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Deliverable 7.2: Impact and ranking of intervention strategies for the target artisanal fermented foods (Other, PUblic)

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1. Introduction

The objective of Deliverable 7.2 is to summarise the effectiveness of selected intervention strategies and/or enhanced process variables in the assurance of the safety of the artisanal foods, as quantified by the process risk models using data on pathogen's occurrence and parameters related to process and microbial kinetics. Whereas information on pathogen's occurrence and process parameters were obtained from the results of Work package 2; the microbial kinetic parameters were determined from the fate studies conducted in Work package 6 and the subsequent predictive microbiology models fitted thereof for the current food production and the bio-intervention strategies under study. The present report compiles the results of simulation process risk models testing selected scenarios with views to defining the most effective (bio-) intervention strategies or control measures that contribute to reducing the exposure of consumers to foodborne hazards.

2. IPB: Impact of intervention strategies in *alheira* sausage

The generic exposure assessment model for artisanal sausage production was evaluated for *S. aureus* in *alheira* raw sausage. The intervention strategies tested were:

#0. Baseline scenario (current production);

#1. Incoming pig meat with better sanitary conditions, lower concentration of *S. aureus* in meat (***MainIngr1***);

#2. Improved washing of casings, one quarter prevalence of contaminated casings (***Pcc***);

#3. Addition of 1.0% annatto extract powder to batter, causing reduction in *S. aureus* concentration (***log10_Intervention***) and slight increase in the pH of the stuffed sausages (***pHf***);

#4. Use of starter culture, causing reduction in *S. aureus* concentration (***log10_Intervention***) and reduction in the pH of stuffed sausages (***pHf***); and

#5. Shorter maceration time, 20% time reduction (***t_mac***).

The outputs of the model was the prevalence and concentration of *S. aureus* in packs of sausages leaving the artisanal production factory. Table 1 presents the prevalence of sausage packs contaminated with *S. aureus* for the baseline scenario and the intervention strategies; whereas

Table 2 compiles the concentration of *S. aureus* in sausage packs in the contaminated fraction only for the baseline scenario and the intervention strategies. The model estimated that *alheira* sausages at the end of production present a mean prevalence of ~0.70, and a median concentration of 2.33 log CFU/g in the contaminated fraction. Such values are in close agreement with the surveys conducted in the factories, which altogether showed a high prevalence of this pathogen in *alheira*. The simulations evidenced that the high prevalence of *S. aureus* in *alheiras* could not be substantially lowered by the different strategies (Table 1), although the median concentration can be significantly reduced by the bio-intervention strategies (Table 2).

Table 1. Effect of interventions on the reduction of *S. aureus* prevalence in *alheira* sausage packs

Scenario	*MainIngr1 (log CFU/g)*	*Pcc*	*Log10_ intervention*	*pHf*	t_mac (h)	Prev	% reduction
Baseline	N(2.60, 0.66)	0.20	0	5.6	Pert(6,12,30)	0.696	-
#1	**N(2.00, 0.66)**	0.20	0	5.6	Pert(6,12,30)	0.696	0%
#2	N(2.60, 0.66)	**0.05**	0	5.6	Pert(6,12,30)	0.635	8.8%
#3	N(2.60, 0.66)	0.20	**Pert(0.4,0.9,1.3)**	**5.8**	Pert(6,12,30)	0.677	3.0%
#4	N(2.60, 0.66)	0.20	**Pert(0.2,0.6,1.2)**	**5.4**	Pert(6,12,30)	0.640	8.0%
#5	N(2.60, 0.66)	0.20	0	5.6	**Pert(4.8,9.6,24)**	0.696	0%

Table 2. Effect of interventions on the reduction of *S. aureus* concentration (log10 CFU/g) in contaminated *alheira* packs. Concentrations in brackets are first and third quantiles of the output distribution.

Scenario	*MainIngr1 (log CFU/g)*	*Pcc*	*Log10_ intervention*	*pHf*	t_mac (h)	Concentration (median)	% reduction
Baseline	N(2.60, 0.66)	0.20	0	5.6	Pert(6,12,30)	2.326 (1.381 – 3.049)	-
#1	**N(2.00, 0.66)**	0.20	0	5.6	Pert(6,12,30)	1.816 (1.175 – 2.549)	12%
#2	N(2.60, 0.66)	**0.05**	0	5.6	Pert(6,12,30)	2.392 (1.564 – 3.1789	0%
#3	N(2.60, 0.66)	0.20	**Pert(0.4,0.9,1.3)**	**5.8**	Pert(6,12,30)	1.020 (0.012 – 1.888)	**56%**
#4	N(2.60, 0.66)	0.20	**Pert(0.2,0.6,1.2)**	**5.4**	Pert(6,12,30)	0.662 (-0.555 – 1.603)	**72%**
#5	N(2.60, 0.66)	0.20	0	5.6	**Pert(4.8,9.6,24)**	2.192 (1.182 – 2.763)	6%

The model demonstrates that the prevalence of *S. aureus* in *alheiras* is high because the product is a mixture of different ingredients, having each of them a given prevalence and load of *S. aureus*. Therefore, the presence of one contaminated ingredient is sufficient to contaminate the whole lot, although not all sausages units will be contaminated. Washing and disinfecting pig casings (#2) and the use of starter cultures (#5) were the strategies that decreased prevalence the most (8.0-8.8%; Table 1).

In the analysis of concentrations, it was found that ensuring that meats have better hygiene quality (a mean of 2.0 log CFU/g *S. aureus*) than they currently have, causes a significant drop in pathogen's concentration in the final product. Therefore, it is important that meats are fresh and consist of big joints, and to avoid the purchase of comminuted meat. This control measure (#2) is far more effective than shortening the maceration period (#6), which is a stage where growth can occur. The two most efficient bio-intervention strategies (the starter culture and the plant extract) were selected according to the results of the fate studies (WP5 and WP6). Among all the interventions tested in the simulations, the two bio-intervention strategies were the most effective in reducing the numbers of *S. aureus* in the final product. According to the simulations, the use of annatto extract reduces the median concentration from 2.33 log CFU/ to 1.020 log CFU/g in the final product; whereas the use of the starter culture selected (WP5) decreases further the median number until 0.662 log CFU/g.

3. IPB: Impact of intervention strategies in goat's raw milk cheese

The generic exposure assessment model was evaluated for *L. monocytogenes* in goat's raw milk cheese. The intervention strategies tested were:

#0. Baseline scenario (current production);

#1. Thermisation of goat's raw milk prior to cheesemaking, causing a reduction in the mean concentration of the pathogen (***Bulk***);

#2. Addition of 1.0% spearmint extract powder to curd, causing reduction in *L. monocytogenes* concentration (***log10_Intervention***);

#3. Use of starter culture, causing an increase in the initial concentration of lactic acid bacteria (***LAB0***) and a reduction in the specific growth rate of *L. monocytogenes* (***mumax_ref_LM_mean***); and

#4. Thermisation of goat's raw milk (#1) and use of starter culture (#3).

