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Deliverable 2.3: Report on risk factors leading to increased contamination in the artisanal food chains (**R**eport, **PU**blic)

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1. Introduction

Deliverable 2.3 is linked with Task 2.3 of the ArtiSaneFood project, whose objective is to analyse the longitudinal data obtained from the microbiological and physicochemical surveys of raw materials, intermediate and final products, and the microbiological surveys of processing environments, conducted in Task 2.2. The present report compiles the variables or risk factors that lead to an increased contamination of the artisanal end products, as identified by adjusting both generalised linear models and stepwise regressions to the longitudinal data.

2. Methodology

Generalised linear models can be useful in the identification of variables that have a significant contribution to an increased contamination in the finished products. Nonetheless, the identification of the variables will greatly rely on the number of batches that were tracked. Such variables, which can be intrinsic properties of raw materials and mid-products, environmental factors or processing variables, will be referred to as *risk factors*. Specific processing risk factors will be addressed in the fate studies of pathogens (WP5).

Longitudinal data sets obtained from IPB, UCO, AUA, ISBST/UMA and UNIBO were adjusted to generalised linear models of the form:

$$Y_{isf(b)} = \left(\beta_0 + u_f + v_{f(b)}\right) + Stage_s + Stage_s(IP_i) + \varepsilon_{isf(b)}$$

where:

$Y_{sf(b)}$: counts in log CFU/g of a given microbial group determined in the sample *i*, taken at the end of the processing stage *s*, belonging to batch *b* from factory *f*;

β_0: model intercept that can take shifts u_f according to factory *f*, and shifts $v_{f(b)}$ according to batch *b* within factory f;

$Stage_s$: Processing stage *s* from where the sample was taken;

IP: Intrinsic property determined in the sample *i*, taken at the end of the processing stage. *IP* can be pH, water activity (aw), moisture content or lactic acid concentration; and

$\varepsilon_{isf(b)}$: error of the microbial count determined in the sample *i*, taken at the end of the processing stage *s*, belonging to batch *b* from factory *f*.

This model has the advantage that allows the determination of between-factory and between-batch variability in the concentration of a given microorganism. The between-factory variability is estimated from the squared standard deviation of the normal distribution for the random effects u_f; whereas the between-batch variability (within a factory) is determined from the squared standard deviation of the normal distribution for the random effects $v_{f(b)}$. Errors are also assumed to follow a normal distribution.

If the model was fitted to *Salmonella* presence/absence data, the same model was used, but with the logit link function of binomial family. $Y_{sf(b)}$ was then defined as presence (1) or absence (0) of *Salmonella* in at least one of the samples taken from a batch.

In addition, stepwise regression analysis was run on the microbial concentration of the final product for total viable counts (TVC), *S. aureus* or *Enterobacteriaceae* (response variable) with potential explanatory variables, such as pH, aw, moisture content or lactic acid concentration in raw materials and mid-products, initial microbial load in raw materials, microbial load in environmental elements, batch duration, etc. Values of response and explanatory variables were calculated as within-batch means.

3. Risk factors leading to increased contamination in Portuguese artisanal products (IPB)

The generalised model revealed that the evolution in pH and aw has a significant impact on TVC counts during the first stage of maturation of alheiras (between batter and mid-maturation). This can be deduced by the significant terms for pH:Mid-maturation (p=0.002) and aw:Mid-maturation (p=0.038) in Table 1. Half-maturated alheiras that presented lower pH were significantly associated with higher TVC counts. This characterises optimal fermentations whereby sausage pH rapidly drops due to the rapid development of lactic acid populations (TVC also retrieve mesophilic lactic acid bacteria). On the other hand, half-maturated sausages with higher aw presented higher TVC counts, and the opposite, sausages with lower aw presented lower TVC counts. This inhibition might be an effect of dehydration, as opposed to fermentation. Similarly, finished alheiras of higher moisture content (p=0.048) presented higher TVC counts. Putting this together, in order to keep TVC high, the first days of maturation can be considered critical because fermentation should occur swiftly, yet dehydration should be slow.

