively (Tables 22 and 23).

Table 22. Results obtained from the analysis of control samples of fresh soft cheese stored at 10°C for 20 days.

Time (days)	TCC (log cfu/g)	LAB (log cfu/g)	pH	a_w
0	6.73±0.17	0.92±0.38	5.27±0.06	0.993±0.007
6	8.11±0.65	5.00±0.34	5.31±0.19	0.995±0.001
7	7.92±0.17	5.80±0.42	5.30±0.09	0.992±0.002
8	7.76±0.43	6.59±0.49	5.17±0.08	0.992±0.004
9	8.26±0.67	7.95±0.53	4.96±0.01	0.987±0.007
10	8.55±0.09	7.06±0.39	4.76±0.03	0.994±0.001
11	8.84±0.36	7.15±0.31	4.73±0.08	0.995±0.002
12	8.39±0.29	7.32±0.20	5.07±0.02	0.996±0.002
13	8.52±0.52	7.31±0.58	5.06±0.08	0.985±0.001
14	8.39±0.29	7.36±0.23	5.03±0.04	0.992±0.002
15	7.89±0.31	6.76±0.28	5.10±0.02	0.996±0.001
17	8.43±0.60	7.10±0.69	4.95±0.05	0.997±0.001
18	8.94±0.44	7.77±0.48	5.09±0.13	0.996±0.001
19	9.09±0.36	7.93±0.43	5.16±0.14	0.990±0.002
20	8.82±0.27	7.69±0.25	5.11±0.08	0.991±0.001

At 10°C, the pH slightly decreases from 5.3 to 5.1 whereas the a_w increased from 0.993 to 0.997.

Table 23. Results obtained from the analysis of contaminated samples of fresh soft cheese stored at 10˚C for 20 days

Time (days)	TCC (log cfu/g)	LAB (log cfu/g)	LM* (log cfu/g)
0	6.80±0.12	1.35±0.23	3.07±0.14
6	7.84±0.80	5.00±0.34	3.55±0.29
7	8.12±1.36	4.57±1.24	3.25±0.19
8	7.96±0.45	5.88±1.01	3.44±0.06
9	7.59±0.14	7.40±0.28	3.07±0.12
10	7.73±0.11	6.40±0.10	2.82±0.17
11	8.42±0.46	6.61±0.79	2.59±0.11
12	8.46±0.19	7.14±0.30	2.90±0.19
13	8.58±0.51	6.85±0.51	3.00±0.11
14	8.46±0.19	6.37±0.75	2.97±0.12
15	8.79±0.16	7.51±0.40	2.84±0.61
17	7.95±0.48	6.74±0.72	2.84±0.08
18	8.45±0.66	7.24±0.65	2.90±0.11
19	8.87±0.53	7.17±0.59	2.95±0.42
20	8.38±0.57	7.07±0.67	2.85±0.22

*LM= *Listeria monocytogenes*

In general, growth rates of TCC and LAB increased with the increase in storage temperature. However, *L. monocytogenes* maximum population density slightly decreased at 10°C in comparison to 3°C. This could be associated with the competition between the pathogen and LAB (i.e., Jameson effect). The decrease of pH was higher at 10°C due to the higher LAB growth rate.