Results are shown in Table 3. The model simulated the baseline scenario for *L. monocytogenes* in goat's raw milk cheese; and resulted in a high final concentration in cheese at a median of 3.8 log CFU/g; although at a frequency of contamination of 5% (prevalence). The use of goat's raw milk either of better hygienic quality or through thermisation (Scenario #1; assuming a small reduction of the pathogen in 1.5 log in the incoming raw milk) would cause a reduction of 28% of *L. monocytogenes* population in the final product; which turned out to be more effective than the single use of spearmint extract (Scenario #3; reduction of 16%). A far better intervention strategy turned out to be the use of the starter culture with antilisterial activity (Scenario #3) since it would reduce the final concentration of *L. monocytogenes* in contaminated cheeses to -0.193 log CFU/g; this is, a reduction of over 100%. If raw milk was mildly heat treated, in addition to using an ad-hoc starter culture, further reduction in *L. monocytogenes* concentration would be attained (-1.097 log CFU/g or 0.08 CFU/g); however, it is not clear how milk thermisation would affect the sensory properties and acceptability of this traditional product. The use of the ad-hoc starter culture on its own appears therefore as a preferable strategy.

Table 3. Effect of interventions on the reduction of *L. monocytogenes* concentration (log10 CFU/g) in contaminated goat's raw milk cheeses. Concentrations in brackets are first and third quantiles of the output distribution.

Scenario	*Bulk (log CFU/g)*	*Log10_ intervention*	*Mumax_ref_ LM_mean*	LAB0 *(log CFU/g)*	Concentration (median)	% Reduction
Baseline Raw	**N(-2.0, 0.1)**	0	0.78	Pert(4.0, 4.5, 6.0)	3.799 (3.264 – 4.385)	-
#1	**N(-3.5, 0.1)**	0	0.78	Pert(4.0, 4.5, 6.0)	2.736 (2.211 – 3.231)	28%
#2	N(-2.0, 0.1)	**Pert(0.3, 0.8, 1.5)**	0.78	Pert(4.0, 4.5, 6.0)	3.183 (2.556 – 3.800)	16%
#3	N(-2.0, 0.1)	0	**0.70**	**Pert(7.0, 8.2, 9.0)**	-0.193 (-0.755 - 0.515)	**105%**
#4 (#1 & #3)	**N(-3.5, 0.1)**	0	**0.70**	**Pert(7.0, 8.2, 9.0)**	-1.097 (-1.495 - -0.607)	130%

4. Anses/CNIEL: Impact of intervention strategies in artisanal cheese

The quantitative risk assessment model developed on shiga-toxin producing *Escherichia coli* in raw milk cheese was used to assess different interventions (Table 4).

The first intervention is associated to milk quality assessment at farm level according to *E. coli* (hygienic indicator). In the baseline scenario (close to current situation), farms are monitored every 10 days (*pmilk*=10%). If the concentration of *E. coli* in raw milk of farm that is above 50 cfu/ml (*lmilk*) is not used for cheese production.

Increasing the milk testing for hygienic microbiological criteria (*pmilk* scenarios #1 and #2) has little effect of risk. A 10% reduction (risk reduction factor = 1.1 for scenario #2)) can be obtained by testing farm on average every 3.33 days instead of every 10 days.

Changing the microbiological limit (*lmilk*) that defines "selected"/"unselected" farms has no significant impact (scenarios #3, #4).

Increasing the percentage of batches of cheese tested (*pcheese*) from 10 to 100% allows to divide the risk by a 1.8 factor (scenario #6).

Increasing the number of samples (*nsample)* from 1 to 5 has no significant impact on risk reduction.

Combining every control measures (more farm testing for hygiene, with a lower microbiological limit, every batch testing, with n=5) allows to divide by a factor 3.7 the risk compared to the baseline.

Table 4. Effect of interventions for raw milk cheese on the reduction of STEC infection risk

Scenario	*pmilk*	*lmilk*	*pcheese*	*nsample*	Risk (STEC)	Risk reduction
Baseline	10%	50	10%	1	2.2E-05	_
#1	20%	50	10%	1	2.2E-05	not significant
#2	30%	50	10%	1	1.9E-05	1.1
#3	10%	25	10%	1	2.1524E-05	not significant
#4	10%	10	10%	1	2.1524E-05	not significant
#5	10%	50	50%	1	2.1524E-05	not significant
#6	10%	50	100%	1	1.2221E-05	1.8
#7	10%	50	10%	3	2.1524E-05	not significant
#8	10%	50	10%	5	2.1524E-05	not significant
#9	30%	10	100%	5	5.8939E-06	3.7

5. UCO: Impact of intervention strategies in *salchichon*

The generic exposure assessment model for artisanal sausage production was evaluated for *L. monocytogenes* in Spanish *salchichon*. The intervention strategies tested were:

#0. Baseline scenario (current production);

#1. Improved quality control of raw materials: reduction of LM concentration in raw meat (***MainIngr1***);

#2. Improved quality control of the casings. Reduced proportion of contaminated units ***(Pcc***);

#3. Addition of ad-hoc starter culture of selected lactic acid bacteria, causing reduction in *L. monocytogenes* concentration (***log10_Intervention***);

#4. Decreased maceration temperature (***T_mac***); and

#5. Improved quality control of raw materials (#1) and addition of ad-hoc starter culture (#3).

Table 5. Effect of interventions on the reduction of *L. monocytogenes* prevalence in *salchichon* sausage packs

Scenario	*MainIngr1 (log CFU/g)*	*Pcc*	*Log10_ Inter-vention*	t_mac (h)	T_mac (ºC)	Prev	% reduction
Baseline	N(0.915,0.102)	0.105	Pert(0, 0, 0)	Pert(336,336, 360)	Pert (13.9; 15.95; 16.9)	0.197	-
#1	**N(0.5; 0.102)**	0.105	0	Pert (336; 336; 360)	Pert (13.9; 15.95; 16.9)	0.197	0%
#2	N(0.915; 0.102)	**0.05**	0	Pert (336; 336; 360)	Pert (13.9; 15.95; 16.9)	0.148	25%
#3	N(0.915,0.102)	0.105	**Pert(0, 0.55, 1.1)**	Pert(336,336, 360)	Pert (13.9; 15.95; 16.9)	0.197	0%
#4	N(0.915; 0.102)	0.105	0	Pert (336; 336; 360)	**Pert (12.9; 13.5; 15)**	0.197	0%
#5 (#1 & #3)	**N(0.5; 0.102)**	0.105	**Pert(0, 0.55, 1.1)**	Pert (336; 336; 360)	Pert (13.9; 15.95; 16.9)	0.197	0%

According to the current production of *salchichon* (baseline scenario), an estimate of 19.7% of the sausage packs would be contaminated with *L.monocytogenes* (at least one cell present), at a median of 1.309 log CFU/g (Table 5 and 6). It is therefore necessary to diminish the level of contamination through intervention strategies. Hygienised pig casings was the only intervention

strategy (#2) that diminished the prevalence of this pathogen (Table 5); whereas the addition of ad-hoc starter culture of selected lactic acid bacteria, (#3) and decreasing the maceration temperature (#4) were the most effective single intervention strategies. Using starter culture or decreasing maceration temperature would bring about similar levels of reduction of the concentration of *L. monocytogenes*, of about 38-40% (Table 6).