In terms of between-batch variability (within a factory), this value (0.160^2=0.026) was lower than the variability between factories (0.574^2=0.329) (Table 1).

Table 1: Parameter estimates of the generalised linear model assessing the overall effects of processing stage, Aw, pH and moisture on TVC in alheira sausage during production, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Factory	0.574	-
Batch in Factory	0.160	-
Residual	0.659	-
Effect of pH		
Stage		
Batter	3.339 (9.733)	0.733
Mid-maturation	12.77 (2.135)	<.0001
Finished product	10.04 (1.542)	<.0001
pH		
Batter	0.103 (1.633)	0.950
Mid-maturation	-1.184 (0.377)	**0.002**
Finished product	-0.403 (0.284)	0.160
Effect of aw		
Stage		
Batter	13.85 (30.20)	0.648
Mid-maturation	-45.69 (24.70)	0.068
Finished product	1.733 (16.35)	0.916
aw		
Batter	-9.992 (30.47)	0.744
Mid-maturation	52.58 (25.02)	**0.038**
Finished product	6.334 (16.65)	0.705
Effect of moisture content		
Stage		
Batter	2.159 (3.249)	0.508
Mid-maturation	6.956 (2.170)	0.002
Finished product	4.994 (1.809)	0.007
Moisture		
Batter	0.030 (0.054)	0.580
Mid-maturation	-0.014 (0.042)	0.736
Finished product	0.067 (0.021)	**0.048**

The generalised model adjusted to the *S. aureus* data revealed that the pH of batter was the only intrinsic property directly affecting the development of this pathogen. Batters of higher pH tended to have higher *S. aureus* counts (p=0.018; Table 2). The levels of *S. aureus* in alheira did not differ significantly between factories, reason as to why the between-factory variability was virtually zero. The between-batch variability in *S. aureus* was estimated as $0.444^2=0.197$ (Table 2). Likewise, for total coliforms the variability between factories was negligible, whereas the between-batch variability was $1.651^2=2.726$ (Table 3). Among the three intrinsic properties tested, moisture content was the only one affecting the total coliforms counts. The higher the moisture content in the finished product, the most likely to have higher counts of total coliforms (p=0.001; Table 3). This means that sufficient dehydration taking place in sausages during the second stage of maturation (Mid-maturation to finished product) should ensure a significant inactivation of coliforms.

Table 2: Parameter estimates of the generalised linear model assessing the overall effects of processing stage, Aw, pH and moisture on *S. aureus* in alheira sausage during production, as well as estimates of between-batch variability

Parameters	**Estimate (SE)**	**Pr > |t|**
Random effects (σ)		
Factory	0.001	-
Batch in Factory	0.444	-
Residual	0.590	-
Effect of pH		
Stage		
Batter	-19.35 (9.336)	0.039
Mid-maturation	3.252 (2.065)	0.119
Finished product	2.258 (1.420)	0.115
pH		
Batter	3.760 (1.567)	**0.018**
Mid-maturation	-0.006 (0.371)	0.987
Finished product	0.156 (0.270)	0.567
Effect of aw		
Stage		
Batter	66.06 (29.75)	0.029
Mid-maturation	9.272 (25.29)	0.715
Finished product	32.10 (16.10)	0.050
aw		
Batter	-63.80 (40.02)	0.097
Mid-maturation	-6.128 (25.61)	0.811
Finished product	-29.59 (16.40)	0.075
Effect of moisture content		
Stage		
Batter	4.958 (3.139)	0.118
Mid-maturation	0.659 (2.146)	0.759
Finished product	5.951 (1.702)	0.001
Moisture		
Batter	-0.036 (0.053)	0.500
Mid-maturation	0.051 (0.042)	0.233
Finished product	-0.066 (0.038)	0.091

Through stepwise regression analysis, it was possible to identify a series of variables that drive the TVC and *S. aureus* concentration in the finished alheiras (Table 4 and 5). On a batch level, higher TVC in finished alheira was linked to higher TVC in batter, higher TVC in meat, and higher TVC in casings.

