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Short Communication

Microbial spoilage of portuguese *chouriço* along shelf life period

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Abstract

Microbial flora of portuguese *chouriço* (*Alentejano* (A) and *Ribatejano* (R)) with abnormal sensorial characteristics along shelf life was studied. Mesophilic anaerobic bacteria, enterococci, mesophilic sporeformers, coliforms, coagulase-positive staphylococci, sulphite reducing clostridia, *Clostridium perfringens,* moulds and yeasts were the most representative in both types of *chouriço*.

Key words: spoilage microbial flora, Abnormal sensorial characteristics, Portuguese *chouriço*, Returned product.

Portuguese dry smoked sausage - *chouriço* - is a meat product with *sui generis* characteristics. The nutritional value, the long shelf life period (120 days) and the processing technology make it a very well accepted and consumed product in Portugal. Portuguese *chouriço* is intensively smoked through the combustion of hard wood (cork tree and holm oak) (Matos *et al.*, 2008) and is considered a *shelf stable product* at temperature of 20 ± 5 °C (Matos *et al.*, 2007). However, *chouriço* with abnormal sensorial characteristics may occur along shelf life period (Matos *et al.*, 2005). Product loses the marketability and it is normal practice to return it to the processing industry which will credit the client by the corresponding value. Problems such as the potential risk for the public health; the industry "brand image" depreciation; the compromised market position and economic losses are of great concern. In order to prevent these problems it is extremely important to study which factors have influence on the stability of the product to ensure the foregoing quality and safety of the meat product during the producer-defined shelf life period. In this context it was evaluated the microbial flora of two types of Portuguese dry smoked sausage, *chouriço* type *Alentejano* and type *Ribatejano*, which were returned to the meat processing industry along shelf life period (packaged in modified atmosphere, 45% CO_2 / 55% N_2, and kept at 20 ± 5 °C) with abnormal sensorial characteristics (colour and texture changes, fungi development, slime and drip loss).

The experiments were conducted in a large meat plant certified according ISO 22000 (ISO 22000, 2005) standard. Two types of Portuguese smoked dry sausage (*Alentejano*, A and *Ribatejano,* R) were studied. The recipe of these *chouriços* consisted in pork, red pepper paste, water, salt, garlic paste, olive oil, spices, sugar, liquid smoke, additives (sodium nitrite, E250 and commercial sausage sodium polyphosphate, E452(i)) for type A, and pork, red pepper paste, water, salt, garlic paste, spices, white wine, sugar, liquid smoke, additives (sodium nitrite, E250 and commercial sausage sodium polyphosphate, E452(i)) for type R. Pork meat and fat were minced (± 20 mm) and mixed with the formulation ingredients. The batter was stuffed into salted pork intestine (Ribatejano type sausage) and into beef dry casing (Alentejano type sausage) and then dried and smoked for about three days (weight loss ca 40%). The thermal treatment was divided in two phases. First phase was conducted in a smoke chamber with temperature, relative humidity (RH) and smoke addition automatically controlled. This first phase included 1) 60 min at 50 °C, 2) 90 min at 53 °C and 3) 90 min at 55 °C. During these treatments smoke was given continuously. In the second phase, product was submitted to one traditional chimney at the same industry in which smoke was produced from firewood (oak wood). The product was stabilised for one day at 17-19 °C and at 75-85% (RH) before packaging.

Final product was packaged separately in modified atmosphere with 45% of CO_2 and 55% of N_2. Package ma-

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terial was a Combitherm film, 70-300 mm, PA/EVOH/PE/SY – coextruded, laminate [EVOH (ethylene vinyl alcohol), PE (polyethylene) and PA (polyamide polymer)]. Shelf life period was defined as 120 days at 20 ± 5 °C. The study was based in 60 samples (27 from product A and 33 from product type R), each sample was composed by a mixture of three sausages (a total of 180 sausages) randomly drawn from those sausages which returned to the industry with abnormal sensorial characteristics. Samples were not peeled for the accomplished analysis.

