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Deliverable 2.2 – Part A: Report on Physicochemical and Microbiological Parameters of Raw Materials, Intermediate and Final products under Categorised Storage Conditions; Production Environments under Categorised Hygiene and Sanitation Conditions (**R**eport**, PU**blic; Month 27)

Partners: Alessandra de Cesare, Gerardo Manfreda, Antonio Valero Díaz, Panagiotis Skandamis, Spyridon Kintzios, Fouad Achemchem, Nourhene Boudhrioua Mihoubi, Vasco Cadavez, Ursula Gonzales-Barron

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1. Introduction: D2.2 summaries the results of activities performed under Tasks 2.2 and 2.3

The objective of Task 2.2 was to conduct tracking surveys of the microbiological and physicochemical parameters of raw materials, intermediate and final products as well as environmental data from processing facilities producing the artisanal products addressed in the project. This information will serve to elucidate microbial contamination routes, and to identify specific processing risk factors to be addressed in D2.3 and WP5. The objective of Task 2.3 was submitting the isolates collected in the artisanal foods investigated in the project to whole genome sequencing (WGS) to map each isolate to a strain level and investigate its pathogenic, virulence and antibiotic resistance properties. All sequenced genomes will be made publicly available in databases, as ENA-EBI, NCBI, Enterobase, to be used to improve global surveillance of foodborne pathogens. Some of the strains isolated from the artisanal products will be used in the preparation of pathogenic inocula for the fate studies in WP5. The protocols applied by all ArtiSaneFood Partners to collect the results described below are described in D1.1. The flow diagrams of the products addressed by each Partner are described in D2.1.

2. Microbiological and physicochemical parameters of raw materials, intermediate and final products as well as environmental data from processing facilities producing alheira sausage and goat raw milk cheese (IPB)

The factory surveys were conducted in two artisanal manufacturers of alheira sausage in the district of Bragança, auditing 6 batches of production. The microbiological, physicochemical and manufacture surveys of alheira involved sampling of raw meat, ingredients, casings, batter, half maturated product and finished product; and they were finished in June 2020. In addition, a separate experiment not contemplated in the original proposal was carried out, whereby the artisanal alheira from 16 companies throughout Bragança district were characterised in terms of both physicochemical and microbiological attributes.

The physicochemical attributes measured were water activity (aw), pH and proximate composition; whereas the microbiological attributes included counts of mesophiles, lactic acid bacteria on MRS and M17 agars, *C. perfringens*, *S. aureus*, *L. monocytogenes* and presence of *Salmonella* spp. Bidimensional maps of pairwise principal components 1, 2 and 3 of the similarity and differences in quality of the alheiras produced by the regional producers is presented in Figures 1 and 2. It can be noticed in Figure 1 that some artisanal producers such as Vinhais 1, 3, 4 and Bragança 4 produced microbiologically safe

alheiras, yet with a higher fat content. In some batches, alheira sausages did not meet the food safety criterion set by Regulation (EC) No 2073/2005 for *Salmonella* spp.

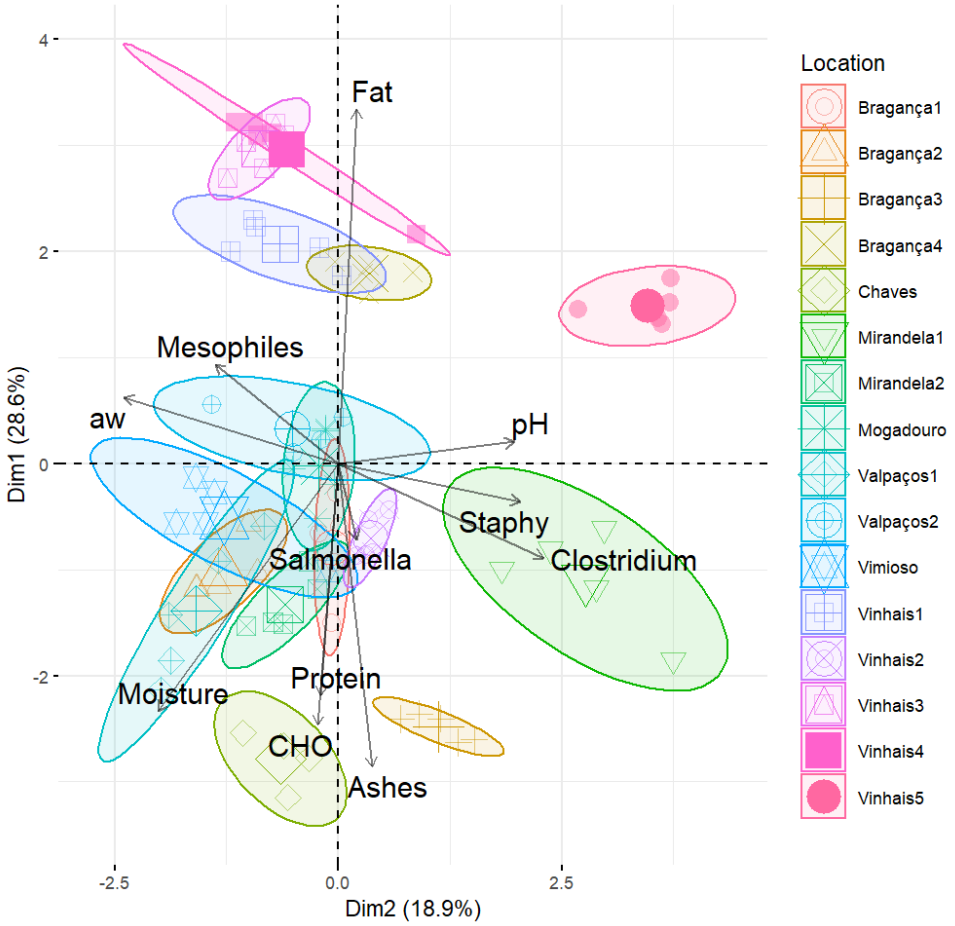

Figure 1: Bi-dimensional map of principal components 1 and 2 of physicochemical and microbiological características of alheira sausages commercialised by artisanal producers of Trás-Os-Montes Portuguese region. Staphy: *S. aureus* counts; Clostridium: presumptive *C. perfringens* counts; CHO: content of carbohydrates; aw: water activity.

Figure 2 evidenced two trends: first, that alheiras of higher water content or water activity tended to have a higher concentration of mesophiles; and second, alheiras of higher pH were associated to higher *S. aureus* counts. A hierarchical clustering analysis allowed the determination of three types of quality for alheiras: (i) mid-levels of acidity, low fat content, high protein content, less fermented and less contaminated (with hygiene indicators microorganisms); (ii) high acidity, high fat content, low protein content, highly fermented and less contaminated; and (iii) low acidity, mid fat content, high protein content, less fermented and more contaminated.

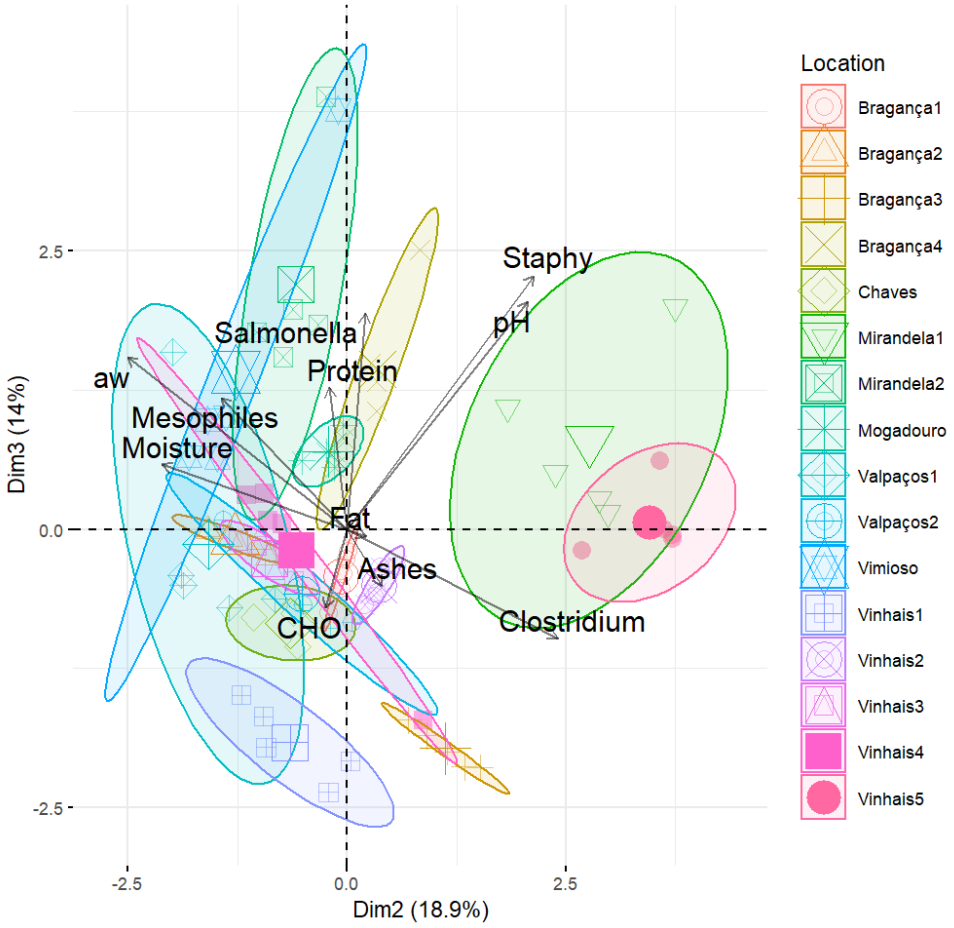

Figure 2: Bi-dimensional map of principal components 2 and 3 of physicochemical and microbiological características of alheira sausages commercialised by artisanal producers of Trás-Os-Montes Portuguese region. Staphy: *S. aureus* counts; Clostridium: presumptive *C. perfringens* counts; CHO: content of carbohydrates; aw: water activity.

In relation to the results from the tracking studies in two alheira artisanal establishments (BRA4 and VIN5), the evolution of the physicochemical properties (pH, aw and moisture content) and the microbiological characteristics (counts of total mesophiles, *S. aureus* and *C. perfringens*) during processing are shown as box plots in Figures 3 and 4, respectively. These box plots evidence the variability between lots within the same artisanal producer

VIN5 produced alheiras that presented lower pH (5.14; 95% CI: 4.97 – 5.32), lower aw (0.9784; 95% CI: 0.9753 – 0.9814) and lower moisture content (40.7%; 95% CI: 39.4 – 41.9%) than those produced by BRA4 (5.22; 95% CI: 5.04 – 5.39; 0.9845; 95% CI: 0.9815 – 0.9875; 46.4%; 95% CI: 45.1 – 47.7); since these two artisanal producers had different a processing duration and different mechanisms of drying. A faulty fermentation process was identified for BRA4 in their lot 3, by which, overall, alheiras did not acidify sufficiently, but they dehydrated too much (Figure 3).

The microbiological profile of the sampled alheiras was also quite different between artisanal producers, and between lots within the same producer (Figure 4). According to the microbiological survey, alheiras presented high levels of mesophiles, which was expected for being a fermented product, and acceptable levels of coliforms, *S. aureus* and *C. perfringens*.

Mesophiles counts in alheiras increased during processing, being higher for the end products from BRA4 (8.62 log CFU/g; 95% CI: 8.27 – 8.97 log CFU/g) than VIN5 (7.28 log CFU/g; 95% CI: 6.93 – 7.63 log CFU/g) (Figure 4). Whereas levels of *E. coli* were below the limit of quantification (<0.70 log CFU/g) in the mid-products and products from both artisanal producers, the total coliforms counts in the alheira product from BRA4 (5.00 log CFU/g; 95% CI: 3.76 – 6.24 log CFU/g) was higher than that of VIN5 (3.32 log CFU/g; 95% CI: 2.08 – 4.55 log CFU/g). As shown in Figure 4, on a batch basis, *S. aureus* was found to either decrease or increase during processing, which is likely to be due to the quality of the fermentation process itself and to the existence of contamination events during mixing or stuffing. Overall, the mean levels of this pathogen remained low in the final product, and did not differ significantly between artisanal producers (3.13 log CFU/g; 95% CI: 2.76 – 3.50 log CFU/g for BRA4 versus 3.00 log CFU/g; 95% CI: 2.63 – 3.37 log CFU/g for VIN5). *C. perfringens* counts slowly increased from batter to end product (Figure 4), yet mean values in the end product were low for both artisanal producers (1.10 log CFU/g; 95% CI: 0.85 – 1.35 log CFU/g for BRA4 versus 1.25 log CFU/g; 95% CI: 1.00 – 1.50 log CFU/g for VIN5).

In addition to the sampling of mid-products and products, ingredients used for the elaboration of alheiras were also microbiologically investigated in the establishments of both artisanal producers, as well as environmental elements in one of the producers. Results are shown in Tables 1 and 2, respectively.

