THE INFLUENCE OF THE STARTER CULTURES IN THE PRODUCTION OF REGIONAL PORTUGUESE SAUSAGE – MICROBIOLOGICAL, PHYSICAL AND CHEMICAL PROPERTIES

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Background

Nowadays, the use of starters in the production of sausages is an interesting subject. These cultures are important in improvement and maintenance of color and flavor,. They contribute to the preservation of different sausages allowing best safety, and homogeneity of cured meat products. Starters in meat products are used since 1921 (Liepe, 1983; Lahti *et al.*, 2001; Papamanoli *et al.*, 2002).

Starters aren't used in the production of sausage in Portugal but some research has been made in this area (Elias *et al.*, 2000).

Objectives

The main objective of this work is to know the influence of autochthonous strains of *Staphylococcus xylosus* and *Lactobacillus sake* on microbiological physical and chemical properties of one typical kind of sausage, "Paio de Barrancos", produced in the southeast of Portugal.

Methods

"Paio de Barrancos", is a regional and traditional Portuguese sausage with a cilindrical form (diameter: 4 -5cm; length: 25-30cm); raw material is meat and fat from Alentejano pig breed. Two factories were studied. Factory A doesn't use smoke during the curing time. Factory B use smoke from holm-oak wood. At both factories the process conditions for the ripening period are the same (2 days; temperature: 3-5ºC; relative humidity: 90-95%). However the curing period conditions at factory A (30 days; temperature 10-12ºC; relative humidity 78-80%) are different from those of factory B (8 days, temperature 30-35ºC and smoking; 21 days, temperature 17-18ºC).

Four 25Kg portions of meat and fat were prepared in each of those factories. Three out of them were inoculated, one with 10^8 cells/g of *Staphylococcus xylosus*, other with 10^8 cells/g of *Lactobacillus sake*, and anoother one with 10^8 cells/g of *Staphylococcus xylosus* and 10^8 cells/g of *Lactobacillus sake*. The fourth portion, the control, wasn't inoculated. Those portions are refereed respectively as inoculation modality 1, 2, 3 and 4.

Microbiological analysis: mesophyles (Tryptone Glucose Extract Agar medium, Merck; 30ºC during 48 hours); Psicrotrophics (Tryptone Glucose Extract Agar medium, Merck; 6ºC during 10 days); yeast (Yeast Extract Agar, Oxoid, with 0,5% of ciclohexamide, 25ºC during 5 days); *Enterobactereaceae* (Violet Red Bile Glucose Agar medium, 37ºC during 48 hours); *Streptococcus* Group D (Kanamicin Aesculin Azide Agar medium, Oxoid, 37ºC during 48 hours); *Micrococci* (Manitol Salt Agar, Oxoid, 30ºC during 72 hours; *Lactobacilli* (Man, Rogosa and Sharp, Oxoid, 30ºC during 72 hours); Aerobic bacteria spores (previous inactivation of vegetative strains, 80ºC during 10 minutes, and the other conditions as refereed for mesophyles); *Clostridium* sulphite-reducers (previous inactivation of vegetative strains, Sulphite Polimixin Sulphadiazine Agar, Merck, 44,5ºC during 72 hours).

Physical and chemical analysis: colour (L*a*b*) measured trough a colorimeter Minolta CR-210b. pH according the portuguese standard NP-3441 (1990). Water activity measured with Rotronic Hygroscop DT using a probe WA-40.

The program STATISTICA was used to perform Anova analysis and for means comparison test (LSD).

Results and discussions

According to the microbiological results, both factories exhibit similar behavior. The anova analysis only found significant differences for yeast, *Lactobacilli* and *Enterobactereaceae* (Table 1). Considering the sanitary aspects, in factory B the values of *Enterobactereaceae* were smaller in all the modalities of inoculation comparing with control (Table 2). In factory A the difference between control and other portions were not so pronounced. The microbiological results obtained in this research didn't show clearly the advantage of using these starters in these products. Although, other research work done before (Elias *et al.,* 2000), using the very same strains and the same products, exhibits clearly advantage of the inoculation with *Staphylococcus xylosus* when analyzed the mechanical and sensorial properties.

The Anova of the physical and chemical properties (Table 3) made clear the difference between modalities of inoculation considering the parameter of colour a* and pH, in factory A, and the parameter a* and b* in factory B.

The values of a* are higher in the products of both factories when inoculated with *L.sake* pure or in mixture with *S.xylosus* (Table 4). These results apparently in contradiction with others that indicate *Micrococci* as responsible for improving the colour in sausage, can be due to the lactic fermentation and the production of pseudocatalise by *L. sake* (Carrascosa-Santiago, 2001).

The parameter b* exhibit lower values in the control samples. This can be justified by the fact of turning yellow of the intramuscular fat (very characteristic of the products studied), consequence of lipolitic process more intense in the inoculated products.

The factory A, that doesn't use smoke, revelead lower values of pH in all the modalities of inoculation with *L.sake* (modalities 1 and 2). The factory B, probably due to the action of smoke, didn't show any differences among different modalities of inoculation.