Higher moisture contents in batter and, therefore, in the finished product, produced higher counts of mesophiles in alheiras. This was also previously pointed out by the generalised linear model. Higher counts of *S. aureus* in casings and in half-maturated sausages were also linked to higher TVC in finished alheiras (Table 4).

Table 3: Parameter estimates of the generalised linear model assessing the overall effects of processing stage, Aw, pH and moisture on total coliforms in alheira sausage during production, as well as estimates of between-batch variability

Parameters	**Estimate (SE)**	**Pr > |t|**
Random effects (σ)		
Factory	0.001	-
Batch in Factory	1.651	-
Residual	1.028	-
Effect of pH		
Stage		
Batter	33.19 (23.55)	0.165
Mid-maturation	-2.870 (4.147)	0.492
Finished product	3.919 (2.869)	0.178
pH		
Batter	-5.318 (3.979)	0.187
Mid-maturation	1.064 (0.738)	0.156
Finished product	0.047 (0.532)	0.930
Effect of aw		
Stage		
Batter	10.77 (68.74)	0.877
Mid-maturation	116.1 (60.73)	0.062
Finished product	-13.79 (31.68)	0.665
aw		
Batter	-9.145 (70.52)	0.897
Mid-maturation	-114.8 (81.62)	0.108
Finished product	18.34 (32.35)	0.573
Effect of moisture content		
Stage		
Batter	-5.198 (5.939)	0.386
Mid-maturation	-3.343 (4.445)	0.455
Finished product	-10.17 (4.243)	0.020
Moisture		
Batter	0.117 (0.099)	0.245
Mid-maturation	0.122 (0.084)	0.155
Finished product	0.320 (0.093)	**0.001**

Table 4: Stepwise selection of process variables and intrinsic characteristics contributing to the TVC in finished alheira sausage

Selected variables*	**Partial R^2 (%)**	**F-value**	**Pr > F**
TVC in batter (+)	90.4	37.62	0.004
TVC in meat (+)	59.1	5.758	0.074
TVC in casings (+)	44.2	3.165	0.150
S. aureus in mid-matured sausage (+)	40.3	2.697	0.176
S. aureus in casings (+)	58.5	5.560	0.076
Moisture in batter (+)	53.0	4.516	0.100
Moisture in end product (+)	75.2	12.15	0.025

(*) Positive (+) or negative (-) association between variables

As with the generalised linear model, the stepwise regression analysis also evidenced the positive relationship between pH in batter and *S. aureus* counts in finished alheira (Table 5). In addition, contaminated casings (represented by high *C. perfringens* counts and TVC in casings) had also a significant contribution to the increased counts of *S. aureus* in finished alheiras. Finally, it was also interesting to see that *S. aureus* could be present in pepper, and this bacterium could survive throughout processing to increase the probability of recovering *S. aureus* in finished alheira. In the case of the within-batch *Salmonella* presence in the finished alheira, no single process variable or intrinsic characteristic was found to be determinant (results not shown).

Table 5: Stepwise selection of process variables and intrinsic characteristics contributing to *S. aureus* counts in finished alheira sausage

Selected variables*	**Partial R^2 (%)**	**F-value**	**Pr > F**
pH in batter (+)	59.0	5.759	0.074
C. perfringens in casings (+)	59.4	5.839	0.073
TVC in casings (+)	44.5	3.213	0.147
S. aureus in pepper (+)	42.5	2.954	0.160

(*) Positive (+) or negative (-) association between variables

In the case of the results from the tracking study conducted on goat's raw milk cheese, the generalised linear models did not find any statistical effect of the intrinsic properties (pH or aw) on the counts of mesophiles, *S. aureus* and *Listeria* spp in any of the sampled production stages. Whereas pH did not have any impact on the counts of *Clostridium* spp. at any production stage, aw was found to have an effect on *Clostridium* counts in cheese right after moulding (p=0.001) and cheese after 20 days maturation (p=0.022; Table 6). This indicates that between moulding and the first 20 days of maturation, cheeses that remained with higher water activity tended to have higher populations of *Clostridium* spp.