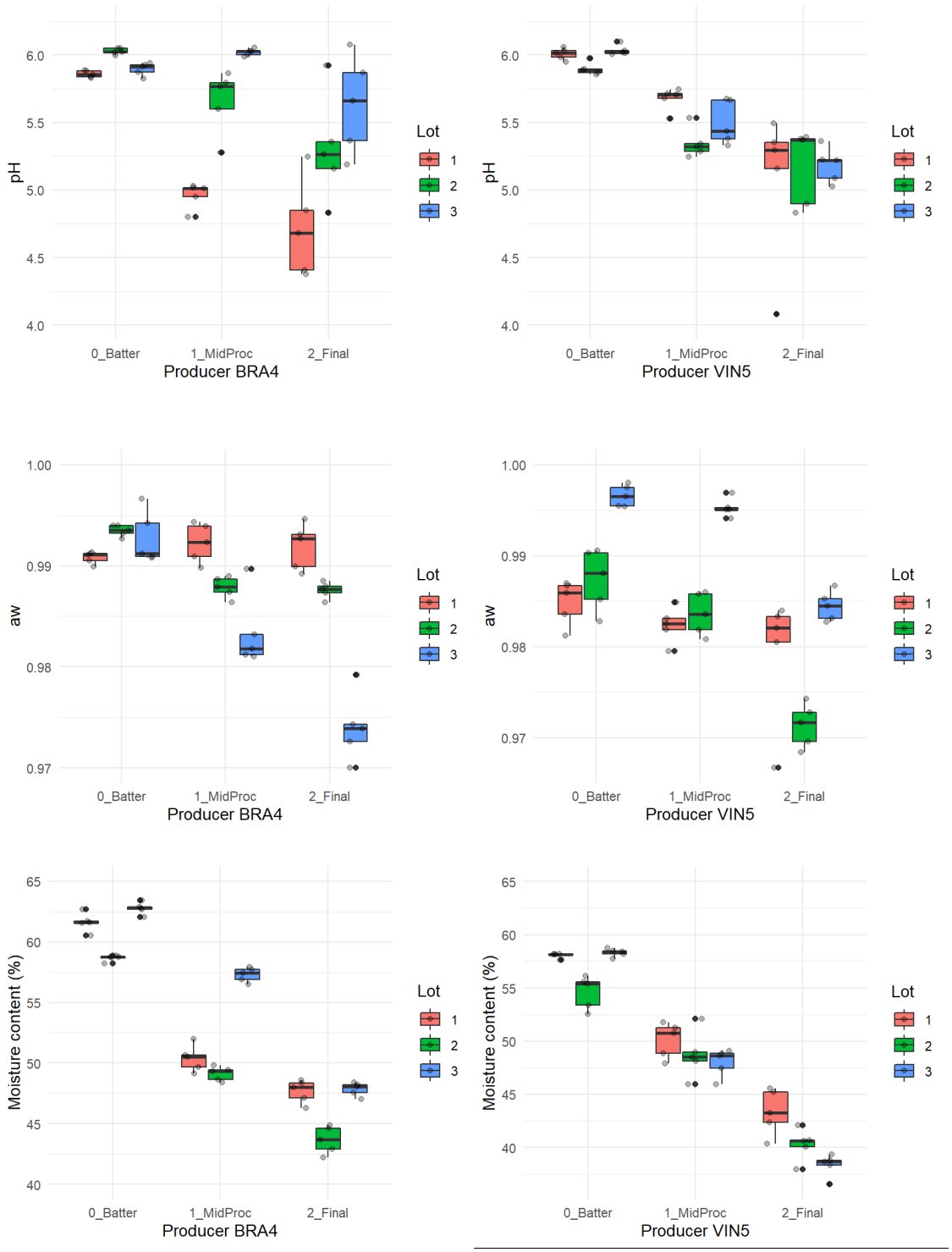

Figure 3: Box plots of the evolution of pH, water activity (aw) and moisture content of alheira by processing stage (batter, mid-processing and final product) showing differences between lots for the two artisanal producers BRA4 and VIN5

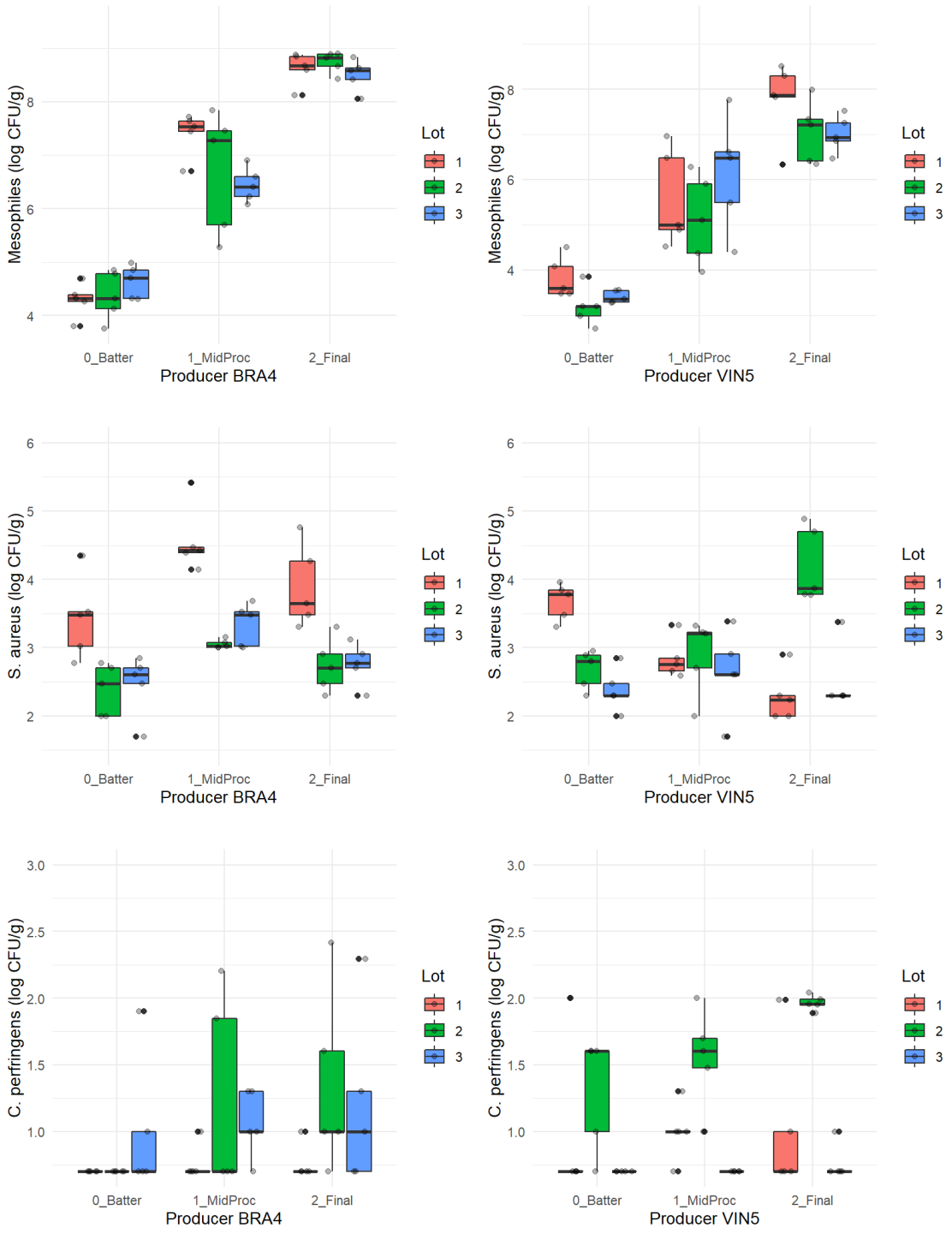

Figure 4: Box plots of the evolution of the counts of total mesophiles, *S. aureus* and *C. perfringens* during processing showing differences between lots for the two producers BRA4 and VIN5

Cooked meats sampled from both the artisanal producers BRA4 and VIN5 presented low mean counts of mesophiles (3.17 and 3.84 log CFU/g), total coliforms (1.57 and 2.13 log CFU/g) and *C. perfringens* (<0.70 log CFU/g). Although raw meat was subject to cooking for over 1 hour, *S. aureus* was still detected in cooked meats (2.98 log CFU/g for BRA4 and 4.03 log CFU/g for VIN5), because being manually shredded, they are susceptible to being contaminated from operators. It was found out that dry paprika was an ingredient that could introduce some contamination in the batter as *S. aureus* and *C. perfringens* were therein detected (Table 1). Casings, despite having being washed and soaked in vinegar, presented moderate levels of mesophilic bacteria (5.67 log CFU/g for BRA4 and 4.91 log CFU/g for VIN5), total coliforms (3.72 log CFU/g for BRA4 and 2.56 log CFU/g for VIN5), *S. aureus* (4.53 log CFU/g for BRA4 and 3.67 log CFU/g for VIN5) and *C. perfringens* (3.26 log CFU/g for BRA4 and 3.19 for VIN5). Thus, casings may represent an important source of contamination if they are not properly sanitised. These results highlighted that the casing washing procedures should be improved by both artisanal producers.

Table 1: Microbial quality of ingredients used in the elaboration of alheiras by artisanal producers. Mean and 95% confidence interval are shown.

Microbial group	BRA4	VIN5
Mesophiles [log CFU/g]		
Washed casings	5.67[a] [4.86 – 6.48]	4.91[a] [4.10 – 5.72]
Cooked meat	3.17[a] [2.36 – 3.98]	3.84[a] [3.03 – 4.65]
Paprika	6.08[a] [5.27 – 6.89]	4.92[b] [4.11 – 5.73]
Total coliforms [log CFU/g]		
Washed casings	3.72[a] [1.08 – 6.35]	2.56[a] [-0.08 – 5.20]
Cooked meat	1.57[a] [-1.07 – 4.21]	2.13[a] [-0.51 – 4.77]
Paprika	3.10[a] [0.46 – 5.74]	2.97[a] [0.33 – 5.61]
E. coli [log CFU/g]		
Washed casings	0.91[a] [0.63 – 1.18]	0.89[a] [0.63 – 1.16]
Cooked meat	<0.70	<0.70
Paprika	<0.70	<0.70
S. aureus [log CFU/g]		
Washed casings	4.53[a] [3.26 – 5.81]	3.67[a] [2.40 – 4.95]
Cooked meat	2.98[a] [1.70 – 4.26]	4.03[a] [2.76 – 4.26]
Paprika	1.70[a] [0.42 – 2.97]	3.55[b] [2.27 – 4.82]
C. perfringens [log CFU/g]		
Washed casings	3.26[a] [1.96 – 4.55]	3.19[a] [1.90 – 4.49]
Cooked meat	<0.70	<0.70
Paprika	1.57[a] [0.27 – 2.86]	1.41[a] [0.11 – 2.90]

[a,b] Different superscript letters in a row indicate significant differences (α=0.05)

According to Table 2, some of the environmental elements swabbed presented levels of hygiene indicators higher than others; namely, the cleaning cloth and prickers and spoons. On the other hand, the working table and meat chopping boards were well sanitised; however the stuffing machine presented a

high level of *S. aureus*. None of the environmental elements investigated tested positive for *Salmonella* spp.

Table 2: Mean levels of hygiene indicator microorganisms in environmental elements sampled in the facilities of the artisanal producer BRA4

More contaminated elements	Cleaning cloth (log CFU/cloth)	Prickers, spoons (log CFU/element)	
Mesophiles	6.05	3.26	
Coliforms	5.62	3.87	
Escherichia coli	2.90	ND	
Staphylococcus aureus	2.60	1.70	
Salmonella spp.	Negative	Negative	
Better sanitised elements	Working table (log CFU/cm^2)	Chopping board (log CFU/cm^2)	Stuffing machine (log CFU/cm^2)
Mesophiles	2.27	1.27	<1.00
Coliforms	1.26	1.21	<1.00
Escherichia coli	ND	ND	ND
Staphylococcus aureus	ND	ND	**5.40**
Salmonella spp.	Negative	Negative	Negative

*ND: not detected

Physicochemical and microbiological surveys of raw milk, recently-pressed cheese, maturing cheese and final product have been completed for four batches of production. All batches of raw milk cheese met the food safety criterion for *Listeria monocytogenes* in ready-to-eat foods.

3. Microbiological and physicochemical parameters of raw materials, intermediate and final products as well as environmental data from processing facilities producing Salchichón sausage and goat raw milk cheese (UCO)

The surveys have been completed in four processing industries of cheese and cured meat. The products selected were artisanal raw milk goat cheese and Iberian raw-cured sausage (Salchichón). A longitudinal study was carried out with a total of 12 sampling visits (February – December 2020), corresponding to 3 sampling occasions per company. Environmental sampling consisted of at least 10 food and non-food contact surfaces in 6 different areas during each visit.

Regarding cheese companies, surfaces were taken from the raw milk tanks, storage boxes, cutters and cutting boards, fermentation tanks, ripening cameras, moulds, scales, food operators' hands and conveyors belts. For meat industries, the surfaces analysed corresponded to the hopper and exit channel of the filling machine and mincers, mixer blades, manufacture trolleys, storage, and ripening-drying room

racks, cutting saws, scales and food operators' hands. Five samples of raw material (raw goat milk or meat batter) and five samples of final products belonging to the same batch (raw milk cheeses and salchichón) were collected in each visit.

The results derived from samplings in the dairy companies, namely companies "A" and "B", are shown in Table 3. For raw milk samples, averages microbial counts were above 5.45 for TMA; 5.45 for LAB; 4.29 log CFU/mL for total coliforms; and 4.74 log CFU/mL for moulds and yeasts, being higher in samples from company B. Raw milk cheeses samples yielded LAB counts greater than 7 log CFU/g in all cases. The cheeses from company A presented pH values within the range 4.68-5.21, while pH values of cheeses from company B varied from 4.89 to 5.49. Regarding aw values, measures from 0.897 to 0.955 were observed in cheeses from company A, and values ranging from 0.917 to 0.969 in cheeses from company B. Differences between microbial counts and physicochemical parameters of both raw materials and end products may be associated to a series of factors, including microbiological quality of raw materials, ripening times and other factors related to the production processes.

Table 3: Summary of the microbiological survey performed in dairy companies (log CFU/mL for raw milk and log CFU/g for cheeses).

Company	Sample	Total mesophilic aerobic	Total coliforms	Entero-bacteria	Positive coagulase staphylo-cocci	Lactic acid bacteria	Moulds and yeasts
A	Raw milk	6.04 ± 1.93	4.93 ± 1.25	3.47 ± 0.01*	4.90 ± 0.45	6.80 ± 1.29	5.16 ± 1.10
	Cheese	8.03 ± 0.91	2.98 ± 1.27	3.47 ± 0.01*	4.43 ± 0.28*	8.28 ± 1.18	4.33 ± 1.11
B	Raw milk	5.45 ± 1.64	4.29 ± 0.63	3.04 ± 0.64*	3.62 ± 0.86	5.45 ± 1.84	4.74 ± 1.04
	Cheese	8.55 ± 1.47	3.63 ± 0.65*	3.67 ± 0.30*	4.57 ± 0.88	8.86 ± 1.82	5.08 ± 0.74*

Mean ± standard deviation from data obtained after evaluation of three different batches is reported.
*Not detected in all samples analysed.