Should be distinguished the lower values of a_w in the products inoculated with *S. xylosus*, which are responsible for a shorter time of cure in these products.

Conclusions

The microbiological evaluation puts enhances the lower number of *Enterobactereaceae* in the modalities of inoculation studied when compared with the control. The values of a_w should also be distinguished in products inoculated with S. xylosus, often refereed as responsible for a shorter time of cure in these products.

References

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Table 1 - Microbiological analysis - Variance analysis for inoculation modality factor in two factories

	Mesophilic Bacteria	Yeasts	*Lactobacilli*	*Micrococci*	Aerobic Bacteria	*Enterobactereaceae*	D Streptococci	Sulphite Reducing
					Spores			Clostridia Spores
FACTORY A								
F value	1,211	12,639	6,194	0,899	0,421	2,479	7,998	0,600
p value	0,348	0,001	0,009	0,470	0,741	0,111	0,003	0,627
Significant level	N.S.	***	**	N.S.	N.S.	N.S.	**	N.S.
FACTORY B								
F value	1,045	5,243	4,151	0,981	2,309	1,931	12,319	0,268
p value	0,408	0,015	0,031	0,434	0,128	0,178	0,001	0,847
Significant level	N.S.	*	*	N.S.	N.S.	N.S.	***	N.S.

Significant level : * for $p < 0,05$; ** for $p < 0,01$; *** for $p < 0,001$; NS - no significant

Table 2 - Microbiological analysis - means for different inoculation modality and two factories

Inoculation Modality	Mesophylic Bacteria (C.F.U./g)	Yeasts (C.F.U./g)	*Lactobacilli* (C.F.U./g)	*Micrococci* (C.F.U./g)	Aerobic Bacteria Spores (nº/g)	*Enterobacte-reaceae* (C.F.U./g)	D *Streptococci* (C.F.U./g)	Sulphite Reducing Clostridia Spores
FACTORY A								
1	$20x10^7$	$49x10^4$ a	$115x10^6$ a	$13x10^5$	$1150x10^2$	2400	$16,8x10^4$ a	2
2	$42x10^7$	$333x10^4$b	$138x10^6$ a	$36x10^5$	$1450x10^2$	3400	$0,04x10^4$ a	1
3	$37x10^7$	$58x10^4$ a	$413x10^6$ b	$59x10^5$	$1425x10^2$	825	$257x10^4$ b	1
4	$15x10^7$	$101x10^4$a	$0,8x10^6$ a	$15x10^5$	$1100x10^2$	2625	$0,35x10^4$ a	1
FACTORY B								
1	$11x10^7$	$11x10^4$ a	$50x10^6$ a	$1501x10^5$	$4x10^2$	1450	$0,23x10^4$ a	2
2	$11x10^7$	$4x10^4$ b	$69x10^6$ ab	$140x10^5$	$4x10^2$	0	$450x10^4$ a	2
3	$10x10^7$	$1,2x10^4$ b	$106x10^6$ b	$340x10^5$	$7x10^2$	75	$1563x10^4$ b	2
4	$6x10^7$	$12x10^4$ a	$35x10^6$ a	$21x10^5$	$5x10^2$	7125	$8,5x10^4$ a	2

Inoculation modality: 1-*L. sake + S. xylosus; 2 –L. sake;* 3-*S. xylosus*; 4- Control
At the same factory and in the same column, different letters represent means significant different
Results of Sulphide Reducing Clostridia Spores: 1 means < 1 spore/g; 2 means > 1 spore/g and < 10 spores/g

Table 3 - Physical and chemical analysis - variance analysis for inoculation modality factor in two factories

	Colour parameters			pH	aw
	L*	a*	b*		
FACTORY A					
F value	0,261	5,345	1,404	27,228	1,993
p value	0,853	0,003	0,254	0,000	0,174
significant level	N.S.	**	N.S.	***	N.S.
FACTORY B					
F value	1,411	9,693	12,722	2,213	1,186
p value	0,252	0,000	0,000	0,100	0,356
significant level	N.S.	***	***	N.S.	N.S.

significant level :** for $p<0,01$; *** for $p<0,001$; N.S.- no significant

Table 4 - Physical and chemical analysis – means for different inoculation modality and two factories.

Inoculation	Colour Parameters			pH	aw
Modality	L*	a*	b*		
FACTORY A					
1	38,8	17,1 abc	14,1	5,7 a	0,813
2	39,3	18,6 b	15,4	5,7 a	0,775
3	37,8	15,8 cd	14,1	5,82 b	0,688
4	39,1	14,2 d	10,8	5,76 c	0,734
FACTORY B					
1	37,3	15,9 a	9,0 a	5,54	0,762
2	41,0	16,5 a	12,9 b	5,59	0,737
3	38,3	12,8 b	8,1 ac	5,50	0,714
4	40,2	12,9 b	6,3 c	5,56	0,738

At the same factory and in the same column, different letters represent means significant different