BEHAVIOUR OF THREE PATHOGENS IN PORTUGUESE CHOURIÇO INNOCULATED WITH SELECTED INDIGENOUS LACTIC ACID BACTERIA.

L. Patarata[1], M.J. Fraqueza*[2], M. Elias[3], M. Rua-Pinto[1], A. Esteves[1], A.S. Barreto [2], and M.C. Martins[1]

[1] University of Trás-os Montes e Alto Douro, CECAV, 5000-911 Vila Real, Portugal, [2] Facultulty of Veterinary Medicine, CIISA, UTL, Av. da Universidade Técnica, 1300-477, Lisbon, Portugal, [3] University of Évora, ADCA, Apartado 94, 7002-554, Évora. Email:mjoaofraqueza@fmv.utl.pt

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Introduction

In Portugal there is a large variety of traditional dry fermented meat products. Among these, chouriço is made with pork and fat grossly grounded, with a seasoning varying according to the region, but salt, garlic, pimiento paste and wine are frequently used. The batter is stuffed into pork casings, and the product is smoked and dry-cured for a few weeks. In the manufacture of these non heated sausages, besides the effects of the low pH, the reduced water activity and the protective effect of nitrite and/or nitrate, there is strong evidence that the presence of lactic acid bacteria plays a role in the safety of these products more than the reduction of pH (Hugas, 1998). Even with centuries of experience manufacturing these products, occasionally foodborne outbreaks related to fermented sausages and other non-heated ready-to-eat meat products occur (Moore, 2004).

The aim of this work was to evaluate the effect of starter cultures prepared from indigenous microflora on the behaviour of three pathogens frequently considered hazards in the preparation of not heated ready-to-eat sausages: *Salmonella* spp., *S. aureus* and *L. monocytogenes,* under different experimental conditions.

Materials and Methods

Bacterial strains and preparation of inoculum: Three strains of *Salmonella* spp., *L. monocytogenes* and *S. aureus* were used. One reference and two strains isolated from meat products or its production environment. Freshly prepared suspensions of cells in NaCl 0.85% were prepared to achieve the level of inoculation required. *S. equorum*, *L. sakei*, *L. plantarum* were prepared basically as the pathogens.

Experiments on model sausages: After mincing the meat, model sausage were prepared (100 g each) in polyethylene bags, sealed and incubated at the regimen temperature.

A factorial design was performed for the three effects in study: Salt level (2%; 4%); Starter culture (control, St1 – *L. sakei* + *S. equorum*; St2 – *L. plantarum* + *S. equorum*); Temperature regimen (constant 22ºC; oscillating 12 h at 10ºC and 12 h at 18ºC). For each one of the 12 possible experimental units, 3 replicates were prepared. Samples were contaminated with the pathogens and incubated for 2 h, 3 and 7 days of incubation.

Experiments on sausages: A challenge test was performed using sausages prepared with meat and pork fat, salt, pimiento paste, garlic paste and water. Meat and fat were chopped and other ingredients were added to the batter. After 10 min of mixture, bacteria were added. First the mix of pathogens to a level of ca. 2 log ufc/g (mixing during 5 min) and diveded in two portions: one control and one batch with a starter culture - (St1) to a level of inoculation of ca. 6 log cfu/g. After 24h at 4±2ºC the batter was stuffed in casings and smoked overnight. Sausages were then dried at 15ºC, 85% RH during the first week, and 80% the second week. Samples were drawn for analysis (in triplicate) at 4 h, 2, 7 and 14 days.

Microbial counting: Were performed in Compass *Listeria* (Biokar 06508), Compass *Salmonella* (Biokar 06608) and Baird Parker with RPF (Biokar 074). Lactic acid bacteria were enumerated in MRS (Cultimed 413785).