Results of the microbiological survey performed with raw meat samples and salchichón sausages from two different companies, namely "C" and "D", are shown in Table 4. Average counts of total coliform and *Enterobacteriaceae* in raw meat samples were higher than 3 log cfu/g. For salchichón, the average total coliform counts were 2.73 (company C) and 3.15 (company D), while for enterobacteria average

counts of 4.06 and 2.82 log CFU/g were observed in samples from company C and D, respectively. Salchichón samples from company D presented pH values ranging from 5.77 to 6.76, and aw from 0.926 to 0.938. In contrast, the final products from company C presented less variability between batches with a range of pH from 5.08 to 5.37 and 0.870 to 0.897 for aw. Differences between microbial counts and physicochemical parameters of both raw materials and end products may be associated to a series of factors, including microbiological quality of raw materials, ripening times and other factors related to the production processes.

Table 4: Summary of the microbiological survey performed in meat companies (log CFU/g).

Company	Sample	Total mesophilic aerobic	Total coliform	Entero-bacteria	Positive coagulase staphy-lococci	Lactic acid bacteria	Moulds and yeasts
C	Raw meat	5.19 ± 1.75	4.44 ± 0.78*	3.55 ± 0.33*	3.98 ± 0.97*	4.25 ± 1.65	3.38 ± 1.75
	Salchichón	8.14 ± 1.83	3.15 ± 0.77	4.06 ± 0.33	ND	7.65 ± 2.12	6.16 ± 1.71
D	Raw meat	5.37 ± 1.52	3.68 ± 0.80	3.56 ± 0.63*	3.67 ± 0.37	5.47 ± 1.65	4.66 ± 0.66
	Salchichón	8.35 ± 1.40	2.73 ± 0.85*	2.82 ± 0.89*	3.48 ± 0.44	8.61 ± 1.47	3.99 ± 0.87

Mean ± standard deviation from data obtained after evaluation of three different batches is reported.
*Not detected in all samples analysed.
ND = Not detected.

Environmental samples were also collected during visits at each factory: a total of 12 food contact surfaces and air samples were collected at different points in the facilities. Food contact surfaces were tested for the presence of *L. monocytogenes* and enumeration of TMA, positive coagulase staphylococci and enterobacteria, while the air samples were evaluated for enumeration of TMA and yeasts and moulds. The results of food contact surfaces and air samples obtained in dairy companies are shown in Tables 5 and 6, respectively. The highest TMA concentration results from the different food-contact surfaces were obtained for conveyors and cutting board from the company A. In general, the company B presented higher concentrations of positive coagulase staphylococci and enterobacteria than the company A, reaching results of 600 and 228 CFU/cm^2, respectively.

Table 5: Microbiological results of food contact surfaces from dairy companies (CFU/cm^2).

Company	Surface	Enterobacteria	Positive coagulase staphylococci	Total mesophilic aerobic
A	Raw milk tank	<1	<1 - 5.6	<10 - 4
	Fermentation vat	<1	<10	<10 - 334
	Conveyor's belt	<1->30	<10	<10 - 30000
	Clean moulds	0.12 - <1	<10	4-85
	Slicer blades	0.28 – 2.4	1.6 -32	37- >300
	Cutting board	<1	<1-52	>300 - 28000
	Storage boxes	<1	<10	<10 - >300
	Manipulator hands	0.28 - <1	1.6 - 208	<10 - 227
B	Raw milk tank	<1	<1	12 - 154
	Fermentation vat	<1	<1 – 88	40 - 60
	Clean moulds	<1 - 6.56	<1	4 - <100
	Slicer blades and Cutting board	<1 – 228	<1 – 600	172 - 1180
	Storage boxes	<1	<1 – 360	36 - 1600
	Manipulator hands	0.4- <1	<1 – 60	6 - 38

Table 6: Microbiological results of air samples from dairy companies (CFU/m^3)

Company	Air sample	Total mesophilic aerobic	Moulds and yeasts
A	Milk reception room	15 – 130	25 - >300
	Brine room	ND – 38	89 - >300
	Oreo chamber	ND – 50	11 - 17700
	Ripening chamber	ND – 5	3 - >300
	Packing room	21 – 40	11- >300
B	Production plant	8 - >1310	10 - 1307
	Oreo chamber	7 – 28	12 - 770
	Ripening chamber	68 – 270	16 - >1310
	Packing room	30 – 203	19 - >1310

For environmental samples, higher counts of moulds and yeasts were found compared to TMA, reaching concentrations of 1.77×10^4 CFU/m^3 in the airing chamber of the company A. The highest total TMA counts were found in company B. The results of food contact surfaces and air samples obtained in meat companies are shown in Tables 7 and 8, respectively. In general, the food contact surfaces of company C presented higher loads of all the microbial groups evaluated compared to company D. Higher counts of TMA were found in air samples from meat companies compared to moulds and yeasts (Table 8). The highest TMA counts were found in the packaging and cutting rooms from both companies C and D.

Table 7: Microbiological results of food contact surfaces from meat companies (CFU/cm^2)

Company	Surface	Enterobacteria	Positive coagulase staphylococci	Total mesophilic aerobic
C	Cutting saw	<1 - >120	<1 - >2500	38 - 23040
	Mincer	<1 - >120	<1 - 2272	2100 - 46600
	Mixer	<1 - >120	<1 - >1200	16 - >12000
	Stuffer	<1 – >120	<1 - >2500	106- 120000
	Ripening room	<1 - >120	<1 - >2500	153 - 13000
	Packing room	<1 - >120	<1 – 52	<1 - 7900
	Manipulator hand	<1 - >120	<1 – 180	28 -416
D	Cutting saw	<1 - >120	<1 – 24	<10 - 540
	Mincer	<1	<10	<10 - 210
	Mixer	<1 – 5	<10	<10 – 40
	Stuffer	<1	<10	24 - <100
	Trolley	<1	<10	<10 - 120
	Ripening room	<1 -2	<1 ->300	<10 - >12000
	Packing room	<1	<1 - >300	4 -2260
	Manipulator hand	<1 -18	12 -260	54 -200
	Storage box	<1 – 4,8	<1 – 16	85 - >300

Table 8: Microbiological results of air samples from meat companies (CFU/m^3)

Company	Air sample	Total mesophilic aerobic	Moulds and yeasts
C	Meat raw material storage room	6 - 56	6 - 12
	Production room	47 – 64	27 – 59
	Ripening room	2 – 10	4 – 21.5
	Packaging room	30 – 525	18 - 31
	Cutting room	17 - 153	18 - 32
D	Meat raw material storage room	21 – 233	ND - 7
	Production room	34 – 230	15 -45
	Ripening room	5.5 – 140	2 - 110
	Packaging room	72 – 582	6 – 113
	Cutting room	77 – 298	13 - 88

ND: Not detected.

4. Microbiological and physicochemical parameters of raw materials, intermediate and final products as well as environmental data from processing facilities producing Katiki cheese and Noumboulo sausage (AUA)

Samples of the two targeted products (Katiki cheese and Noumboulo sausage) were collected and analysed microbiologically and physicochemically. More specifically, samples were collected from 3 independent producers in order to better characterise the two products. Then, samples were collected only from the 2 artisanal food companies stated in Task 2.1. Triplicate product samples (final products) were further collected at 2 independent time intervals and analysed within 24 h. From each sample of 200 g, sub-samples of 25 g were used for the microbiological analysis of technological and spoilage flora, as well as the enrichment for detecting *L. monocytogenes* and *Salmonella* spp.

The results of the microbiological analysis of technological and spoilage flora as well as the pH and water activity values of Katiki cheese are shown in Table 9. A significant variation on the total microbiological flora between the products of the two different producers examined was observed. Specifically, regardless sampling time, Product 2 was characterised by higher microbial loads compared to Product 1. Lactic acid bacteria were the dominant species in both Katiki products tested, followed by populations of yeasts and moulds. All Katiki cheese samples were enriched and tested for the presence of pathogens (*L.*

monocytogenes, *Salmonella* spp. and *E. coli* O157:H7). None of the pathogens were detected in any of the samples. The latter is in accordance with the physicochemical results of the products, as the pH and aw values of both products tested meet the food safety criterion set by Regulation (EC) No 2073/2005 for '*Listeria monocytogenes* in ready-to-eat foods unable to support the growth of *Listeria monocytogenes* (Products with pH ≤ 4.4 or aw ≤ 0.92, products with pH ≤ 5.0 and aw ≤ 0.94).

Table 10 shows the results of the microbiological analysis of technological and spoilage flora as well as the pH and water activity values of Noumbulo sausages. The total microbiological flora as well as the physicochemical characteristics of the three products varied significantly. Specifically, Product 1 was characterised by higher microbial loads compared to the other two products. Therefore, this product (Product 1) was selected to be further examined. Regardless sampling time, lactic acid bacteria were the dominant species in all of producers 1 Noumbulo sausages tested, followed by populations of yeasts and moulds.

None of the examined pathogens (*L. monocytogenes*, *Salmonella* spp. & *E. coli* O157:H7) were detected in any of the samples tested. In contrast to Katiki cheese (Table 9), the pH and aw values of the Noumbulo sausage tested (Product 1) marginally meet the food safety criterion set by Regulation (EC) No 2073/2005 for '*Listeria monocytogenes* in ready-to-eat foods unable to support the growth of *Listeria monocytogenes* (Products with pH ≤ 4.4 or aw ≤ 0.92, products with pH ≤ 5.0 and aw ≤ 0.94).

Table 9: Counts (Mean log CFU/g ± sd) of the observed microbial association and the physicochemical characteristics (pH & aw values ± sd) of Katiki cheese samples produced by different producers.

Visit	Producer	TVC	Lactic Acid Bacteria	Yeasts/Moulds	Entero-bacteriaceae	Coagulase positive *Staphylococci*	pH	a_w
V1	**P1**	5.26 ±0.00	5.20 ±0.00	5.01 ±0.00	<1	<2	4.35 ±0.03	0.943 ±0.03
	P2	8.10 ±0.01	7.59 ±0.01	6.41 ±0.01	<1	<2	4.27 ±0.07	0.930 ±0.07
V2	**P1**	6.26 ±0.34	6.29 ±0.38	5.54 ±0.04	<1	<2	4.30 ±0.05	0.947 ±0.05
	P2	8.38 ±0.23	8.15 ±0.04	5.49 ±0.35	<1	<2	4.15 ±0.06	0.937 ±0.05
V3	**P2**	7.81 ±0.02	7.56 ±0.08	5.66 ±0.03	<1	<2	4.39 ±0.03	0.946 ±0.03

Table 10: Counts (Mean log CFU/g ± sd) of the observed microbial association and the physicochemical characteristics (pH & aw ± sd) of Noumbulo sausage samples produced by different producers.

Visit	Producer	TVC	Lactic acid bacteria	Yeasts/Moulds	Entero-bacteriaceae	Coagulase positive *Staphylococci*	pH	a_w
V1	P1	7.16 ±0.00	7.09 ±0.00	4.58 ±0.00	<1	<2	5.27 ±0.00	0.873 ±0.00
	P2	5.13 ±0.00	5.08 ±0.00	4.08 ±0.00	<1	<2	5.33 ±0.00	0.878 ±0.01
	P3	4.87 ±0.00	4.49 ±0.00	2.95 ±0.00	<1	<2	5.44 ±0.00	0.853 ±0.00
V2	P1	6.34 ±0.04	6.42 ±0.08	5.36 ±0.05	<1	<2	5.12± 0.05	0.949 ±0.03
V3	P1	6.70 ±0.92	6.63 ±1.11	5.09 ±0.94	<1	<2	5.26 ±0.06	0.953 ±0.04

5. Microbiological and physicochemical parameters of raw materials, intermediate and final products as well as environmental data from processing facilities producing Merguez sausage and Jben cheese (UIZ)

The analyses of samples of Merguez sausage and Jben cheese were conducted from October 2019 until October 2020. Due to the interruption of production of Jben cheese due to COVID-19 outbreak, the tracking surveys of Jben cheese was resumed from August 2020 and ended in December 2020 (farrowing of the goats of Ait Momo cooperative).

Tables 11 and 12 summarise the microbiological profile of the Moroccan traditional products. Approximately 80% of samples of Merguez sausage and 64% in Jben goat cheese contained bacteria above the maximum limits established by the Moroccan regulatory standards for meat and dairy products. The presence of pathogens was evaluated in both type of Moroccan products using ISO standardized methods for searching *L. monocytogenes*, *Salmonella* and *S. aureus*. Many dairy and meat samples were positives for the presence of at least one pathogen. From Merguez sausages, *Salmonella* spp., *L. monocytogenes*, and coagulase-positive staphylococci were isolated in 6, 13 and 1 sample, respectively, out of 25 samples. Out of 14 Jben cheese samples, these pathogens were recovered from 4, 8 and 8 samples respectively. Suspected pathogens isolated from both traditional products were confirmed and kept for use in WP5.