Table 6. Effect of interventions on the reduction of *L. monocytogenes* concentration (log10 CFU/g) in contaminated *salchichon* sausage packs. Concentrations in brackets are first and third quantiles of the output distribution.

Scenario	*MainIngr1 (log CFU/g)*	*Pcc*	*Log10_ Inter-vention*	t_mac (h)	T_mac (ºC)	Concentration (median)	% reduction
Baseline	N(0.915,0.102)	0.105	Pert(0, 0, 0)	Pert(336,336, 360)	Pert (13.9; 15.95; 16.9)	1.309 (0.480 – 2.188)	-
#1	**N(0.5; 0.102)**	0.105	0	Pert (336; 336; 360)	Pert (13.9; 15.95; 16.9)	1.118 (-1.903 – 1.893)	15%
#2	N(0.915; 0.102)	**0.05**	0	Pert (336; 336; 360)	Pert (13.9; 15.95; 16.9)	1.677 (0.944 – 2.437)	-28%
#3	N(0.915,0.102)	0.105	**Pert(0, 0.55, 1.1)**	Pert(336,336, 360)	Pert (13.9; 15.95; 16.9)	0.781 (-0.151 – 1.633)	40%
#4	N(0.915; 0.102)	0.105	0	Pert (336; 336; 360)	**Pert (12.9; 13.5; 15)**	0.816 (-0.061 – 1.576)	38%
#5 (#1 & #3)	**N(0.5; 0.102)**	0.105	**Pert(0, 0.55, 1.1)**	Pert (336; 336; 360)	Pert (13.9; 15.95; 16.9)	**0.051 (-0.642 – 0.679)**	**96%**

According to the model, the combination of Scenarios #1 and #4 constitute the best strategy to diminish the current levels of *L. monocytogenes* in *salchichon*, which would attain an average of 96% reduction (Table 6). Figure 1 represents the distribution of pathogen's counts in contaminated packs of *salchichon*, with an estimated median of 1.125 CFU/g (0.228 – 4.775 CFU/g), which is well below the limit of 100 CFU/g at the end of the shelf life. It should be kept in mind that the distribution shown in Figure 1 is applicable only to the contaminated fraction, which is expected to occur at a probability of 0.197.

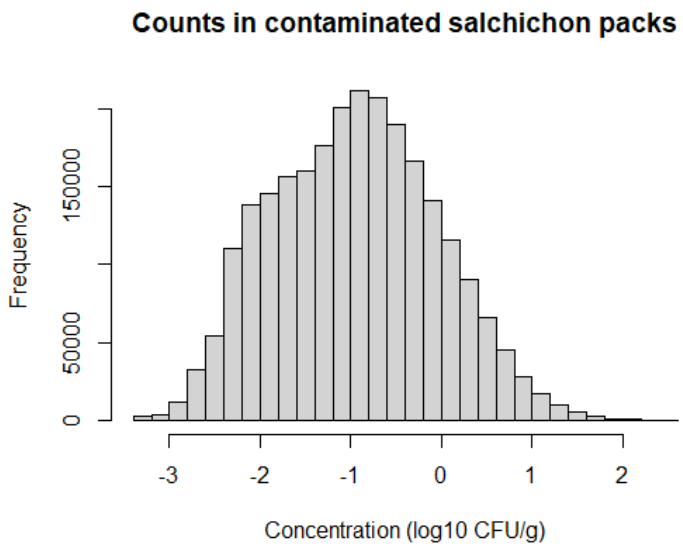


Figure 1. Prediction of the concentration of *L. monocytogenes* (CFU/g) in packs of *salchichon* sausage (contaminated fraction) produced using raw meat of good microbiological quality and ad-hoc starter culture (median: 1.125 CFU/g [0.228 – 4.775 CFU/g])

6. UCO: Impact of intervention strategies in goat's soft cheese

The generic exposure assessment model for artisanal cheesemaking was evaluated for *L. monocytogenes* in Spanish goat's soft cheese. The intervention strategies tested were:

#0. Baseline scenario (current production);

#1. Improved quality control of raw materials, causing a reduction in the prevalence of *L. monocytogenes* in milk (***P***);

#2. Shortening of the maturation time (***t_mac***);

#3. Decreasing the maturation temperature (***T_mac***); and

#4. Shortening maturation time (#2) and decreasing maturation temperature (#3).

For the Spanish goat's soft cheese, no intervention strategy related to bio-preservation was tested in the generic cheesemaking model. According to the simulation results, shortening the

maturation time of the cheese (Scenario #2) was the most effective intervention strategy among the singles ones (#1, #2, #3), reaching a reduction in the concentration of *L. monocytogenes* of approximately 66%. Reducing the prevalence of *L. monocytogenes* in milk was as efficient as reducing the temperature of maceration (44-46% reduction in counts; Table 7). The combined strategy (#4) of decreasing both temperature and time of maceration would bring about a reduction in microbial concentration of 85% (median = 0.344 log10 CFU/g [-0.767 – 1.498 log10 CFU/g].

It is noteworthy mentioning that the application of a bio-intervention strategy such as the use of selected lactic acid bacteria would further reduce the prevalence and concentration of *L. monocytogenes* in cheeses; yet this strategy was not tested in the Spanish cheeses. The simulation model showed that the addition of lactic acid until reaching a final concentration of 0.25% (w/w) in cheese would represent an interesting intervention strategy that would bring the occurrence of *L. monocytogenes* down up to a median of -0.752 log10 CFU/g [-1.398 – 0.422 log10 CFU/g]) (Figure 2).

Table 7. Effect of interventions on the reduction of *L. monocytogenes* concentration (log10 CFU/g) in contaminated goat's soft cheese. Concentrations in brackets are first and third quantiles of the output distribution; prevalence (Prev) of contaminated cheeses also included

Scenario	*P*	*Log10_ intervention*	*t_mat (h)*	*T_mat* (ºC)	Concentration (median)	% Reduction
Baseline	0.3	Pert (0,0,0)	Pert (154.08; 168; 480)	Pert (4; 6.5; 9)	2.202 (0.146 – 4.573) Prev=0.254	-
#1	**0.024**	Pert (0,0,0)	Pert (154.08; 168; 480)	Pert (4; 6.5; 9)	1.197 (-0.020 – 2.823) Prev=0.250	46%
#2	0.3	Pert (0,0,0)	**Pert (120; 150; 180)**	Pert (4; 6.5; 9)	0.742 (-0.484 – 2.533) Prev=0.254	66%
#3	0.3	Pert (0,0,0)	Pert (154.08; 168; 480)	**Pert (4; 5; 8)**	1.231 (-0.478 – 2.830) Prev=0.254	44%
#4	0.3	Pert (0,0,0)	**Pert (120; 150; 180)**	**Pert (4; 5; 8)**	**0.344 (-0.767 – 1.498)** Prev=0.254	**85%**