For alheiras sausages, the analysis of the tracking data allowed the selection of three variables that will be used in the simulation studies to be conducted in WP7: (i) initial pH of batter (to be changed per simulation), (ii) initial concentration of *S. aureus* in batter (to be changed per simulation); and (iii) maturation time.

With regards to goat's raw milk cheese, no significant processing stage or intrinsic property was found to constitute an important risk factor leading to higher counts of *S. aureus*, *Clostridium* spp. or *Listeria* spp.

Table 6: Parameter estimates of the generalised linear model assessing the overall effects of processing stage, Aw and pH on *Clostridium* spp. in goat's raw milk cheese during production, as well as estimates of between-batch variability

Parameters	**Estimate (SE)**	**Pr > |t|**
Random effects (σ)		
Batch	0.922	-
Residual	0.451	-
No effect of pH		
Effect of aw		
Stage		
Milk	10.29 (21.14)	0.627
Moulded cheese	-59.18 (17.73)	0.001
Maturated 20 days	-26.27 (12.12)	0.003
Maturated 40 days	18.81 (10.20)	0.069
Maturated 60 days	-1.584 (14.84)	0.915
aw		
Milk	-9.420 (21.34)	0.660
Moulded cheese	62.44 (17.93)	**0.001**
Maturated 20 days	28.82 (12.36)	**0.022**
Maturated 40 days	-17.93 (10.54)	0.093
Maturated 60 days	2.950 (15.56)	0.850

4. Risk factors leading to increased contamination in Spanish artisanal products (UCO)

Based on the generalized linear model estimates, the physicochemical parameters of goat's raw milk cheese (pH and Aw) did not show a statistically significant relationship with *Enterobacteriaceae* counts ($p>0.05$) (Table 7). Since intrinsic properties did not directly affect the development of *Enterobacteriaceae*, other external factors as hygienic-sanitary conditions, processing variables or manufacturing practices could have affected their counts, which would explain the microbial differences found in the final counts.

Same strategy of statistical analysis was developed for the counts observed for the microbial group *Staphylococcus* spp. in the cheese product. In this case, the generalised model revealed that the pH was the only physicochemical parameter having a significant impact on *Staphylococcus* spp. counts ($p \leq 0.05$; Table 8). According to the results, the higher the cheese pH, the higher the counts of *Staphylococcus* spp. As a ripened cheese, the decrease of pH observed during cheese maturation, especially during the first stage due to lactic acid production by the lactic acid bacteria (LAB), could favour the decline in *Staphylococcus* spp. counts over the production processes.

The levels of *Staphylococcus* spp. in goat's raw milk cheese did not differ significantly ($p > 0.05$) between different production batches (within a factory), which was evidenced by the between-batch factory variability (nearly 0).

Table 7: Parameter estimates of the generalised linear model assessing the overall effects of Aw, pH on *Enterobacteriaceae* counts in goat's raw milk cheese, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Batch	0.587	-
Stage in Batch	0.001	-
Residual	0.277	-
Effect of pH		
Finished product	-0.360 (1.137)	0.760
Effect of Aw		
Finished product	3.061 (4.236)	0.494

Table 8: Parameter estimates of the generalised linear model assessing the overall effects of Aw, pH on *Staphylococcus* spp. counts in goat's raw milk cheese, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Batch	0.001	-
Stage in Batch	0.900	-
Residual	0.385	-
Effect of pH		
Finished product	0.531 (0.206)	**0.016**
Effect of Aw		
Finished product	8.276 (6.967)	0.249

The effects of intrinsic properties of the cured meat product "salchichon" on microbial counts were also statistically tested by a generalised linear model. In this case, physicochemical parameters (pH and Aw) did not have statistically significant relationships with both *Enterobacteriaceae* and *Staphylococcus* spp. counts ($p > 0.05$) (Table 9 and 10). Likewise, for both microbial groups, the variability between batches (within factory) was very low. These results indicate standardised hygienic-sanitary conditions of both raw materials used for sausage production and food contact surfaces.