Table 11: Microbiological quality (log CFU/g) of some samples of Moroccan traditional products and their pH

Batch	TVC	TC	FC	*E. coli*	YM	LAB (M17)	LAB (MRS)	Staph	pH
Merguez 01	8.30	6.27	4.37	4.57	8.21	7.09	8.11	6.24	5.82
Merguez 02	8.72	6.37	5.37	5.48	8.01	7.73	7.34	6.36	6.19
Merguez 03	8.14	6.75	5.32	5.75	8.01	8.71	7.47	6.19	6.84
Jben 01	5.45	Nd	Nd	Nd	7.18	7.48	5.48	3.04	4.6
Jben 02	7.38	2	2	2	3.60	7.96	6.08	4.93	4.2
Jben 03	8.95	5.93	5.91	2.18	7.96	9.26	8.04	3.46	4.0

TVC: Total viable counts; TC: Total coliforms; FC: Faecal coliforms; YM: yeasts and moulds; LAB: lactic acid bacteria; Staph: *Staphylococcus aureus*; Nd: Not detected

Table 12: Summary of microbiological profile of traditional products (log CFU/g)

	Mean value ± SD	Min. value	Max. value
Total aerobic flora	7.88 ± 0.70	5.71	8.72
Total coliforms	5.12 ± 0.91	2.30	6.75
Fecal coliforms	4.61 ± 0.73	2.69	5.78
E. coli	4.94 ± 0.68	3.51	**6.01**
Yeasts and moulds	7.90 ± 0.58	6.69	**8.78**
Staphylococci	6.12 ± 0.63	5.00	**7.62**

6. Microbiological and physicochemical parameters of raw materials, intermediate and final products as well as environmental data from processing facilities producing Lben milk, dried Merguez sausage and sheep meat Kaddid (ISBST/UMA)

Lben milk, dried Merguez sausage and sheep meat Kaddid are among the oldest ethnic traditional products in Tunisia. For each of the three artisanal food products, samples of raw materials, intermediate and final products were analyzed for physicochemical properties and microbiological parameters. The results presented in Tables 13-14 indicate that the environment and the different surface points of sheep meat Kaddid show low contamination. At the end of this work, the results of the microbiological analyses revealed the total absence of total coliforms, fecal coliforms and staphylococci. The degree of contamination of surfaces changes from one point to another. However, the surface of the cutting board has the highest degree of contamination since it contains the highest load.

Table 13: Microbiological surface analysis (CFU/100 cm²)

Environ. element	Cutting board	Worktop 1	Worktop 2	Worktop 3	Knife
TVC	125	65	38	40	101
Staphylococci	0	0	0	0	0
Yeasts and molds	41	40	62	28	16
Faecal coliforms	0	0	0	0	0
Total coliforms	0	0	0	0	0

Table 14: Results of the environmental analysis (CFU/m³)

Environmental scan	Entrance to the butcher's shop	Near equipment	Near equipment	In front of the window
Yeasts and molds	47	52	24	49
FAMT	91	90	89	76

The second part of the work consisted in carrying out a physicochemical (Table 15) and microbiological (Table 16) characterization of fresh meat, salted and spicy meat and dried meat. All analyses were repeated three times. In this context, the physicochemical analyses considered as the basic analyzes: pH, moisture, water activity (aw), ash content, lipid, chloride, water content in protein and TBVN.

Table 15: Physicochemical parameters (FM: Fresh meat; SSM: spicy salted meat; DM: dried meat;* g/100g de MF ; **mg N2/100g)

Parameter	FM	SSM	DM
pH	5.52 ± 0.01	5.48 ± 0.01	5.33 ± 0.01
Aw	0.895 ± 0.001	0.82 ± 0.00	0.6 ± 0.01
moisture	74.36 ± 0.33	67.26 ± 1.3	18.88 ± 0.7
Ash content	1.7 ± 0.38	22.21 ± 0.6	56.62 ± 1.8
Chloride	1.02 ± 0.53	4.6 ± 1.3	4.71 ± 0.2
Lipid	7.3 ± 0.5	3.49 ± 0.3	1.60 ± 0.14
TVBN	12.36 ± 0.4	15.26 ± 0.4	10.08 ± 2.7

Table 16: Microbiological parameters (log CFU/g)

Stage	TVC	Y M	TC	FC	LB	ST	LM	SAL
Before salting	2.85 ± 0.3	3.36 ± 0.7	2.29 ± 0.5	0	1.73 ± 0.19	3.29 ± 0.04	0	0
After salting	2.82 ± 0.5	3.33 ± 0.28	2.65 ± 0.32	0	2.15 ± 0.16	3.46 ± 0.5	0	0
After drying	2.6 ± 0.2	3.08 ± 0.11	1.87 ± 0.36	0	1.79 ± 0.29	2.82 ± 0.08	0	0

Total Viable Counts; Y M: yeasts and molds; TC: total coliforms; FC: fecal coliforms; LB: Lactic acid bacteria; St: staphylococci; LM: *Listeria monocytogenes*; Sal: *Salmonella*.

Results showed that total viable counts and yeasts and molds counts were under 100 CFU/m^3 recommended by the BPPH (200 CFU/m^3) (Osimani et al., 2014). These points are rated non-critical and are not in contact with food handling areas. The results confirm that all the zones belong to class C and therefore comply with the BPF standard. Thus, the quality of meat products is ensured.

The results of the Table 16 show that the drying step has significantly reduced ($p < 0.05$) the initial microbial load of fresh meats, this result is due to the effect of reducing the activity of water. Chabbouh et al. (2013) found that spicing step have significantly affected the microbiological quality of a Tunisian Kaddid. Statistical results showed that spicing and slating steps are critical points of the Kaddid process since there is no significant reduction ($p > 0.05$) of the number of microorganisms after these two steps.

Sampling of **fermented beef sausage** was performed at 5 different stages of sausage production, Samples, namely raw meat, batter, stuffed sausage, semi dry sausage, dried sausage, and stored sausage were collected. In addition, samples were collected from equipment surfaces, machines, and spices mix. The samples of spices were taken just prior to use for production. Samples of batters were taken from the cutter before stuffing. Sausage samples were taken just after each process (stuffing, drying, and storage). All meat, batter and sausage samples were taken from the same runs. Samples from the surfaces of equipment and tables were taken at the before the start of the workday after cleaning and sanitising. Results concerning the total viable counts, *Enterobacteriaceae*, LAB and yeasts and molds during the preparation of dry sausages are reported in Figure 5.

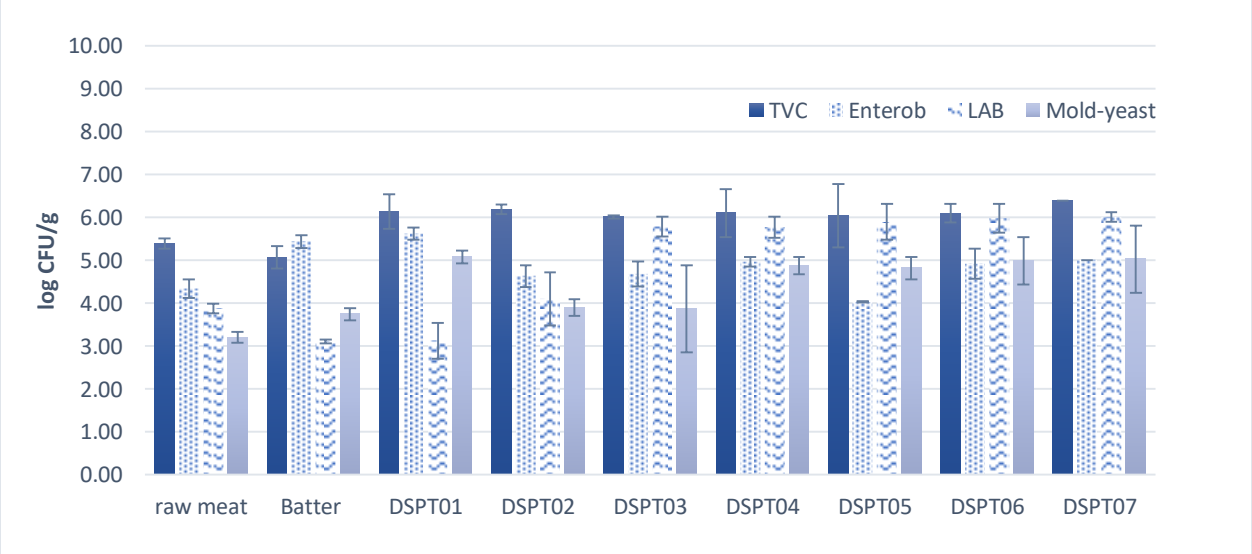

Figure 5: Evolution of microbial population during the preparation of dry sausages
***DSPT01: raw sausages; DSPT02, DSPT03, DSPT04: sausages during natural drying; DSPT05, DSPT06, DSPT07: sausages during storage.**

The microbiological analysis revealed significant differences between samples at different step of the preparation of the sausages. The differences between raw meat, batter and raw sausages could be related mainly to the addition of spices and the use of natural casings in the stuffing step. Indeed, the level of *Enterobacteriaceae* and TVC increased significantly from 4.34 ± 0.22 log CFU/g and 5.39 ± 0.12 log CFU/ g in raw meat to 5.62 ±0.14 log CFU/g and 6.19 ± 0.40 log CFU/g in spiced sausages, respectively. The LAB count was 3.13 ± 0.42 log CFU/g for raw sausages against 5.9 ± 0.33 log CFU/g for dried ones. However, the maximum level was noted after two weeks of storage (DSPT07) suggesting that ripening phase is still active despite there is no significant difference of aw values during this phase (Figure 6). Many studies observed a slight decrease of LAB during ripening probably due to the decrease of fermentable carbohydrates (Lorenzo & Franco, 2012) and the decrease of water activity (Spaziani, Del Torre & Stecchini, 2009).

LAB was the dominant microflora at the end of the drying; this result confirms the good adaptation of LAB to the meat environment and their faster growth rates during this natural fermentation of sausages, which is also correlated to the significant decreases of pH values from 5.87±0.15 to 5.51±0.01. At the beginning of the drying step, the number of yeasts and molds varied significantly from about 5.08±0.15 log CFU/g to reach about 4.87±0.55 log CFU/g at the end. Yeasts and molds were affected by the drying conditions and parameters. This variation can be due to the competition among LAB, yeasts, and molds (Al-ahmad et al., 2014). Furthermore, during storage, the counts increased gradually in all samples as expected due to the initial microbial load. In overall processing, microorganism counts were within the values found in Essid et al. (2018). Moreover, these levels were not harmful for human health and the microbial load of the final product was within critical limits.

As for the pathogens, both *S. aureus* and *L. monocytogenes* were found in the raw meat (Table 17) with high levels of TVC, *Enterobacteriaceae*, yeast and molds the meat was considered as in poor quality. As observed for the pathogens, *S. aureus* levels decreased during the drying process. Although this pathogen is considered a biological hazard in dry meat products due to its potential ability to grow in low aw products, the results of the present study indicate that this pathogen is unable to grow or survive during the manufacturing process. However, only *L. monocytogenes* survived during the whole processing to the final product.

Table 17: Microbiological results for pathogens of the artisanal dry Merguez (in 25 g samples)

Sample	*Staphylococcus aureus*	*Listeria monocytogenes*
Raw meat	Presence	Presence
Batter	Presence	Presence
Raw Sausages	Absence	Presence
Dried sausages	Absence	Presence
Dried sausage 8 days old	Absence	Presence

Microbial counts of equipment surfaces (cutting board, knife, hashing machine and the traditional filler machine) were just below the standard limit value. The levels varied from 6.85 log CFU/cm^2 to 3.29 log CFU/cm^2 and from 3.83 log CFU/cm^2 to 2.69 log CFU/cm^2 for TVC and *Enterobacteriaceae*, respectively. Generally, microbial quality of food and cleaning and sanitation program in the plant is associated with microbial load of equipment surfaces. In alignment with the results found, high microbial count causes an increase in the microbial count of the product at processing stages.

Figure 6 shows the results of the water content and water activity along the different processing stages of dry, naturally fermented sausage. The moisture content in all samples significantly ($p<0.5$) decreased (ranged from 77.29% to 12.5%) upon the storage period.

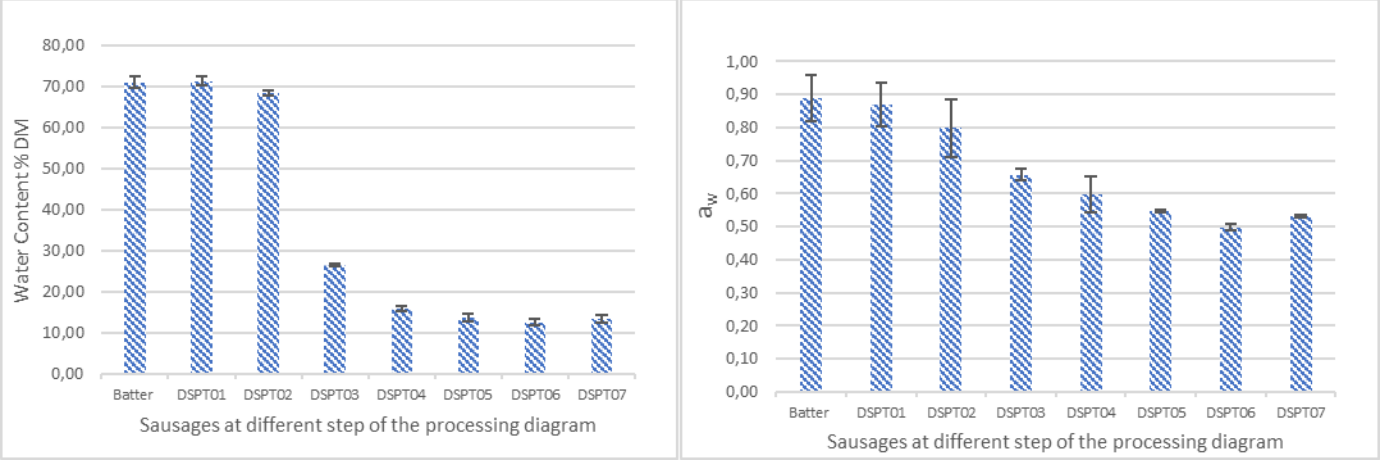

Figure 6: Water content and water activity during drying and storage step of dry merguez production.