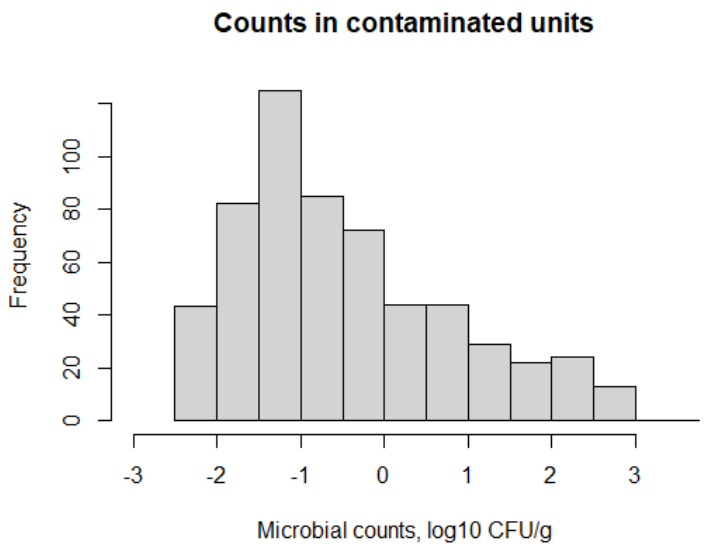


Figure 2. Prediction of the concentration of *L. monocytogenes* (CFU/g) in Spanish goat' soft cheeses (contaminated fraction) if produced with lower maturation time and temperature (#3 and #4) and with added lactic acid until 0.25% (median: -0.752 log10 CFU/g [-1.398 – 0.422 log10 CFU/g])

7. ISBST-UMA: Impact of intervention strategies in dry *Merguez*

The generic exposure assessment model for artisanal sausage production was evaluated for *L. monocytogenes* in Tunisian dry *Merguez* sausage. The intervention strategies tested were:

#0. Baseline scenario

#1. Use of spices of certified microbiological quality; therefore having a lower concentration of lower concentration of *L. monocytogenes* (***MainSpices***)

#2. Addition of 1 mg/mL mint extract powder to batter, causing reduction in *L. monocytogenes* concentration (***log10_Intervention***).

In the elaboration of the Tunisian *Merguez* sausage, it was found that the most contaminated ingredient is the spices. However, the process risk model simulation showed that, given the low dose at which the spices are added to the batter, improving their microbiological quality (i.e., a reduction of concentration in ~1.5 log10 CFU/g), would cause only a marginal decrease of 8% in

L. monocytogenes concentration in the final product (Table 9). On the other hand, the use of mint extract powder would decrease the pathogen's concentration in 51%, up to a median of 0.410 log10 CFU/g (0.176 – 0.634 log10 CFU/g).

Table 8. Effect of interventions on the reduction of *L. monocytogenes* prevalence in dry *Merguez* sausage packs

Scenario	*MainIngr1 (log CFU/g)*	*MainSpices (log CFU/g)*	*Log10_ intervention*	t_mac (h)	Prev	% reduction
Baseline	N(0.70, 0.56)	N(2.09,1.23)	Pert(0,0,0)	Pert(18,24, 26)	0.382	-
#1	N(0.70, 0.56)	**N(0.5, 0.2)**	Pert(0,0,0)	Pert(18,24, 26)	0.382	0%
#2	N(0.70, 0.56)	N(2.09,1.23)	**Pert(0.075,0.436,0.789)**	Pert(18,24, 26)	0.381	0.2%

Table 9. Effect of interventions on the reduction of *L. monocytogenes* concentration (log10 CFU/g) in contaminated dry *Merguez* sausage packs. Concentrations in brackets are first and third quantiles of the output distribution.

Scenario	*MainIngr1 (log CFU/g)*	*MainSpices (log CFU/g)*	*Log10_ intervention*	t_mac (h)	Concentration (median)	% reduction
Baseline	N(0.70, 0.56)	N(2.09,1.23)	Pert(0,0,0)	Pert(18,24, 26)	0.845 (0.649 – 1.029)	-
#1	N(0.70, 0.56)	**N(0.5, 0.2)**	Pert(0,0,0)	Pert(18,24, 26)	0.778 (0.522 – 0.942)	8%
#2	N(0.70, 0.56)	N(2.09,1.23)	**Pert(0.075,0.436,0.789)**	Pert(18,24, 26)	0.410 (0.176 – 0.634)	**51%**

Thus, it is advisable for artisanal food producers to consider the use of certified spice ingredients as well as the modification of their formulations with mint extract at doses that do not affect the organoleptic characteristics of the *Merguez* sausage. Figure 3 illustrates the concentration of *L. monocytogenes* in contaminated packs of *Merguez* sausage that would be attained if interventions #1 and #2 would be applied. Notice that concentrations would be very low (median: 2.13 CFU/g [1.043 – 3.41 CFU/g]), and well below the legislation limit of 100 CFU/g at the end of shelf life.

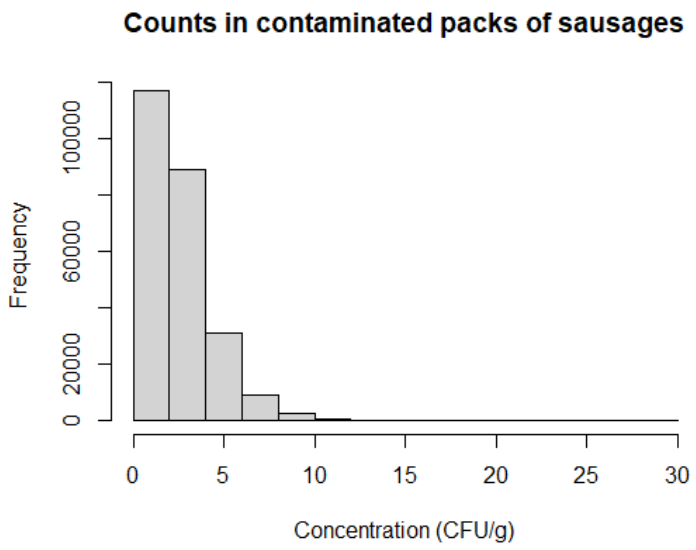


Figure 3. Prediction of the concentration of *L. monocytogenes* (CFU/g) in packs of dry *Merguez* sausage (contaminated fraction) produced using spices of good microbiological quality and adding mint extract powder to the batter (median: 2.13 CFU/g [1.043 – 3.41 CFU/g])

8. ISBST-UMA: Impact of intervention strategies in *Lben* milk

The generic exposure assessment model for artisanal cheese-making was evaluated for *L. monocytogenes* in *Lben* milk. However, since it is not a cheese product, but a fermented milk product, the stage of maturation was replaced by a shorter fermentation period. The intervention strategies tested were:

#0. Baseline scenario (current production); and
#1. Addition of 1 mg/mL citrus extract powder to milk, causing reduction in *L. monocytogenes* concentration (***log10_Intervention***).