Table 9: Parameter estimates of the generalised linear model assessing the overall effects of Aw, pH on *Enterobacteriaceae* counts in salchichon, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Batch	0.001	-
Stage* in Batch	0.653	-
Residual	0.190	-
Effect of pH		
Finished product	-0.040 (0.195)	0.893
Effect of aw		
Finished product	0.124 (0.857)	0.887

(*) Stage: Batter and finished product

Table 10: Parameter estimates of the generalised linear model assessing the overall effects of Aw, pH on *Staphylococcus* spp. counts in salchichon, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Batch	0.001	-
Stage* in Batch	0.512	-
Residual	0.229	-
Effect of pH		
Finished product	0.077 (0.230)	0.741
Effect of aw		
Finished product	-0.835 (1.834)	0.655

(*) Stage: Batter and finished product

The results relative to the stepwise regression analyses are presented in Table 11. The results evidenced the relevant variables affecting microbial counts on each analysed product. According to the results, as expected, the manufacturing process duration (including ripening) was negatively associated with microbial counts of raw milk cheese. In other words, longer processing times yield products with lower loads of *Staphylococcus* spp. This was also previously evidenced by the generalised linear model. This result consistently supports that the decrease of pH in long ripened cheeses could induce the decline of *S. aureus* population in the final product.

For cured meat product "salchichon", the total viable counts in raw materials used for the product manufacturing influenced *Staphylococcus* spp. counts. In this case, a positive relationship was marked, so that higher TVC in meat batter was associated with higher *Staphylococcus* spp. levels in the final product. These results evidence the importance of using raw materials with good hygienic-sanitary conditions for the elaboration of ready-to-eat cured meat products. Besides, it is also demonstrated the survival ability of *S. aureus* along the processing chain to the final product. Overall, these results will support on the design and performance of fate studies, on the optimisation of process variables and on the development of intervention strategies to control these microorganisms on the evaluated products (WP5, WP6 and WP7).

Table 11: Stepwise selection of process variables and intrinsic characteristics contributing to the counts of *Staphylococcus* spp. in goat's raw milk cheese and salchichon

Product	Selected variables*	Partial R^2 (%)	F-value	Pr > F
Raw milk cheese	Process duration including ripening (-)	40.5	2.717	0.165
Salchichon	TVC in raw materials (+)	51.3	4.216	0.109

(*) Positive (+) or negative (-) association between variables

5. Risk factors leading to increased contamination in Greek artisanal products (AUA)

In both Katiki cheese and Noumbulo sausage, no effect of pH or aw could be found to affect the TVC counts (Tables 12 and 13). However, for the Noumbulo sausage, an inverse relationship between moisture content and TVC counts was found (p=0.001 in Table 13), suggesting that as maturation took place and moisture content became lower, the TVC counts including mesophiles and lactic acid bacteria population increased due to fermentation process. For this sausage, the variability batch-to-batch (within a factory) was lower than between factories (Table 13); however, the opposite was true for Katiki cheese (Table 12).

Table 12: Parameter estimates of the generalised linear model assessing the overall effects of Aw, pH on TVC counts in Katiki cheese, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Factory	0.569	-
Batch in Factory	1.038	-
Residual	0.233	-
Effect of pH		
Finished product	-0.372 (3.699)	0.929
Effect of aw		
Finished product	11.75 (15.27)	0.522

Table 13: Parameter estimates of the generalised linear model assessing the overall effects of Aw, pH on TVC counts in Noumbulo sausage, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Factory	0.754	-
Batch in Factory	0.228	-
Residual	0.415	-
Effect of pH		
Finished product	2.255 (2.401)	0.400
Effect of aw		
Finished product	-6.979 (5.177)	0.249
Effect of moisture content		
Finished product	-0.111 (0.004)	**0.001**

6. Risk factors leading to increased contamination in Tunisian artisanal products (ISBST/UMA)

Ten artisanal producers of fermented cow-milk (Lben) from the region of Ariana (North of Tunisia) were chosen for physicochemical and microbiological characteristics of initial and final products (results not shown). One producer was selected to assess the different steps of the Lben production. Samples were randomly collected from three different batches. For each one, raw milk, coagulum obtained after spontaneous fermentation and Lben obtained after churning were sampled (Figure 1). Results are used to identify risks factors leading to contamination of Lben.