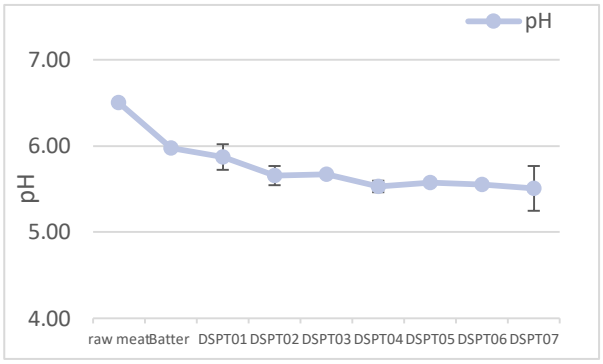

Figure 7: Evolution of pH during different steps of dry sausages processing

The higher retention of moisture registered were in batter and the raw sausages before the start of drying process and hence, their higher mean aw were respectively (0.89 ± 0.07) and (0.87 ± 0.07). The significant decrease of both parameters was observed at the middle of the natural drying process. This increase is also correlated with the increase of LAB, and thus with a decrease of pH values from 5.98 ± 0.01 to 5.57 ± 0.01 as reported in the Figure 7.

The rapid pH drop in the sausages recoded in drying step is necessary early in fermentation in order to inhibit the proliferation or the development of *S. aureus* (Gonzales-Barron et al., 2015) which was in concordance with our results in pathogens evaluation. The aw value of the final product stabilised and reached 0.53 ± 0.01 after one week of storage. Moreover, fat and ash contents of the final dry product were respectively 21.32 ± 1.28% dw and 3.67 ± 0.04% dw.

As for colour, the colour development was significantly affected by the different processing stages ($P < 0.05$). The quality parameters Lightness (L*), redness (a*) values underwent a decrease through the drying and storage periods of different samples studied (Figure 8). However, yellowness (b*) was the only colour parameter that was not modified ($p > 0.05$) by the dehydration or drying process. Indeed, for L* values, a decrease was observed during ripening as sausage became darker due to weight loss (Olivares et al. 2010). The same observation for a* values was noted; a decrease was observed during the drying, followed by a slight increase.

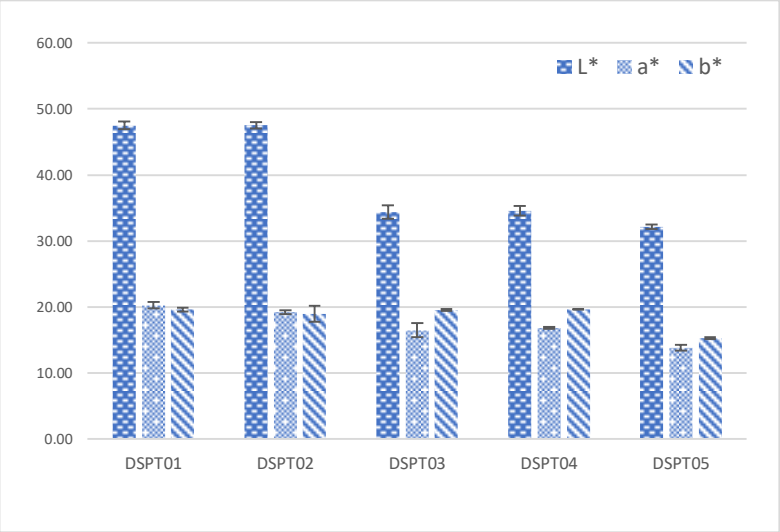

Figure 8: Evolution of L*, a* and b* color parameters along the different processing stages of Tunisian dry sausage

The variation of the parameter color a* during ripening of dry fermented sausages is linked to the formation of a small amount of nitroso myoglobin pigment because of the production of lactic acid (Perez-Alvarez, Sayes-Barbare, Fernandez-Lopez, & Aranda-Catala, 1999). Our results agree with those found by Essid et al. (2018), Casaburi et al. (2007) and Olivares et al. (2010). However, yellowness (b*) was the only color parameter that was not modified ($p > 0.05$) by the dehydration or drying process. Similar results were reported by Aleson-Carbonell, et al. (2004).

The quality of the final product is closely related to the quality of raw material (meat, spices, and casings), the ripening that takes place during drying and finally the storage conditions. This process of drying, which confers to the product its, firmness, color, and flavor, characterised by a complex interaction of chemical and physical reactions associated with the microbiological development of the mainly the batter flora.

According to the results, processing, handling, and storing conditions may influence the microbiological and physicochemical quality as the raw material, followed by work surfaces and equipment. The presence of pathogens *S. aureus* and *L. monocytogenes* were detected in the raw meat, mid and final product; and is is considered a hazard in the process, and suggest the application of good manufacturing procedures in processing facilities together with selection of raw materials of good quality. In addition to high levels of

Enterobacteriaceae counted in different samples; the fermentation/ripening phase was not controlled even with relatively stable aw at the final product.

Lben is a traditional fermented milk, which plays an important role in daily diet of Arabic countries which is traditionally made by spontaneous fermentation of raw milk at ambient temperature for up to 24 h after which, it is stirred and ready for consumption. Manufacturing of this dairy product includes a multitude of complex enzymatic and chemical reactions having technological consequences mainly dictated by lactic acid bacteria (LAB) (Sarhir et al., 2019). The potential role of stakeholders influencing Lben quality was explored. Ten small producers were randomly selected along the milk chains and systematically considered for our study. Samples were collected into sterile bottles at each stage of the processing diagram. Physicochemical parameters: Fat, SNF, protein, lactose, salts were measured by ultrasound with the Lacto scan Ultrasonic Milk Analyzer (Milkotronic, Ltd, Bulgaria) according to the directions of the manufacturer, pH was measured by a digital pH meter, water activity with water activity meter (LabMaster-aw, novasina) and acidity was analysed according to the Afnor official methods. Viscosity was measured by Brookfield viscosimeter; and Whiteness index was calculated after measuring color parameter L, a* and b* according to formula presenting below:

$$W = 100-[(100-L)\,2 + (a2 + b2)]1/2$$

The environment characteristics: temperature and residual humidity are measured at the time of sampling with a hygro-thermometer. One producer was chosen for sampling at different steps of production: sample of raw milk was collected at day 0, and on the next day after about 24 h curdled milk before churning and skimmed fermented milk were sampled. Swabbing procedure was adopted to control stainless steel milk container, churning machine, container for lben storage, plastic bag for packaging and air control by sedimentation.

General aspects of hygiene and good manufacturing practices were lacking from collection of milk to selling of final product; for example, hand-washing and cleaning were not observed, storage and selling were performed in improper cleaning containers. Despite all, the study participants used mechanical churning to separate fermented milk from butter and mostly the same manipulator did reception and processing which can reduce cross contamination from manipulator. Approximately most of vendors declared to use spontaneous fermentation practices depending on environmental conditions. During the summer season, the warm room temperature (~30˚C) sustained the spontaneous fermentation and delivered lben in about 20-24 h. In the rainy season, the room temperature was relatively low and

fermentation took much longer, so some of them was initiated via slow heating of milk to accelerate fermentation time.

Regarding the effect of environment conditions, T° and RH were controlled for each producer chosen for sampling and represented by boxplot graphics (Figure 9). We can observe that for the two parameters, the variation was high for both time of sampling for raw milk and after its processing. A high variation between producers was observed in the step of final fermented milk. From that, we can state that the quality of fermented milk can be different under the effect of environmental conditions which are generally not controlled by producers.

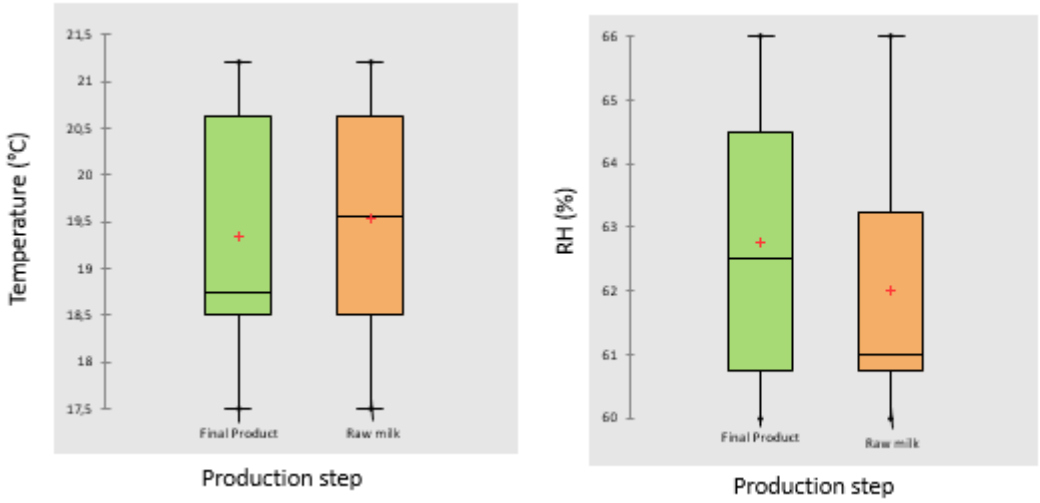

Figure 9. Evolution of environmental temperature and relative humidity in Lben throughout production showing variability within and between artisanal producers.

Sampling results for 2 batches are presented in Table 18. Data showed that pH decrease significantly under the effect of fermentation, and acidity values increase with no difference in water activity between raw, intermediate and final product. Fat, SNF protein and lactose content were also decreased after fermentation and separation of lben from raw butter for that fat content. These modifications in composition under spontaneous fermentation were demonstrated in several studies (Samet bali et al, 2010; Ben karroum et al, 2004).

The initial bacterial load of lactic acid bacteria, yeasts and molds increased while passing from raw (1.08E+07 CFU/ml for LAB and 2.86E+06 CFU/ml for yeasts), curdled milk (2.84E+08 CFU/ml for LAB and 5.55E+06 CFU/ml for yeasts) and lben as final product (2.72E+08 CFU/ml for LAB and 6.80E+06 CFU/ml for yeasts).

Table 18: Physicochemical and microbiological parameters in different steps of milk processing

Lot	Stage	Tempera-ture (ºC)	RH (%)	Type of sample	Units	TVC	Coliforms	S. aureus	Yeast &moulds	Lactic bacteria	Listeria	pH	aw	lactic acid (°D)	DM(%)	Fat (%)	SNF (%)	Density	Lactose(%)	Salts (%)	Protein (%)	Wihtness index	Viscosity (Cp)
Batch 1	Milk reception	20,5	63	stainless steel milk container	CFU/Swab	2,50E+02	1,68E+02		NA	NA	NA												
		20,5	63	Cow milk	CFU/ml	2,69E+05	1,55E+05	0,00E+00	2,86E+06	1,08E+07	Pos (<10 ^2)	6,667	0,974	15,7	11,959	4,52	8,43	27,815	4,57	0,75	3,515	73,352	26
	After fermentation	19,5	65	churning machine	CFU/Swab	1,37E+02	3,30E+01	NA	NA	NA	NA												
		19,5	65	curdled milk after fermentation (before churning)	CFU/ml	2,70E+08	8,70E+04	0,00E+00	5,55E+06	2,84E+08	Pos (<10 ^2)	4,844	0,97455	73,75	11,4775	4,83	7,75	36,36	3,855	0,69	3,275	73,912	-
		19,5	65	plastic container for lben storage	CFU/Swab	2,89E+02	2,18E+02	NA	NA	NA													
		16,5	65	Air control	CFU/Petri dish	2,20E+01	0,00E+00	NA	NA	NA													
	Packaging	19,5	65	Lben just before packaging	CFU/ml	3,06E+07	1,90E+05	0,00E+00	6,80E+06	2,72E+08	Pos (<10 ^2)	4,863	0,97515	65,75	10,4476772	2,07	6,17	30,63	2,295	0,77	3,1	77,827	87
		19,5	65	plastic bag for packaging	CFU/Swab	2,92E+02	0,00E+00	NA	0,00E+00	NA													
Batch 2	Milk reception			stainless steel milk container	CFU/Swab	2,50E+02	1,50E+02	NA	0,00E+00	NA	NA												
		18,5	67	Cow milk	CFU/ml	2,40E+08	9,20E+04	0,00E+00	6,45E+07	8,80E+07	Pos (<10 ^2)	6,38	0,974	18,25	11,885	4,975	8,1	26,575	4,65	0,48	2,97	77,19	26
	After ferme			churning machine	CFU/Swab	1,40E+01	0,00E+00	NA	2,50E+01	NA	NA												

	ntation	19,1	64	plastic container for lben storage before saling	CFU/Swab	1,85E+02	2,20E+01	NA																
	Packaging	19,1	64	Lben just before packaging	CFU/ml	1,82E+08	1,92E+05	2,06E+01	6,45E+07	2,63E+08	Pos (<10^2)	4,593	0,974	87,5	10,977	1,285	8,05	25,315	4,29	0,81	2,95	77,241	88
				plastic bag for packaging/saling	CFU/Swab	1,74E+02	0,00E+00	NA	1,00E+01	NA	NA													

Particularly, the increase of LAB with a final pH around 4 in final product reduces the occurrence of hygienic quality indicators like coliforms and TVC; however, in our case the counts are still higher after fermentation. This may be linked to cross contamination through air environment, utensils and handlers. Our findings indicated the presence of coliforms by surface swabbing in the churning machine (3.30 E+01 CFU/swab) and in containers for lben storage (2.18E+02 UFC/swab). This suggested the lack of hygiene in practices and the type of material used for processing. The most critical steps in the process are fermentation, churning, storage and packaging. In addition, since heating of milk was not observed, the bacteria recorded in raw milk can persist in the final fermented milk even at pH value around 4.

7. Microbiological and physicochemical parameters of raw materials, intermediate and final products as well as environmental data from processing facilities producing Squacquerone cheese and salame gentile (UNIBO)

Two artisanal food products and related food processing plants were monitored: squacquerone di Romagna DOP and salame gentile. Based on the flowcharts, raw materials, intermediate and final products were sampled along with the processing environment. In particular, for salame gentile, 420 samples of raw materials as well as salame gentile at the drying, maturation and storage steps were collected along with environmental samples of surfaces, walls and manholes. For squacquerone di Romagna, 810 samples were tested: pasteurised milk, calf rennet, cheese in the warm room, cheese in the maturation room, cheese at packaging, environmental swabs collected on walls, manhole and packaging material. Additionally, samples of squacquerone di Romagna during storage at 2°C, 8°C and dynamic temperatures of 2°C for the first 5 days and 8°C for remaining 10 days of storage were collected. During storage samples were tested at day 0, 1, 4, 8, 11 and 15. Five sample units per matrix (food and environment) were tested for each batch. Overall, 6 batches were investigated as described in Table 19.