The simulation run for the baseline scenario showed that the levels of *L. monocytogenes* in *Lben* milk were below 50 CFU/ml in the contaminated fraction of 5% (Figure 4, left); therefore, the level of contamination appeared as in-control by the artisanal manufacturers. For that reason, the

only bio-intervention strategy tested was the incorporation of optimised citrus peel extract. As shown in Table 10, the sole application of such bio-preservative would reduce the median concentration in 160%, reaching a median concentration of -0.409 log10 CFU/ml (-0.750 - -0.111 log10 CFU/ml). The predicted distribution of *L. monocytogenes* concentration in *Lben* jars formulated with citrus peel extract (Figure 4, right) shows that *L. monocytogenes*, when present (at a probability of 4.9%) would reach very low concentrations of up to 10 CFU/ml.

Table 10. Effect of interventions on the reduction of *L. monocytogenes* concentration in contaminated jars of *Lben* milk. Concentrations in brackets are first and third quantiles of the output distribution.

Scenario	*Bulk (log CFU/ml)*	*Log10_ intervention*	*Mumax_ref_ LM_mean*	LAB0 *(log CFU/ml)*	Concentration (median)	Mean reduction
Baseline	N(0.76, 0.03)	Pert(0,0,0)	0.5	Pert (6.07, 6.66, 7.00)	0.652 (0.374 – 0.900)	-
#1	N(0.76, 0.03)	**Pert(0.5, 1, 2)**	0.5	Pert (6.07, 6.66, 7.00)	-0.409 (-0.750 – -0.111)	**160%**

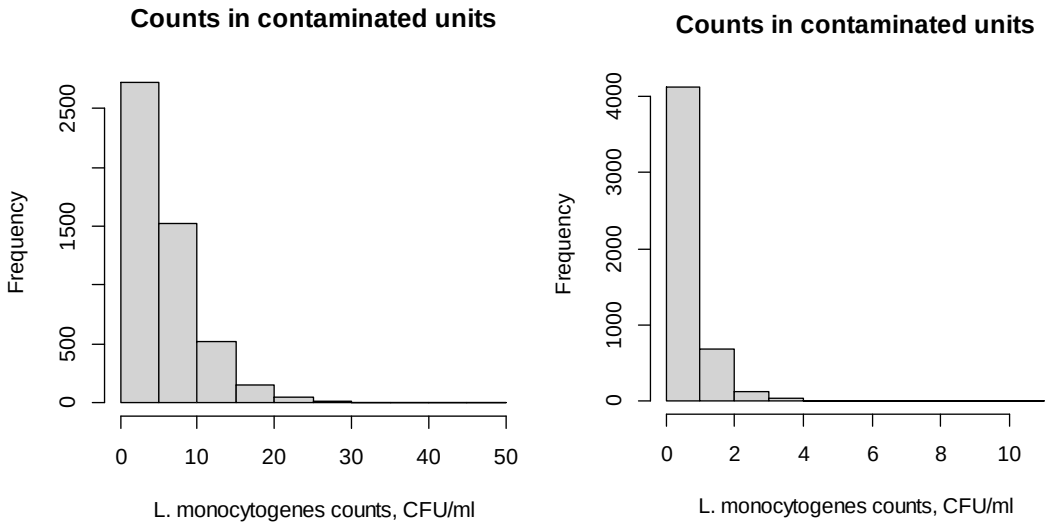


Figure 4. Concentration of *L. monocytogenes* (CFU/ml) in contaminated jars of *Lben* milk in the baseline scenario (left) and under the best bio-intervention strategy of formulating it with citrus peel extract (right). Prevalence=0.049 in both scenarios

9. UIZ: Impact of intervention strategies in *Merguez* sausage

The generic exposure assessment model for artisanal sausage-making was evaluated for *L. monocytogenes* in Moroccan *Merguez* sausage. The intervention strategies tested were:

#0. Baseline scenario (current production);
#1. Improved washing of casings, on quarter prevalence of contaminated casings (***Pcc***);
#2. Addition of 0.3% oregano essential oil to batter, causing reduction in *Salmonella* concentration (***log10_Intervention***); and
#3. Use of starter culture, causing reduction in *Salmonella* concentration (***log10_Intervention***) and reduction in the pH of stuffed sausages (***pHf***).

The three scenarios tested (#1, #2, #3) caused a significant decrease in the prevalence of *Salmonella* in *Merguez* sausage, from 0.200 in the baseline to 0.002-0.077 (Table 11). However, the most important decrease in the concentration of *Salmonella* would be achieved by any of the two bio-intervention strategies: #2 – addition of oregano essential oil, or #3 – use of ad-hoc starter culture. Any of them would produce a median of *Salmonella* concentration of -2.00 log10 CFU/g (Table 12); although the starter culture developed is preferable because it does not have an impact on the sensorial quality of the *Merguez* sausage.

Table 11. Effect of interventions on the reduction of *Salmonella* prevalence in *Merguez* sausage packs

Scenario	*p_MainIngr1*	*Pcc*	*Log10_ intervention*	*pHf*	t_mac (h)	Prev	% reduction
Baseline	12%	0.04	0	5.5	Pert(12,24,48)	0.200	-
#1	12%	0.01	0	5.5	Pert(12,24,48)	0.077	62%
#2	12%	0.04	Pert(2.9,3.2,3.9)	5.5	Pert(12,24,48)	**0.003**	**99%**
#3	12%	0.04	Pert(1.33,3.76,4)	5.4	Pert(12,24,48)	**0.002**	**99%**

Table 12. Effect of interventions on the reduction of *Salmonella* concentration (log10 CFU/g) in contaminated *Merguez* sausage packs. Concentrations in brackets are first and third quantiles of the output distribution.

Scenario	*p_MainIngr 1*	*Pcc*	*Log10_ intervention*	*pHf*	t_mac (h)	Concentration (median)	% reduction
Baseline	12%	0.04	0	5.5	Pert(12,24,48)	-1.523 (-1.823 - -0.979)	-
#1	12%	0.01	0	5.5	Pert(12,24,48)	-1.523 (-2.00 - -0.903)	0%
#2	12%	0.04	Pert(2.9,3.2,3.9)	5.5	Pert(12,24,48)	**-2.000 (-2.301 - -1.602)**	**33%**
#3	12%	0.04	Pert(1.33,3.76,4)	5.4	Pert(12,24,48)	**-2.000 (-2.301 - -1.456)**	**33%**

Figure 5 illustrates the *Salmonella* concentration in *Merguez* sausages, as currently produced by the participating artisanal producers (left), in comparison with the level of contamination in sausages produced using the ad-hoc starter culture (right). Significant decrease would be attained by the selected bio-intervention strategy to levels that in practice would be below the limit of quantification of the *Salmonella* microbiological essays.