Microbial analyses were performed in triplicate. Each sample was prepared from three sausages. Twenty five grams were removed (approximately 8.33 g from each sausage) and mixed into 225 mL of Peptone water (Merck; 7228). Ten fold dilutions were prepared taking 1 mL from the previous dilution into 9 mL in an 0.85% solution of sodium chloride (Merck, 6404). For the enumeration of moulds and yeasts 0.5-mL samples from the dilution tubes were spread onto selective media rose bengal chloramphenicol agar base (RBC; CM549, Oxoid, Basingstoke, UK) supplemented with chloramphenicol (SR78E, Oxoid) and incubated aerobically at 25 °C for 5 days according to official Portuguese standards (NP 2077, 1985). For the enumeration of mesophylic anaerobic bacteria, 1 mL samples from the dilution tubes (previously heated to 80 °C for 10 min) were inoculated onto COLID (BioMerieux) and incubated for 5 days at 30 °C (NP 2262, 1986). Search analysis of *Clostridium perfringens*, sulphite reducing Clostridia and coagulase-positive staphylococci were performed according Portuguese Standard Methods (NP 2260, 1986; NP 2261, 1986; NP 2262, 1986). For search analysis of *Enterococcus* was used ADB medium (Merck 1.01590) incubated at 37 °C for 24 hours with confirmation in VAB (Difco 0606-01-7) at 37 °C for 48 hours; search analysis of coliforms and *E. coli* were performed in COLID medium (BioMerieux) after 48 hours of incubation at 37 °C . Viable counts were calculated as log CFU (mean ± standard deviation, n = 3). Results of search analysis were reported as the