Table 19: UNIBO tested batches

	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Salame	1/7/2020	23/9/2020	7/10/2020	21/10/2020	4/11/2020	18/11/2020
Squacquerone	20/1/2020	18/5/2020	13/7/2020	2/11/2020	25/1/2021	1/3/2021

Total bacteria mesophilic counts (TBC) (ISO 4833-2) were enumerated in all samples as well as pH (ISO 2917) and Water Activity (aw) (ISO 21807). Lactic acid bacteria (LAB) (ISO 15214) and *Enterobacteriaceae* (ENT) (ISO 21528-2) were quantified on raw materials and final products at the end of the production and during storage. The occurrence of *Listeria monocytogenes* (ISO 11290-1),

coagulase positive *Staphylococci* (ISO 6888-1), *E. coli* (Trevisani et al., 2017) and *Salmonella* (ISO 6579) was investigated. Isolates were confirmed by biochemical test (RapID™ ONE System and RapID™ STAPH PLUS System, Thermo Scientific™) and PCR (Wesley et al., 2002; Perelle et al., 2004, Chander et al., 2011, Brakstad et al., 1992). Data were statistically analysed by one-way or two or three-way ANOVA considering batches, storage time and temperature on counts converted in $\log_{10}$ followed by Scheffé test. P value <0.05 were considered statistically significant.

Table 20: TBC and LAB mean values enumerated in the Squacquerone cheese in relation to different batches, time and temperature of storage. Different letters in the same column correspond to statistically different values (p≤0.05)

Factor	TBC ($\log_{10}$ CFU/g)		LAB ($\log_{10}$ CFU/g)	
	Mean	Std. Dev.	Mean	Std. Dev.
Batch				
1	4.154[a]	0.707057	1.823[a]	0.536744
2	5.132[b]	0.683505	2.558[abc]	1.583777
3	5.554[c]	0.113919	2.325[ab]	0.856549
4	6.043[d]	0.941015	3.124[c]	1.139889
5	6.551[e]	0.780658	2.780[bc]	1.606564
6	5.844[cd]	0.537588	2.345[ab]	0.892790
Time of storage				
1 day	5.102[a]	0.951182	1.582[a]	0.532417
4 days	5.478[a]	0.777172	1.959[ab]	0.764834
8 days	5.487[a]	0.786620	2.440[b]	0.942783
11 days	6.114[b]	0.917184	2.979[c]	1.206537
15 days	6.150[b]	0.973384	3.580[d]	1.317220
Storage temperature				
2°C	5.572000	0.731909	2.124[a]	0.889718
8°C	5.796000	1.043367	3.036[b]	1.402515
2-8°C	5.664000	1.074203	2.418[b]	1.143061

Table 21 summarises the mean count of TBC and LAB collected in the artisanal product named squacquerone di Romagna during storage in the six tested batches. Statistically significant differences in TCB mean values were observed between different lots at 2°C up to 4 days of storage but were not detected at the end of the shelf life. On the contrary, the TBC values quantified in the different batches during each time of the storage at 8°C and after dynamic temperature were significantly different (Table 21). LAB were quantified as statistically different in the different lots from the fourth day of storage at the three tested temperature conditions, and these differences were observed up to the end of the shelf life (Table 21).

Table 21: TBC and LAB mean values enumerated in the 6 tested batches of Squacquerone during 15 days of storage at 2°C, 8°C, and dynamic temperature of 2°C (for 5 days) and 8°C (for 10 days). Different letters in the same column per each storage time correspond to statistically different values ($p \leq 0.05$)

Batch label	Storage time (day)	TBC Mean Log_{10} CFU/g			LAB Mean Log_{10} CFU/g		
		2°C	8°C	2-8°C	2°C	8°C	2-8°C
1	1	3.33^{a}	3.56^{a}	3.80^{a}	1.59	2.17	1.76
2	1	4.64^{ab}	4.48^{ab}	4.56^{ab}	1.22	1	1.35
3	1	5.48^{ab}	5.50^{ab}	5.48^{ab}	2.02	1.35	1.75
4	1	-	3.63^{ab}	3.65^{a}	-	1.10	1.28
5	1	6.00^{b}	5.87^{b}	5.96^{b}	1.30	2.13	1.32
6	1	5.77^{b}	5.82^{b}	5.85^{b}	1.77	1.61	1.73
1	4	3.63^{a}	3.62^{a}	3.71^{a}	1.62^{a}	1.37^{a}	1.38^{a}
2	4	4.86^{ab}	4.77^{ab}	4.81^{ab}	1.15^{a}	2.13^{ab}	1.00^{a}
3	4	5.50^{ab}	5.59^{ab}	5.55^{ab}	1.95^{a}	1.79^{a}	1.80^{a}
4	4	6.25^{b}	6.19^{b}	5.97^{b}	3.30^{b}	3.33^{b}	3.28^{b}
5	4	5.93^{b}	5.92^{b}	5.92^{b}	1.53^{a}	1.38^{a}	1.42^{a}
6	4	5.76^{b}	5.83^{b}	5.80^{b}	1.80^{a}	1.96^{a}	1.86^{a}
1	8	4.45	3.89^{a}	3.79^{a}	2.12	1.66^{a}	1.56
2	8	4.74	5.33^{ab}	4.86^{ab}	1.28	3.88^{b}	2.24
3	8	5.46	5.51^{ab}	5.52^{ab}	2.19	3.62^{b}	1.91
4	8	5.97	5.68^{b}	6.20^{b}	1.91	4.46^{b}	2.35
5	8	6.21	6.48^{b}	6.14^{b}	1.45	3.70^{b}	2.13
6	8	5.02	5.16^{ab}	5.07^{ab}	2.44	2.21^{a}	2.06
1	11	5.42	4.50^{a}	4.68^{a}	1.79	1.91^{a}	2.09^{a}
2	11	4.72	6.23^{ab}	5.20^{a}	1.70	5.16^{b}	3.56^{ab}
3	11	5.53	5.61^{ab}	5.64^{a}	2.17	3.06^{a}	2.47^{a}
4	11	6.06	6.24^{ab}	7.40^{b}	1.94	4.40^{b}	3.84^{b}
5	11	6.22	7.33^{b}	7.71^{b}	1.47	4.76^{b}	3.94^{b}
6	11	5.89	6.86^{b}	6.44^{b}	1.72	3.63^{ab}	3.37^{ab}
1	15	5.37	5.58^{a}	3.99^{a}	2.84^{a}	1.75^{a}	1.89^{a}
2	15	5.14	6.43^{bc}	6.20^{bc}	2.58^{a}	5.20^{b}	4.93^{b}
3	15	5.59	5.72^{ab}	5.64^{ab}	2.11^{a}	4.31^{b}	2.40^{a}
4	15	6.05	6.56^{bc}	6.33^{bc}	3.23^{a}	4.36^{b}	3.74^{b}
5	15	6.84	8.03^{d}	7.71^{c}	4.86^{b}	5.31^{b}	4.99^{b}
6	15	6.01	6.50^{bc}	5.93^{ab}	3.22^{a}	3.75^{b}	2.24^{a}

Figures 10 and 11 summarise the increase of TBC and LAB in the six tested batches during storage at the three temperature conditions. Overall, 2°C were associated to a slower increase of both TBC and LAB. In all tested batches, the load of ENT was under or close to the detection limit of 10 CFU/g.

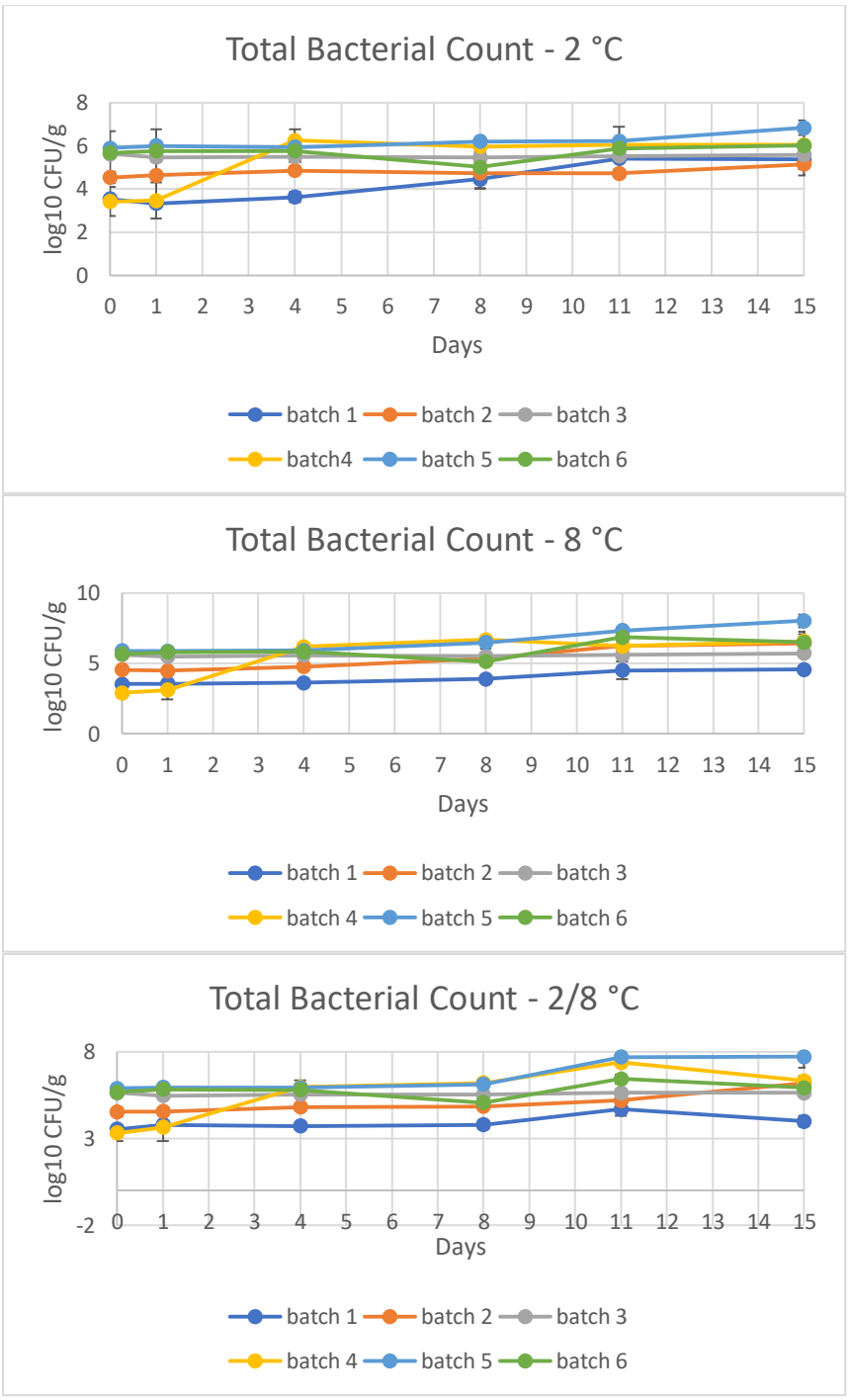

Figure 10: TBC enumeration values in the tested batches of squacquerone di Romagna during 15 days of storage at 2°C, 8°C, and dynamic temperature of 2°C (for 5 days) and 8°C (for 10 days).

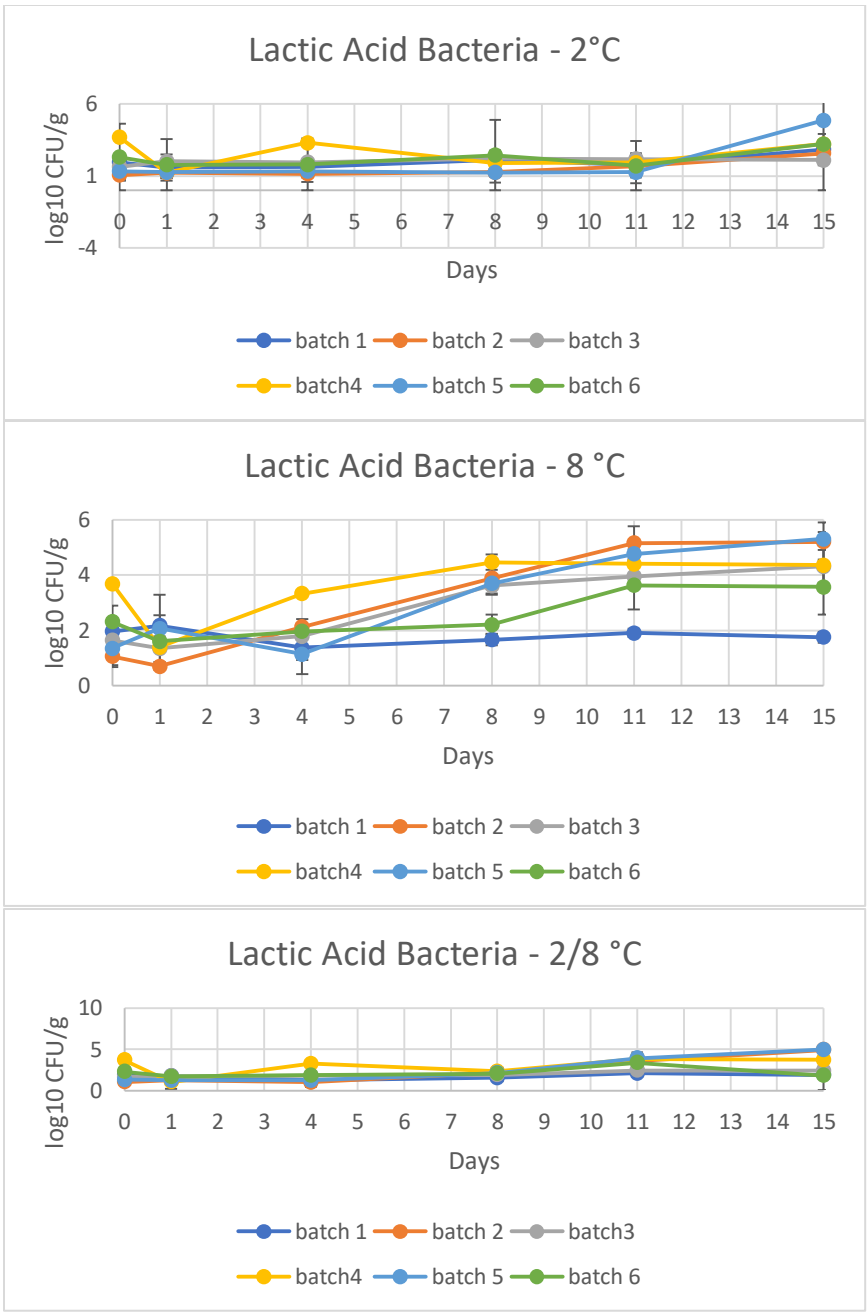

Figure 11: LAB enumeration values in the tested batches of squacquerone di Romagna during 15 days of storage at 2°C, 8°C, and dynamic temperature of 2°C (for 5 days) and 8°C (for 10 days).