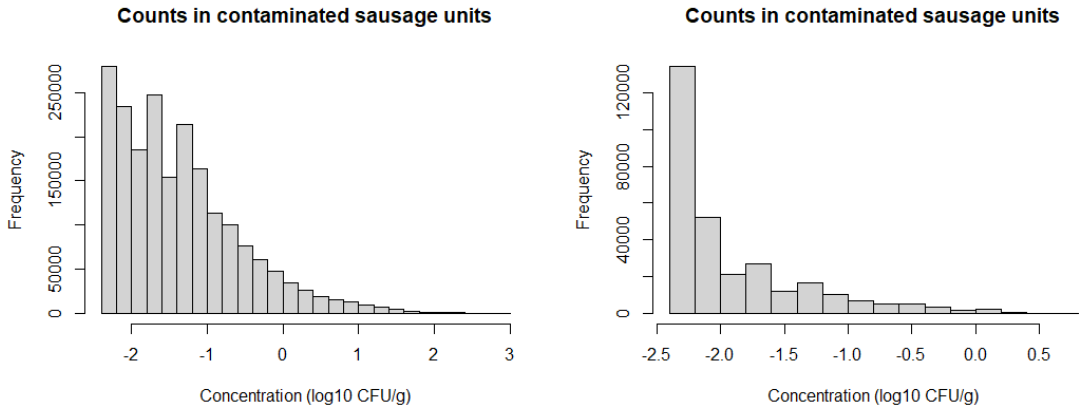


Figure 5. Concentration of *Salmonella* spp. (log10 CFU/g) in contaminated units of *Merguez* sausages in the baseline scenario (left; prevalence 0.20) and under the best bio-intervention strategy of adding an optimised starter culture (right; prevalence 0.02).

10. UIZ: Impact of intervention strategies in *Jben* cheese

The generic exposure assessment model for artisanal cheesemaking was evaluated for *L. monocytogenes* in Moroccan Jben cheese. The intervention strategies tested were:

#0. Baseline scenario (current production);

#1. Improved quality control of raw materials, causing a reduction in the concentration of *L. monocytogenes* in milk (***Bulk***);

#2. Strict control of maturation temperature (***T_mat***);

#3. Using an ad-hoc starter culture that causes mild inactivation (***Log10_Intervention***)

#4. Systematic application of all strategies #1, #2, #3.

Table 13. Effect of interventions on the reduction of *L. monocytogenes* concentration (log10 CFU/g) in contaminated goat's soft cheese. Concentrations in brackets are first and third quantiles of the output distribution; prevalence (Prev) of contaminated cheeses also included

Scenario	*Bulk (log CFU/g)*	*Log10_ intervention*	*LAB0 (log CFU/g)*	*T_mat* (ºC)	Concentration (median)	% Reduction
Baseline	N(1.60,0)	Pert (0,0,0)	Pert (7,7,8)	Pert (13, 17, 20)	2.269 (2.005 – 2.486)	-
#1	**N(0.60,0)**	Pert (0,0,0)	Pert (7,7,8)	Pert (13, 17, 20)	1.283 (0.999 – 1.528)	43%
#2	N(1.60,0)	Pert (0,0,0)	Pert (7,7,8)	**Pert (8, 12, 15)**	1.287 (1.016 – 1.531)	43%
#3	N(1.60,0)	**Pert (0.3, 1.0, 1.2)**	Pert (7,7,8)	Pert (13, 17, 20)	1.351 (1.068 – 1.591)	41%
#4	**N(0.60,0)**	**Pert (0.3, 1.0, 1.2)**	Pert (7,7,8)	**Pert (8, 12, 15)**	**0.383 (0.116 – 0.606)**	**83%**

For the Moroccan Jben cheese, all of the single strategies could reduce the concentration of *L. monocytogenes* in the final product in comparable levels (Table 13). This means that decreasing the concentration of the pathogen in milk in one log (Scenario #1) is as effective as reducing the maturation temperature in 5 ºC (Scenario #2), and in turn, as effective as using the ad-hoc starter (Scenario #3). All these strategies reduce the median level of contamination by 41-43%. For this product, it is therefore necessary to use systematic approach that combines the three strategies (#4). If this is implemented, a reduction in the median microbial concentration of 83% would be

attained. Figure 6 shows a prediction of the current level of *L. monocytogenes* in contaminated *Jben* cheese (left), as produced with the traditional manufacturing processes; and a prediction of the efficiency to control this pathogen if cheeses were produced with goat's milk of better microbiological quality, lower maturation temperature and ad-hoc starter culture (Figure 6, right). The simultaneous application of the three strategies would produce cheeses, that if contaminated, would have a median concentration of 2.41 CFU/g (1.306 – 4.037 CFU/g) or 0.383 log10 CFU/g (0.116 – 0.606 log CFU/g; Table 13).

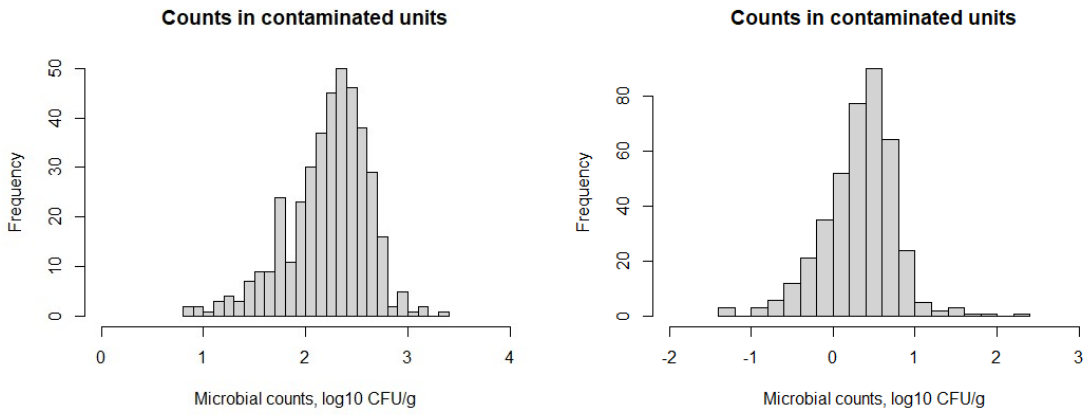


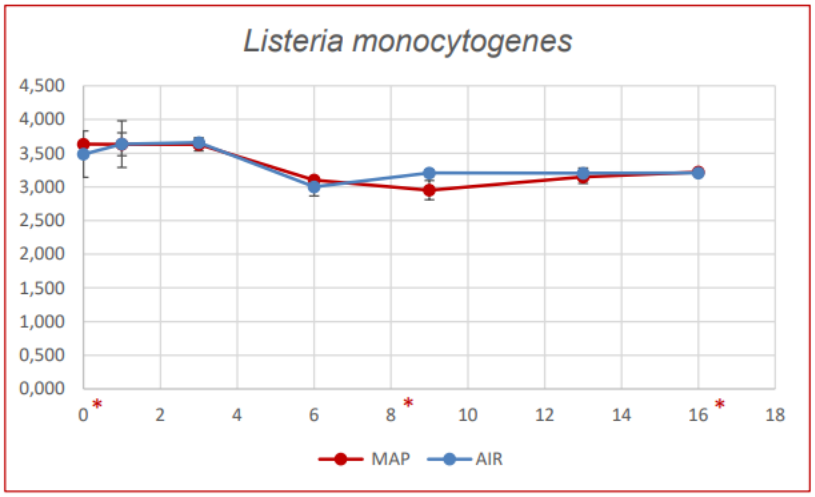
Figure 6. Prediction of the concentration of *L. monocytogenes* (CFU/g) in Moroccan Jben cheese (contaminated fraction) in the baseline scenario (left) and if produced with goat's milk of improved microbiological quality, lower maturation temperature and ad-hoc starter culture (right) (median: 2.41 CFU/g [1.306 – 4.037 CFU/g])