Results of estimated parameters fitted to overall effects of processing stage, aw, pH and lactic acid by linear models are depicted in Table 14. It was found that pH and aw affected significantly *Enterobacteriaceae* counts. Higher values of the two parameters usually led to estimate higher loads of

Enterobacteriaceae in finished Lben (p value = 0.016 for pH and 0.019 for aw). Strongly reducing effect of pH was also reported on *Enterobacteriaceae* counts in products assessed after fermentation and churning.

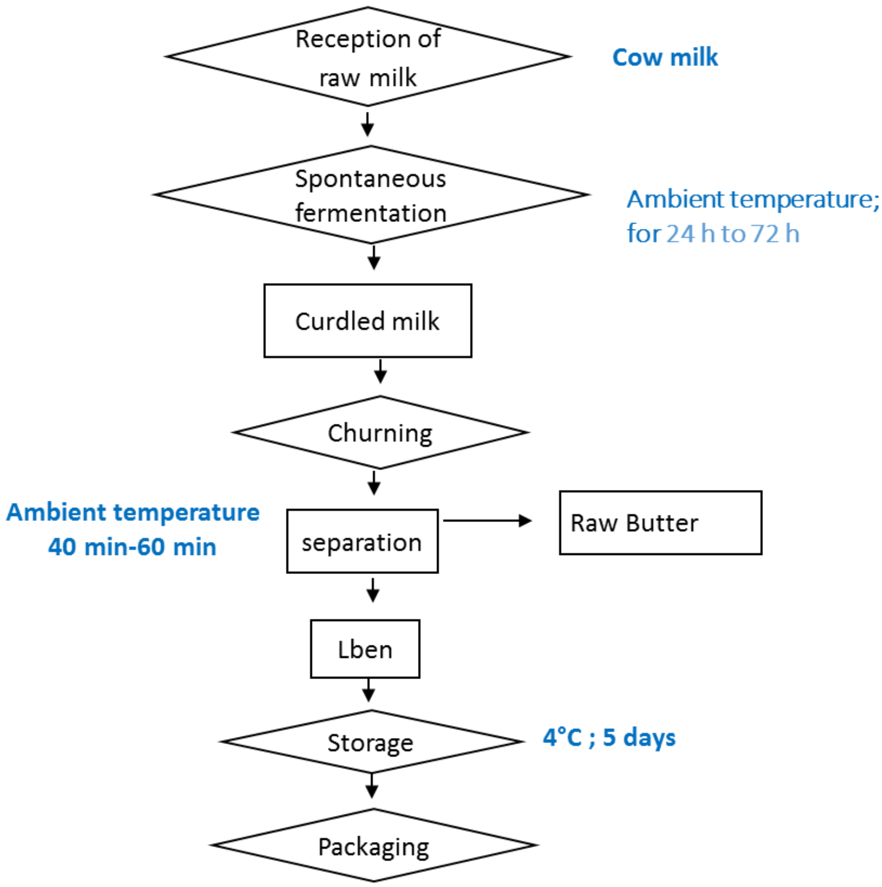

Figure 1: Flowchart of artisanal Lben

The inhibition potential of *Enterobacteriaceae* by the production of lactic acid was implied by the negative estimates values in all steps: -0.533 (raw milk), -0.169 (after fermentation) and -0.075 (after churning) and by the significant p-values recorded for the different processing steps ($p<0.001$).