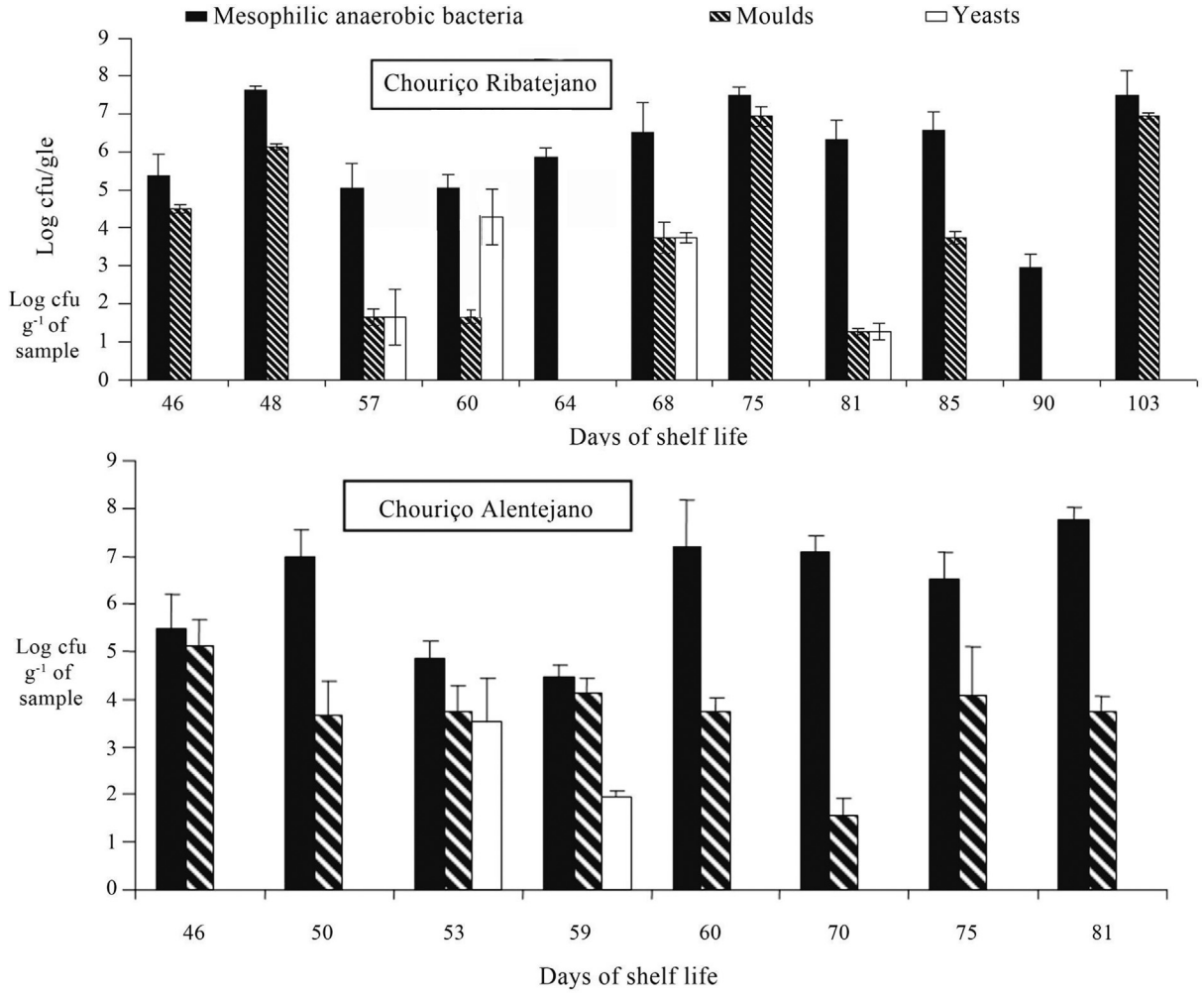

Figure 1 - Mean values ± standard deviation (n = 3) of mesophilic anaerobic bacteria, moulds and yeasts (log CFU g^{-1} of sample), along shelf life period (modified atmosphere package, 45% CO_2 / 55% N_2, at 20 ± 5 °C) of *chouriço* type *Ribatejano* and *Alentejano*.

number of samples containing positive results (presence of the organism).

In samples of *chouriço* R, mesophilic anaerobic bacteria constituted the predominant group, present in 100% of the samples with mean values between 2.96 and 7.63 log cfu/g of sample (Figure 1). For *chouriço* type A, besides the microbial group of mesophilic anaerobic bacteria, moulds were also observed in 100% of the samples (Figure 1). For these microbial groups, mean values (log cfu/g of sample) oscillated between 4.48 and 7.76 and between 1.56 and 6.19, respectively. Yeasts presented a frequency of 36.4% in *chouriço* type R and of 33.3% in A type. From all samples with abnormal sensorial characteristics the predominant flora was mesophylic anaerobic bacteria and in second place moulds. These results could be related with the presence of lactic acid bacteria which is the predominant anaerobic flora (data not shown). Product fermentation promotes the growth of lactic acid bacteria but the presence of moulds and yeasts could indicate package rupture or film permeability to the oxygen. In the present study, yeast counts revealed almost inexistent, only in samples with 57, 60, 68 and 90 days of shelf life for product R and, in samples with 53, 59 and 82 days of shelf life for sausage type A values were detected (Figure 1). These results are in agreement with the results founded by Matos *et al.* (2007) in Portuguese *chouriços*. Encinas *et al.* (2000) also reported in Spanish fermented smoked sausages mean values of yeast counts lower than in non-smoked sausage types and, among all varieties studied by these authors, the lowest mean value founded in sausages hot-smoked was of 16 cfug while in other types of sausages (not-smoked) mean values varied from 1000 to 100000 cfu/g. Smoke does affect yeasts (Leistner, 1995), and factors such as time and temperature influence this effect. Inhibitory effect of garlic on yeasts was reported by Ghamnnoum (1990).

Search analyses, in both types of product, revealed enterococci and mesophilic sporeformers as the predominant microbial groups, with positive results in 100% of the samples (Table 1). In what concerns coliforms, values were superior in *chouriço* type R (positive results in

Table 1 - Number of samples of *chouriço* type *Ribatejano* and *Alentejano* with positive results (presence of the organism) during shelf life period (MAP of 45% CO_2 / 55% N_2, at 20 ± 5 °C).