With regard to environmental samples and samples collected at the processing plant, manhole of the warm room showed the highest level of total bacterial load (Table 22). Moreover, batch 2 showed the highest load of TBC (7.27log$_{10}$ CFU/g respectively) (Table 22). In relation to the physicochemical properties, Figures 12 shows the pH and water activity (a_w) values in Squacquerone di Romagna during

storage at the categorised temperature conditions. Overall, mean pH values displayed statistically significant different values between batches during storage at the tested temperatures (Table 23).

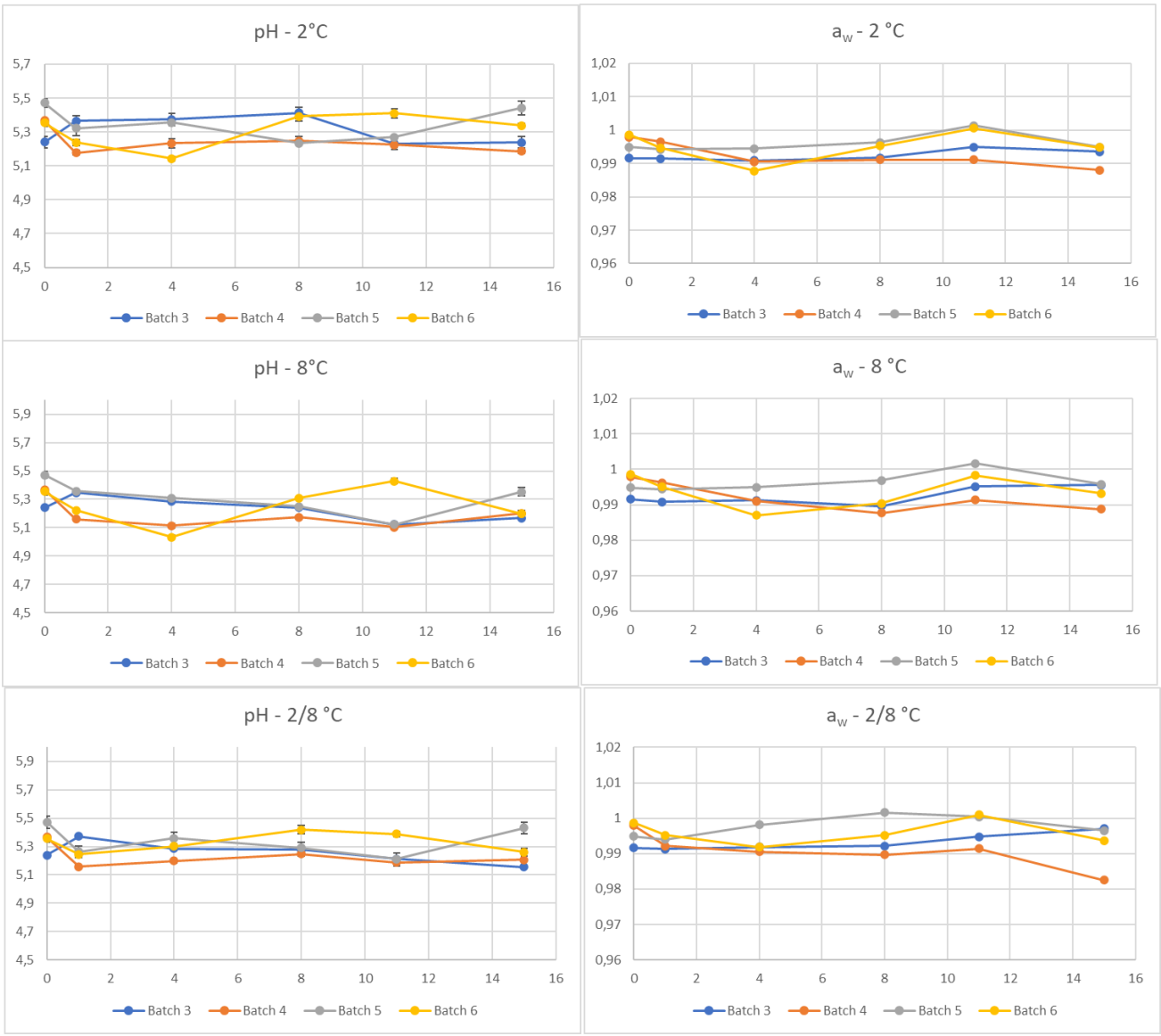

Figure 12: pH and a_w in squacquerone during 15 days of storage at 2°C, 8°C, and dynamic temperature of 2°C (for 5 days) and 8°C (for 10 days).

Table 22: Enumeration of TBC, LAB and ENT in raw materials, intermediate products, packaged squacquerone and environmental samples collected in the Squacquerone production plant (different letters in the same line indicate statistically significant differences, i.e. $p<0.05$).

Sample	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5		Batch 6	
	mean log_{10} CFU/g	SD	mean log_{10} CFU/g	SD	mean log_{10} CFU/g	SD	mean log_{10} CFU/g	SD	mean log_{10} CFU/g	SD	mean log_{10} CFU/g	SD
TBC												
milk before pasteurisation	6.24	0.57	7.37	0.13	6.28	0.22	6.45	0.19	6.56	0.11	5.94	0.11
milk after pasteurisation	2.65^{ab}	0.25	4.15^{ab}	0.09	3.51^{ab}	0,13	3.15^{ab}	0,12	2.36^{a}	0.30	4.18^{b}	1,39
calf rennet	2.14^{ab}	0.55	3.09^{b}	0.20	0.90^{a}	0.82	2.33^{ab}	0,30	2.76^{b}	0.56	3.16^{b}	0.54
warm room -walls	3.96	0.34	4.77	0.20	4.32	0.46	2.00	0.00	2.86	0.43	2	0.63
warmroom- manhole	7.58	0.60	7.79	0.89	7.35	0.28	5.19	0.83	6.88	0.44	6.86	0.61
warm room cheese	3.90	0.25	4.02	0.29	5.03	0.09	5.08	0.18	5.27	0.07	5.49	0.10
maturation room - walls	3.25	1.68	3.00	0.00	2.72	1.90	1.84	0.98	3.75	0.15	2.40	1.59
maturation room - manhole	3.80	0.35	6.10	0.52	4.70	1.55	3.60	0.35	5.40	0.51	4.61	0.32
maturation room - cheese	3.32^{a}	0.28	3.69^{a}	0.27	5.59^{b}	0.09	4.46^{ab}	0.19	5.87^{b}	0.05	5.80^{b}	0.06
packaging -gloves	4.19	0.94	2.86	0.28	2.61	0.45	4.70	0.19	2.69	0.75	3.51	0.63
packaging - manhole	6.25	0.23	6.26	2.00	5.24	0.27	5.74	0.90	4.64	0.68	5.14	0.34
packed cheese	3.53^{a}	0.10	4.54^{ab}	0.26	5.63^{b}	0.06	4.73^{ab}	0.56	5.90^{bc}	0.16	5.67^{b}	0.03
LAB												
warm room - cheese	2.28	0.63	2.65	0.18	2.50	0.82	1.18	0.26	1.14	0.32	1.17	0.23
maturation room - cheese	1.70^{a}	0.00	1.35^{a}	0.20	1.67^{a}	0.19	3.71^{b}	0.02	1.35^{a}	0.41	2.08^{a}	0.46
packed cheese	1.96^{a}	0.24	1.06^{a}	0.39	1.64^{a}	0.27	3.69^{b}	0.09	1.46^{a}	0.44	2.30^{a}	0.59

Table 23: pH and aw values tested in batches 3 to 6 of squacquerone cheese during 15 days of storage at 2°C, 8°C, and dynamic temperature of 2°C (for 5 days) and 8°C (for 10 days). Different letters in the same column per each storage time correspond to statistically different values (p≤0.05).

Batch	Day of storage	pH mean values			aw mean values		
		2°C	8°C	2-8°C	2°C	8°C	2-8°C
3	1	5.364^{b}	5.347^{b}	5.371^{b}	0.991	0.990	0.991
4	1	5.177^{a}	5.161^{a}	5.157^{a}	0.996	0.996	0.992
5	1	5.322^{b}	5.358^{b}	5.264^{ab}	0.994	0.994	0.994
6	1	5.237^{ab}	5.222^{ab}	5.244^{ab}	0.994	0.995	0.995
3	4	5.374^{b}	5.285^{b}	5.284^{ab}	0.990	0.991	0.991
4	4	5.232^{ab}	5.114^{a}	5.198^{a}	0.990	0.991	0.990
5	4	5.356^{b}	5.310^{a}	5.359^{b}	0.994	0.994	0.998
6	4	5.142^{a}	5.033^{a}	5.301^{ab}	0.987	0.987	0.991
3	8	5.412^{b}	5.242	5.278^{ab}	0.991	0.989	0.992
4	8	5.248^{a}	5.174	5.247^{a}	0.991	0.987	0.989
5	8	5.234^{a}	5.248	5.290^{ab}	0.996	0.996	1.001
6	8	5.392^{b}	5.306	5.418^{b}	0.995	0.990	0.995
3	11	5.231^{a}	5.122^{ab}	5.212^{a}	0.994	0.995	0.994
4	11	5.225^{a}	5.104^{a}	5.188^{a}	0.991	0.991	0.991
5	11	5.269^{ab}	5.258^{b}	5.276^{ab}	1.001	1.001	1.000
6	11	5.409^{b}	5.428^{c}	5.388^{b}	1.000	0.998	1.000
3	15	5.238^{ab}	5.168^{a}	5.155^{a}	0.993	0.995	0.997
4	15	5.185^{a}	5.200^{a}	5.206^{a}	0.987	0.988	0.982
5	15	5.441^{c}	5.354^{b}	5.431^{b}	0.994	0.995	0.996
6	15	5.338^{bc}	5.197^{a}	5.260^{a}	0.994	0.993	0.993

With regard to raw materials such as milk, calf rennet and cheese during production, as expected the pH and a_w decreased after the inclusion of the calf rennet (Table 24). At packaging process,the batch 3 and 6 of squacquerone cheese showed the pH value as specified in the Official Product Specification (pH 4.75-5.35).

Table 25 and 26 summarises the mean count of TBC, LAB and ENT collected in the artisanal meat product salame gentile during storage in the six tested batches.

Table 24: pH and water activity (a_w) in raw materials and squacquerone during the production process for different batches. Different letters in the same raw correspond to statistically different values ($p \leq 0.05$).

Stage	pH							
	Batch 3		Batch 4		Batch 5		Batch 6	
	value	SD	value	SD	value	SD	value	SD
Milk before pasteurisation	6.775[a]	0.014	6.897[b]	0.007	6.761[a]	0.010	6.966[b]	0.010
Milk after pasteurisation	6.706[a]	0.006	6.797[ab]	0.023	6.723[ab]	0.006	6.857[b]	0.021
calf rennet	5.087[a]	0.003	5.465[c]	0.002	5.301[b]	0.018	5.617[d]	0.015
cheese at the warm room	5.872	0.022	5.838	0.037	5.810	0.023	5.791	0.023
cheese at the maturation room	5.267[a]	0.013	5.274[a]	0.147	5.537[b]	0.013	5.275[a]	0.018
Cheese at packaging	5.241[b]	0.015	5.368[ab]	0.009	5.471[b]	0.248	5.355[ab]	0.224
Stage	**Water activity**							
	Batch 3		Batch 4		Batch 5		Batch 6	
	value	SD	value	SD	value	SD	value	SD
Milk before pasteurisation	0.996	0.000	0.994	0.000	1.001	0.001	0.996	0.000
Milk after pasteurisation	0.997	0.000	0.995	0.000	1.002	0.000	0.995	0.001
calf rennet	0.826	0.000	0.994	0.000	0.866	0.001	0.853	0.001
cheese at the warm room	0.991	0.001	0.988	0.003	0.995	0.001	0.992	0.000
cheese at the maturation room	0.992	0.000	0.996	0.000	0.996	0.001	0.996	0.000

Table 25. TBC, LAB and ENT values enumerated in Salame gentile in relation to different batches and week of storage. Different letters in the same column correspond to statistically different values ($p \leq 0.05$).