Table 14: Parameter estimates of the generalised linear model assessing the overall effects of processing stage, Aw, pH and lactic acid concentration on *Enterobacteriaceae* counts in Lben during production, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Batch	0.263	-
Residual	0.626	-
Effect of pH		
Stage		
Raw	-89.72 (33.10)	0.021
Before churning	8.898 (39.33)	0.825
After churning	16.92 (12.60)	0.209
pH		
Raw	14.37 (4.976)	**0.016**
Before churning	-0.865 (8.272)	0.919
After churning	-2.476 (2.660)	0.374
Effect of aw		
Stage		
Raw	-104.5 (39.92)	0.026
Before churning	80.77 (43.36)	0.092
After churning	28.608 (34.02)	0.420
aw		
Raw	113.1 (40.88)	**0.019**
Before churning	-77.48 (44.21)	0.110
After churning	-23.94 (34.79)	0.507
Effect of lactic acid concentration [LAC]		
Stage		
Raw	14.72 (1.679)	<.0001
Before churning	18.45 (2.027)	<.0001
After churning	11.38 (1.144)	<.0001
[LAC]		
Raw	-0.533 (0.093)	**<.0001**
Before churning	-0.169 (0.023)	**<.0001**
After churning	-0.075 (0.011)	**<.0001**

Churning step appears to be an important stage affecting the survival/growth of *S. aureus (*p=0.170*)* (Table 15). TVC determined after churning showed positive correlation with *S. aureus* concentration in finished Lben. Temperature affects significantly *S. aureus* counts (P=0.199). The higher the temperature in the processing room, the higher the counts of *S. aureus* in the finished Lben. The final value of pH, aw and concentration of lactic acid bacteria having positive effect on reducing counts of *S. aureus* could be controlled by fermentation duration and starting level of lactic acid bacteria. The analysis of the tracking data allowed the selection of variables that will be used in the simulation studies to be conducted in WP7:

(i) temperature in processing room (to be changed per simulation) and (ii) initial concentration of *S. aureus* (to be changed per simulation).

Table 15: Stepwise selection of process variables and intrinsic characteristics contributing to *S. aureus* counts in finished Lben (processing data)

Selected variables*	**Partial R^2 (%)**	**F-value**	**Pr > F**
TVC after churning (+)	92.5	12.26	0.170
Temperature in processing room (+)	88.6	7.790	0.199

(*) Positive (+) or negative (-) association between variables

7. Risk factors leading to increased contamination in Italian artisanal products (UNIBO)

The generalised model revealed that the evolution in pH has a significant impact on *Enterobacteriaceae* counts at the third week of ripening. This is highlighted by the significant term for pH:ripened 3 weeks (p=0.007) in Table 16. This result is due to decrease of pH between the first and the third week of ripening resulting in a decrease of *Enterobacteriaceae* which then continues up to the week 28 of ripening when *Enterobacteriaceae*, including potential food borne pathogens, reach a concentration under the enumeration level corresponding to 10 CFU/g.

The impact of changes in aw was observed at different steps specifically represented by stuffed, and 10 as well as 18 weeks of ripening. These effects are highlighted by the significant terms for aw:stuffed (p=0.019), aw:ripened 10 weeks (p=0.001) and aw:ripened 18 weeks (p=0.041). At stuffing, the aw of meat was close to 1 and associated to levels of *Enterobacteriaceae* ranging between 4.27 and 5.15 log CFU/g. Later on, during processing, aw tend to constantly decrease reaching values between 0.947 and 0.953 after at 10 weeks of ripening and between 0.854 and 0.871 after 18 weeks of ripening. As a consequence, *Enterobacteriaceae* decreases from initial values between 4.37 and 5.17 log CFU/g at stuffing up to level under 10 cfu/g after 18 weeks of ripening. In terms of within-batch variability (different processing stages of the same batch), this value (0.700^2=0.490) was higher than the variability between batches (0.287^2=0.008) (Table 16).

The generalised model for the cheese tracking study revealed that the evolution in pH has a significant impact on TVC counts in the milk after the pasteurisation process and in the cheese during maturation: the higher the pH in pasteurised milk and in maturated cheese, the higher the TVC counts. This is highlighted by the significant terms for pH:milk post pasteurization (p=0.009) and pH:maturation (p=0.043) in Table 17. In terms of within-batch variability (different processing stages of the same batch), this value ($0.600^2=0.36$) was higher than the variability between batches ($0.400^2=0.16$) (Table 17).