Chouriço type *Ribatejano*	Days of shelf life										
Microorganism[a]	46	48	57	60	64	68	75	81	85	90	103
- *Enterococcus* (0.001 g)	3	3	3	3	3	3	3	3	3	3	3
- Mesophilic sporeformers	3	3	3	3	3	3	3	3	3	3	3
- Coagulase positive staphylococci	3	2	3	3	0	0	0	3	0	3	0
- *Clostridium perfringens* (1 g)	0	0	0	0	0	2	0	3	0	0	0
- Sulphite reducing clostridia (0.01 g)	3	0	0	0	0	3	2	0	0	0	3
- *Listeria monocytogenes* (25 g)	0	0	0	0	0	0	0	0	0	0	0
- *Salmonella* (10 g)	0	0	0	0	0	0	0	0	0	0	0
- Coliforms (0.01 g)	0	3	0	0	0	0	2	0	0	0	3
- *Eschirichia coli* (1 g)	0	1	0	0	0	0	0	0	0	0	0

Chouriço type *Alentejano*	Days of shelf life										
Microorganisms[a]	46	50	53	59	60	70	75	81	82	Microorganisms[a]	46
- *Enterococcus* (0.001 g)	3	3	3	3	3	3	3	3	3	- *Enterococcus* (0.001 g)	3
- Mesophilic sporeformers	3	3	3	3	3	3	3	3	3	- Mesophilic sporeformers	3
- Coagulase positive staphylococci	3	0	0	0	0	2	2	3	3	- Coagulase positive staphylococci	3
- *Clostridium perfringens* (1 g)	0	3	0	0	2	2	0	0	0	- *Clostridium perfringens* (1 g)	0
- Sulphite reducing clostridia (0.01 g)	2	3	3	3	0	3	0	3	0	- Sulphite reducing clostridia (0.01 g)	2
- *Listeria monocytogenes* (25 g)	0	0	0	0	0	0	0	0	0	- *Listeria monocytogenes* (25 g)	0
- *Salmonella* (10 g)	0	0	0	0	0	0	0	0	0	- *Salmonella* (10 g)	0
- Coliforms (0.01 g)	3	0	0	0	0	0	0	0	0	- Coliforms (0.01 g)	3
- *Eschirichia coli* (1 g)	0	0	0	0	0	0	0	0	0	- *Eschirichia coli* (1 g)	0

[a]Reference values according the bacteriological standards for Portuguese food (Ribeiro, 1974): *Enterococcus*-negative in 0.001 g of product sample; sulphiye reducing clostridia and coliforms-negative in 0.01 g of product sample; *Eschirichia coli* and *Clostridium perfringens*-negative in 1 g of product sample; *Listeria monocytogenes* in 25 g and *Salmonella*-negative in 10 g of product sample.

24.2% of the samples). In *chouriço* type A were observed positive results only in 11.1% of the samples. *Escherichia coli* was detected only in one sample of *chouriço* type R. For coagulase-positive staphylococci, sulphite reducing clostridia and *Clostridium perfringens* frequencies of 48%, 63% and 25.9%, respectively, in *chouriço* type A, and of 51.5%, 33.3% and 15.2% in *chouriço* type R, were observed.

Enterococci occur and may compete well in fermented sausages. Opinions about their significance vary as enterococci may enhance sausage aroma and taste by their proteolytic activities, but may also compromise safety if opportunistic pathogenic strains proliferate (Holley *et al.*, 1988). The role of enterococci in Portuguese smoked sausages should be evaluated. Prevalence of staphylococci coagulase-positive was founded in 30 samples (17 samples of R and 13 samples of A sausage). The presence of coliforms and *E. coli* can be explained by the handling of the paste and the stuffing procedure into natural guts. *Clostridium perfringens* and sulphite reducing clostridia were present in 15.2% and in 33.3% for product type R, respectively and, in 25.9% and in 63% of the samples for product type A, respectively. In conclusion, the presence of pathogenic groups represents a health hazard to the consumers in Portugal and reinforced the need to implement urgent measures in meat processing industries and also in market points regarding the stability and safety of these meat products along shelf life period. Factors such as the thermal process, the storage temperature along shelf life, the substitution of natural casings and the final product handling should be considered and improved. However, further studies should be carried out in order to investigate the influence of the normal house flora (useful and noxious) on shelf life and on the applied packaging technology of these types of Portuguese meat products. Storage conditions at market points should also be evaluated.

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