11. UNIBO: Impact of storage in *Squacquerone* cheese

The growth or survival of *L. monocytogenes* in *Squacquerone* cheese was assessed at the following storage scenarios:

#0. Baseline scenario storage for 16 days at 3°C under air (current production);
#1. Storage for 16 days at 3°C under modified atmosphere (MAP);
#2. Storage for 20 days at 10°C under air.

L. monocytogenes did not growth during storage at both 3 and 10°C when the cheese was packed under air and MAP. Therefore, any specific intervention was validated for the Italian cheese. Figures 7 shows the enumeration results for *L. monocytogenes* under MAP and air during storage at 3°C and Figure 8 shows the enumeration results for total bacteria count (TBC) and lactic acid bacteria in the same storage conditions.



	CONTROL (Log_{10} cfu/g)		TEST (Log_{10} cfu/g)	
Days	MAP	AIR	MAP	AIR
0	2.274	2.550	1.915	2.358
1	2.696	2.438	2.679	2.392
3	2.916	2.428	2.641	2.553
6	3.001	2.813	3.155	3.114
9	2.855	2.711	3.473	3.544
13	2.595	2.516	2.815	4.144
16	3.109	3.386	3.065	4.926

Figure 7. Enumeration results of *L. monocytogenes* under MAP and air during storage at 3°C

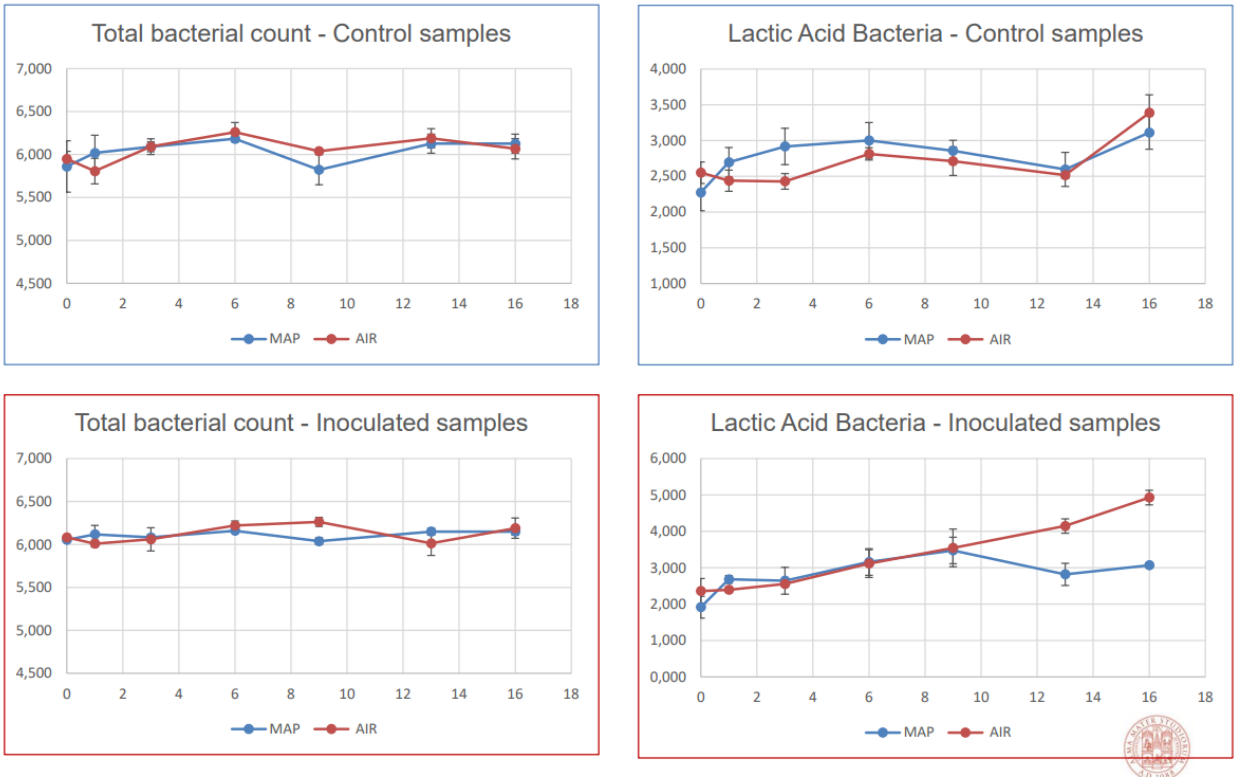
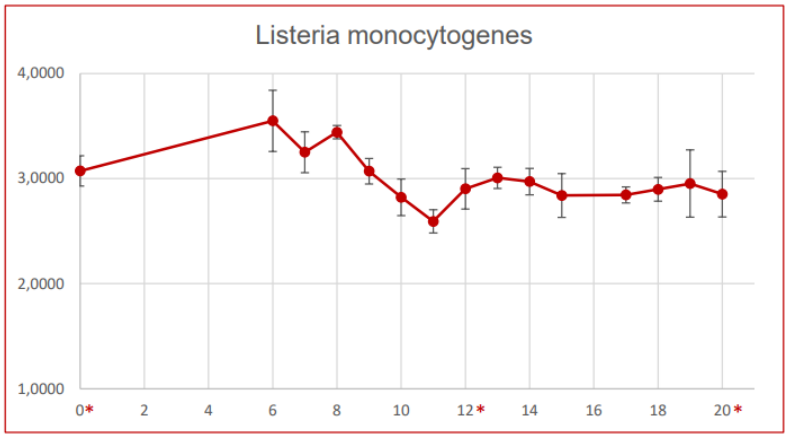


Figure 8. Enumeration results of total bacteria count and lactic acid bacteria under MAP and air during storage at 3°C

Since the pathogen did not growth under air and MAP at refrigeration temperature and the MAP was difficult to keep by the film used by the artisanal food company involved in the project, a second test was performed testing *L. monocytogenes* during storage at 10°C for 20 days under air. Figure 9 shows the enumeration results for *L. monocytogenes* under air during storage at 10°C and Figure 10 shows the enumeration results for total bacteria count (TBC) and lactic acid bacteria at the same conditions.