Factor	TBC ($\log_{10}$ CFU/g)		LAB ($\log_{10}$ CFU/g)		ENT ($\log_{10}$ CFU/g)	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Batch						
1	8.204[ab]	1.983659	7.763000	2.082479	3.293000	1.652073
2	8.476[b]	0.863086	8.096000	0.730862	2.456000	1.788953
3	7.274[ab]	1.707982	6.769000	2.039587	2.413000	1.457636
4	6.890[a]	1.667295	6.341000	2.030350	2.049000	1.297904
5	7.515[ab]	1.435804	6.874000	1.832746	3.050	1.725238
6	7.579[ab]	1.763956	7.380000	2.261663	2.415000	1.197884
Week of storage						
0	4.167[a]	0.391983	2.917[a]	0.339964	3.598[c]	0.961461
1	7.305[b]	1.257594	7.106[b]	1.161728	4.201[c]	1.044141
3	8.247[c]	0.541420	8.303[cd]	0.671374	3.549[c]	1.221711
10	8.818[c]	0.675124	8.750[d]	0.448362	2.602[b]	1.248319
18	8.662[c]	0.311814	7.926[c]	0.577437	1.026[a]	0.380013
28	8.403[c]	0.377652	7.822[c]	0.283921	1.008[a]	0.165990

Table 26: TBC, LAB and ENT mean values enumerated in the six tested batches of salame gentile during 28 weeks of ripening. Different letters in the same column per each storage time correspond to statistically different values ($p \leq 0.05$).

Batch label	Time of ripening (week)	TBC Mean (log_{10} CFU/g)	LAB Mean (log_{10} CFU/g)	ENT Mean (log_{10} CFU/g)
1	0	4.03	3.37	4.63
2	0	-	-	-
3	0	4.15	2.85	2.88
4	0	4.06	2.68	3.64
5	0	4.70	3.13	3.20
6	0	3.91	2.56	3.66
1	1	8.58^{bc}	8.53^{c}	4.56^{ab}
2	1	8.69^{c}	7.95^{c}	5.24^{ab}
3	1	6.15^{a}	5.76^{a}	2.98^{a}
4	1	6.23^{a}	6.06^{ab}	3.57^{ab}
5	1	6.78^{ab}	6.59^{ab}	5.49^{b}
6	1	7.19^{abc}	7.55^{bc}	3.24^{ab}
1	3	8.96	9.08^{b}	4.11^{b}
2	3	7.70	7.68^{ab}	3.65^{ab}
3	3	8.11	8.18^{ab}	4.10^{b}
4	3	7.69	7.47^{a}	1.34^{a}
5	3	8.31	8.37^{ab}	4.84^{b}
6	3	8.72	9.04^{b}	3.25^{ab}
1	10	9.75	9.37	4.41
2	10	8.71	8.96	1.54
3	10	8.57	8.44	2.35
4	10	-	-	-
5	10	8.35	8.28	2.18
6	10	8.71	8.74	2.53
1	18	9.13	8.48	<1.00
2	18	8.41	7.99	<1.00
3	18	8.64	7.76	<1.00
4	18	8.41	7.78	1.00
5	18	8.72	7.04	1.60
6	18	8.66	8.48	<1.00
1	28	8.77	7.78	1.09
2	28	8.87	7.90	<1.00
3	28	8.31	7.83	1.00
4	28	7.93	7.67	1.00
5	28	8.25	7.83	1.00
6	28	8.29	7.92	1.00

The trends of LAB and TBC in salami during the 28 weeks of ripening were similar with a sharp increase in the first week of 3-5 log_{10} CFU/g and 2-4 log_{10} CFU/g, respectively, followed by a substantial maintenance of the load with a pick at 10 weeks of ripening (from 8.28 to 9.34 log_{10} CFU/g depending on batches), and a slight decrease at the end of the ripening period (from 7. 67 to 7.92 log_{10} CFU/g) specifically for lactic acid bacteria (28 weeks) (Figure 13). The initial load of ENT showed great

variations among batches (from 3.1 to 5.2 $\log_{10}$ CFU/g). A decrease trend was observed along the ripening period with values lower than 3 $\log_{10}$ CFU/g already after 10 weeks of ripening (except for batch 1) and values under the detection limit after 18 weeks. Interestingly at 10 weeks of ripening batch 1 showed the highest load for TBC, LAB and ENT (Figure 13).

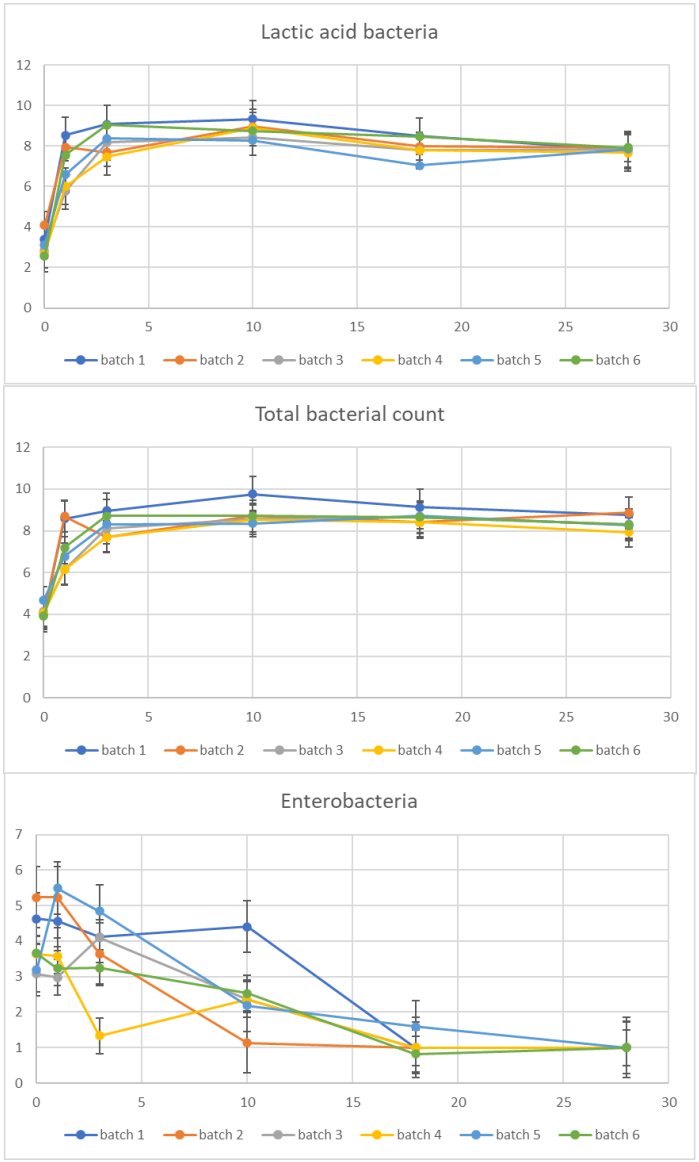

Figure 13: Enumeration of LAB, TBC and ENT in six batches of salami gentile during 28 weeks of ripening.

Regarding environmental samples (Table 27), the loads in both water drainage swabs and walls increased along the flow chart of the food production process from staffing room, to drying and maturation rooms.

The only statistical significant differences between batches were registered at water drainage swab - staffing room.

Table 27: Enumeration of Total Bacterial Count and Enterobacteriaceae in environmental samples collected at the salami manufacturing plant in relation to different batches. Different letters in the same raw correspond to statistically different values ($p \leq 0.05$).

Sample	Batch 1		Batch 2		Batch 3		Batch 4		Batch 5		Batch 6	
	mean (log_{10} CFU/g)	SD	mean (log_{10} CFU/g)	SD	mean (log_{10} CFU/g)	SD	mean (log_{10} CFU/g)	SD	mean (log_{10} CFU/g)	SD	mean (log_{10} CFU/g)	SD
Total bacterial count												
wall swab - staffing room	3.82	0.24	3.32	1.52	5.44	1.40	2.07	1.89	3.37	0.66	1.91	0.65
water drainage swab - staffing room	7.68^{b}	0.08	7.04^{ab}	0.23	4.57^{ab}	0.28	3.65^{a}	2.09	5.79^{ab}	0.49	5.49^{ab}	0.73
surface swab - staffing room	4.28	0.46	4.29	1.70	4.80	0.29	3.92	0.26	4.07	0.05	4.37	0.21
minced meat machine swab - staffing room	3.25	0.86	5.51	1.17	3.23	0.53	2.51	0.53	2.66	0.46	0.99	0.92
wall swab – drying room	4.18	0.35	4.96	0.57	2.99	1.40	3.42	0.80	4.25	0.26	3.87	0.61
water drainage swab - drying room	6.80	0.05	6.99	0.30	7.22	0.28	7.32	0.19	6.85	0.20	7.32	0.15
wall swab - maturation room	6.47	0.18	4.89	0.54	5.94	0.53	5.19	0.65	5.19	0.71	5.07	0.48
water drainage swab - maturation room	6.82	0.36	6.44	0.23	7.07	0.46	7.27	0.84	7.04	0.64	7.68	0.36
Enterobacteriaceae												
surface swab - staffing room	3.36^{b}	0.70	-	-	3.3^{b}	1.29	1.20^{a}	0.30	1.17^{a}	0.24	2.28^{ab}	0.67

A pH decrease was registered after the first week of ripening (Figure 14). Batches 2, 3, 4, 5 and 6 showed pH values of around 5.4 - 5.5. From 10 weeks up to the end of the ripening period a slight increase of the pH up to 5.9-6.2 was observed (Table 28) in line with the slight decrease registered on lactic acid bacteria population. For water activity, a decreasing trend was observed during the ripening period (Figure 15) up to values lower than 0.88 in all batches at 28 weeks of ripening. However, none of the batches reached values below 0.83 and only batch 3 reached values below 0.85 (Table 28).

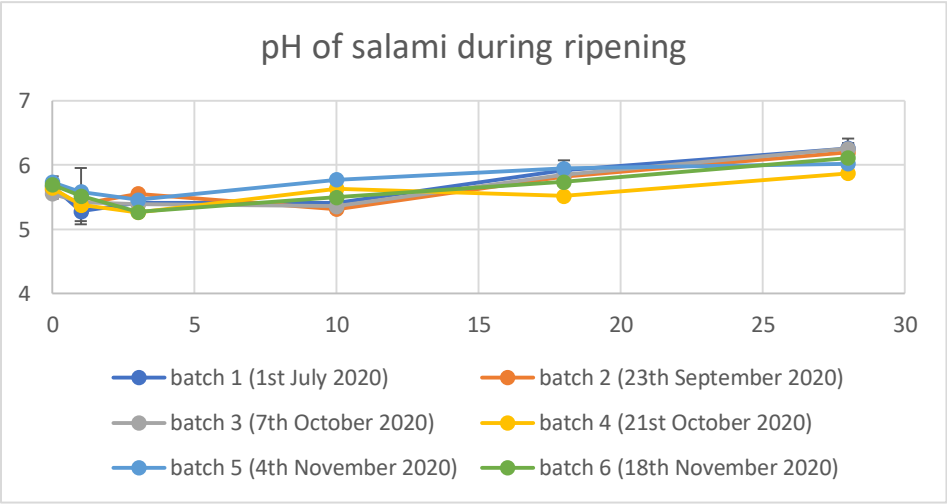

Figure 14: pH values quantified in salame gentile during 28 weeks of ripening in the six tested batches.

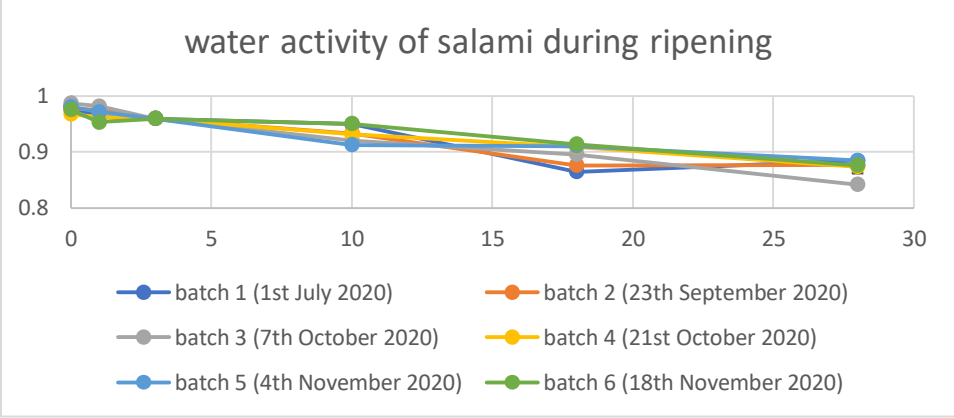

Figure 15: aw values quantified in salame gentile during 28 weeks of ripening in the six tested batches.

Table 28: pH and aw values tested in the six tested batches of salame gentile during 28 weeks of ripening. Different letters in the same column per each time of ripening correspond to statistically different values (p≤0.05)

Batch	Week of ripening	pH	aw
1	0	5.672	0.981
2	0	5.61	0.977
3	0	5.554	0.987
4	0	5.640	0.967
5	0	5.73	0.981
6	0	5.694	0.975
1	1	5.276[a]	0.963
2	1	5.388[ab]	0.975
3	1	5.424[ab]	0.982
4	1	5.377[ab]	0.962
5	1	5.584[b]	0.971
6	1	5.516[ab]	0.953
1	3	5.411[ab]	0.915[a]
2	3	5.550[b]	0.964[b]
3	3	5.386[ab]	0.962[b]
4	3	5.264[a]	0.939[ab]
5	3	5.456[ab]	0.964[b]
6	3	5.268[a]	0.949[b]
1	10	5.408[a]	0.949[b]
2	10	5.314[a]	0.93[ab]
3	10	5.354[a]	0.920[ab]
4	10	-	-
5	10	5.496[b]	0.912[a]
6	10	5.408[a]	0.950[b]
1	18	5.922[ab]	0.864[a]
2	18	5.814[ab]	0.875[a]
3	18	5.847[ab]	0.895[ab]
4	18	5.515[a]	0.908[b]
5	18	5.944[b]	0.91[b]
6	18	5.726[ab]	0.914[b]
1	28	6.258[b]	0.884[b]
2	28	6.195[b]	0.876[b]
3	28	6.254[b]	0.841[a]
4	28	5.870[a]	0.873[b]
5	28	6.021[ab]	0.884[b]
6	28	6.11[ab]	0.876[b]