Results and Discussion

Experiments on model sausages: The results of the experiment performed in a model sausage are presented in Table 1. The main aspect that must be stressed is the loss of control of the growth of *Salmonella* spp. in every combination of conditions. However, as observed by the results of ANOVA, it was sensitive to all the effects under study. The worst situation observed for the growth of that Gram negative pathogen was, as expected, when the environmental condition were more favorable – low amount of salt and constant high temperature – when any starter culture was added. Different situation was observed for the gram positive pathogens. For the most of the combinations of factors, the worst situation was always when no starter culture was added. When starters were present, in 75% of the 16 possible cases (2 pathogens * 2 salt levels * 2 temperatures * 2 Starter cultures) the growth of the pathogens were sustained, as observed through the number of survivors lower than 100. When the means of bacterial counts were compared among the three possibilities of starter cultures (control, St1 and St2), it was observed that the counting of *S. aureus* was similar for both starters, but different from the control. For *L. monocytogenes*, the situation was different, with St2 similar to the control, and the count obtained with St1 significantly different ($p<0.05$).

Experiments on sausages: The microflora had a reduction along the procedure (Table 2). *Salmonella* spp. experienced a slight increase between the first and the second period of analysis, when starter culture was absent. This increase was

favoured by the heating associated to the smoking procedure (ca. 25ºC). In both cases – control and with starter culture – *Salmonella* spp. was not detected in the final product. However, the reduction to below detection level was faster when starter culture was added. The differences in the counting of *Salmonella* spp. among the samples with or without starter culture were significant ($p<0.001$) at day 7. The behaviour observed with *L. monocytogenes* was not as favourable as that observed for *Salmonella* spp., once it was not possible to produce a reduction in the counting of the pathogen. Even so, the addition of the starter culture was advantageous because *L. monocytogenes* counts remained singnificantly lower at days 7 and 14. The effect of using the starter culture in the counting of *S. aureus* was interesting, once that starter microflora stopped the overgrowth of the pathogen during the smoking. The following reduction rendered a sausage with no detectable pathogen at the end of 7 days. The LAB count was always higher, significantly different ($p<0.001$) in samples with no starter culture added.

Table 1: Percentage of survivors of pathogens between 2h and 7 days of incubation in model sausage; results of the analysis of variance of the counting for the effects: salt levels; temperature; starter cultures (and one control) after 7 days of incubation.

Effects					
Salt level	Starter culture	Temperature regimen	*Salmonella*	*S. aureus*	*L. monocytogenes*
2%	St 1	K 22ºC	115.2	97.9	90.3
2%	St 1	O 10-22ºC	122.9	92.8	89.1
2%	St 2	K 22ºC	131.0	107.7	91.8
2%	St 2	O 10-22ºC	220.4	138.0	104.2
2%	control	K 22ºC	130.2	103.5	101.3
2%	control	O 10-22ºC	122.7	96.8	94.8
4%	St 1	K 22ºC	119.0	93.8	95.6
4%	St 1	O 10-22ºC	120.5	101.5	98.2
4%	St 2	K 22ºC	128.8	111.1	101.7
4%	St 2	O 10-22ºC	126.8	153.0	105.2
4%	control	K 22ºC	132.8	107.5	94.4
	Significance of the effects at day 7				
	Salt level (NaCl)		***	***	ns
	Starter culture (St)		***	***	***
	Temperature (T)		**	**	ns

Table 2: Results of the challenge test. Counting of *Salmonella* spp., *S. aureus and L. monocytogenes* in control sausages and inoculated with starter cultures.

Time	*Salmonella* spp.			*S. aureus*			*L. monocytogenes*		
	control	Starter	Sig	control	Starter	Sig	control	Starter	Sig
4 h	2.30	2.62	ns	2.53	2.53	ns	2.26	2.16	ns
2 d	3.60	1.53	ns	3.11	1.33	ns	3.61	1.65	ns
7 d	2.10	0.00	***	1.43	0.00	**	2.95	2.10	**
14 d	0.00	0.00		0.00	0.00		3.27	2.26	***

Conclusions

It is possible to infer that the use of starter cultures in the Portuguese sausage studied results in safer products, due to its contribution in pathogen reduction. Independently of the use of starter cultures, the process of smoking and drying is responsible for an important reduction in *Salmonella* spp. and *S. aureus*. In order to avoid the overgrowth of *L. monocytogenes*, the use of the starter tested obtained from indigenous isolates is determinant.

Acknowledgments

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References

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