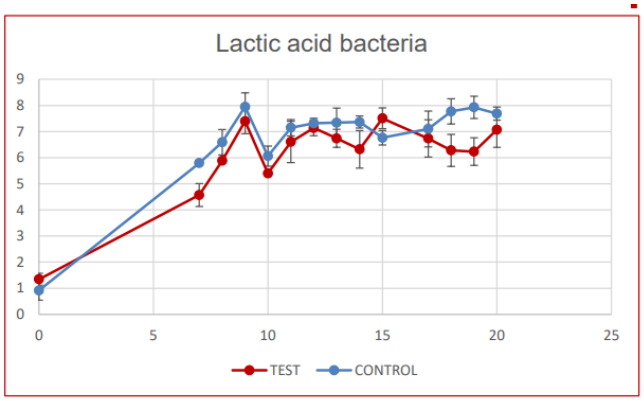
* Listeria not detected in negative controls

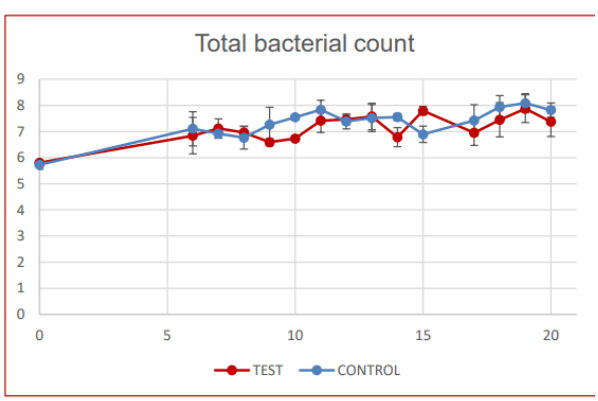
L. monocytogenes

Days	Log_{10} CFU/g	SD
0	3.0730	0.14
6	3.5502	0.29
7	3.2511	0.19
8	3.4416	0.06
9	3.0707	0.12
10	2.8220	0.17
11	2.5927	0.11
12	2.9025	0.19
13	3.0072	0.10
14	2.9714	0.12
15	2.8392	0.21
17	2.8448	0.08
18	2.8976	0.11
19	2.9530	0.32
20	2.8527	0.22

Figure 9. Enumeration results of *L. monocytogenes* under air during storage at 10°C

The results collected for *Squacquerone* were not suitable for model implementation but demonstrated that *L. monocytogenes* does not growth in the tested artisanal cheese at both refrigeration as well as abuse temperature.





Day	TEST Log10 CFU/g	TEST SD	CONTROL Log10 CFU/g	CONTROL SD
0	1.34631	0.23	0.91671	0.38
7	4.57193	0.44	5.79518	0.29
8	5.88149	0.05	6.59339	0.49
9	7.39782	0.48	7.94968	0.53
10	5.39794	0.09	6.05976	0.39
11	6.60654	0.79	7.14784	0.31
12	7.13791	0.30	7.31538	0.20
13	6.74014	0.34	7.34231	0.56
14	6.32006	0.72	7.36492	0.23
15	7.50500	0.40	6.76323	0.28
17	6.73692	0.72	7.10026	0.69
18	6.27937	0.62	7.77249	0.48
19	6.23353	0.53	7.92762	0.43
20	7.07212	0.67	7.68544	0.25

Day	TEST Log10 CFU/g	TEST SD	CONTROL Log10 CFU/g	CONTROL SD
0	5.804094	0.12	5.727804	0.17
6	6.841074	0.70	7.107466	0.65
7	7.120350	0.36	6.919339	0.17
8	6.962989	0.25	6.762067	0.43
9	6.592752	0.14	7.260904	0.67
10	6.730620	0.11	7.547623	0.09
11	7.416922	0.46	7.836320	0.36
12	7.460758	0.19	7.388855	0.29
13	7.579489	0.51	7.518080	0.52
14	6.783249	0.37	7.553021	0.14
15	7.794438	0.16	6.892248	0.31
17	6.953211	0.48	7.426073	0.60
18	7.449067	0.66	7.940124	0.44
19	7.874936	0.53	8.086323	0.36
20	7.375999	0.57	7.820187	0.27

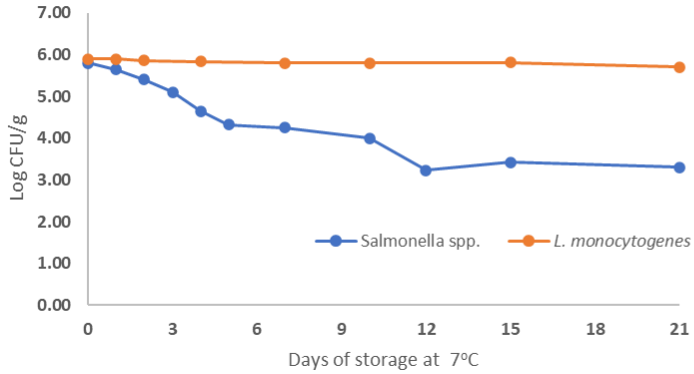
Figure 10. Enumeration results of total bacteria count and lactic acid bacteria under air during storage at 10°C

12. AUA: Impact of storage in *Katiki* cheese

Katiki cheese is a low-pH (4.3 to 4.4) spreadable cheese with increased moisture (ca. 75%) and low salt content (ca.1%). According to EU Reg 2073/2005, Ready to Eat (RTE) products with pH ≤ 4,4 or aw ≤ 0,92, products with pH ≤ 5,0 and aw ≤ 0,94 are automatically considered as unable to support the growth of pathogens such as *L. monocytogenes*. Moreover, preliminary baseline experiments showed that bacterial pathogens such as *L. monocytogenes* will not be able to survive the pasteurization of raw milk during the production of Katiki cheese. Such results are in accordance with previous studies that have demonstrated that *L. monocytogenes'* presence in the final Katiki cheese is almost always due to post-process contamination. As such, AUA team

focused its research on the behavior of *L. monocytogenes* and /or *Salmonella* spp. as postprocessing contaminants, during normal storage conditions (21 days at 7°C under air).

Figure 11 demonstrates the enumeration results (log CFU/g) for *L. monocytogenes* and Salmonella spp. in katiki cheese when stored aerobically at 7°C. None of the tested pathogens was able to grow in the katiki cheese during the aerobic storage at 7°C.



Days of storage	*Salmonella* spp.	*L. monocytogenes*
0	5.80	5.90
1	5.64	5.90
2	5.40	5.86
3	5.10	5.84
4	4.64	5.80
5	4.32	5.80
7	4.25	5.81
10	4.00	5.71
12	3.23	5.68
15	3.42	5.62
21	3.30	5.45

Figure 11. Enumeration results of *L. monocytogenes* and *Salmonella* spp. under air during storage at 7°C

The results collected for *Katiki cheese* were not suitable for model implementation but demonstrated that both pathogens were not able to grow in the tested artisanal cheese at the storage conditions applied (aerobic storage at 7ºC).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