Table 16: Parameter estimates of the generalised linear model assessing the overall effects of processing stage, Aw, pH on *Enterobacteriaceae* counts in salami during production, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Batch	0.287	-
Stage in Batch	0.700	-
Residual	0.540	-
Effect of pH		
Stage		
Stuffed	8.287 (13.38)	0.537
Dried	1.063 (4.357)	0.807
Ripened 3 weeks	-21.34 (9.860)	0.031
Ripened 10 weeks	7.613 (7.266)	0.296
Ripened 18 weeks	8.280 (7.507)	0.272
Finished product	5.959 (8.307)	0.474
pH		
Stuffed	-0.969 (2.269)	0.669
Dried	0.574 (0.801)	0.474
Ripened 3 weeks	4.422 (1.631)	**0.007**
Ripened 10 weeks	-1.130 (1.117)	0.313
Ripened 18 weeks	-1.435 (1.057)	0.176
Finished product	-0.983 (1.129)	0.386
Effect of aw		
Stage		
Stuffed	60.41 (23.87)	0.012
Dried	-2.515 (16.47)	0.879
Ripened 3 weeks	302.3 (355.0)	0.396
Ripened 10 weeks	-32.58 (10.78)	0.003
Ripened 18 weeks	-13.47 (7.056)	0.058
Finished product	12.25 (9.435)	0.196
aw		
Stuffed	57.77 (24.39)	**0.019**
Dried	6.911 (17.00)	0.685
Ripened 3 weeks	-311.25 (369.8)	0.401
Ripened 10 weeks	37.55 (11.55)	**0.001**
Ripened 18 weeks	16.21 (7.880)	**0.041**
Finished product	-12.87 (10.80)	0.235

Table 17: Parameter estimates of the generalised linear model assessing the overall effects of processing stage, Aw, pH on TVC counts in Squacquerone cheese during production, as well as estimates of between-batch variability

Parameters	Estimate (SE)	Pr > |t|
Random effects (σ)		
Batch	0.400	-
Stage in Batch	0.600	-
Residual	0.347	-
Effect of pH		
Stage		
Milk pre pasteurisation	19.42 (10.11)	0.058
Milk post pasteurisation	-33.16 (13.61)	0.017
Storage warm room	8.380 (10.06)	0.407
Maturation	-3.930 (4.980)	0.431
Finished product	-2.991 (7.742)	0.707
pH		
Milk pre pasteurisation	-1.915 (1.476)	0.198
Milk post pasteurisation	5.386 (2.010)	**0.009**
Storage warm room	-0.542 (1.727)	0.754
Maturation	1.753 (0.732)	**0.043**
Finished product	1.566 (1.444)	0.281
No effect of aw		

Through stepwise regression analysis (Table 18), it was possible to identify drying as the key step in processing salame able to result in a highest concentration of TVC in the final product. As far as squacquerone is concerned (Table 19), the initial TVC level at day 1 of shelf life as well as aw at day 4 and pH values at the end of the shelf life were identified as the key variables impacting on higher levels of TVC in the final product after 15 days of storage.

Table 18: Stepwise selection of process variables and intrinsic characteristics contributing to the total viable counts (TVC) in finished salami (processing data)

Selected variables*	Partial R^2 (%)	F-value	Pr > F
TVC after drying (+)	88.8	28.72	0.006

(*) Positive (+) or negative (-) association between variables

Table 19: Stepwise selection of process variables and intrinsic characteristics contributing to the total viable counts (TVC) in finished cheese (shelf life data, storage temperature was clustered)

Selected variables*	Partial R^2 (%)	F-value	Pr > F
TVC at day 1 (+)	23.5	3.194	0.089
Aw at day 4 (+)	20.1	10.38	0.018
pH at day 15 (+)	48.2	14.54	0.009

(*) Positive (+) or negative (-) association between variables

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