MICROBIAL GENERA OF DECOMPOSITION ANALYSED IN TWO TYPES OF PORTUGUESE SMOKED DRY SAUSAGES WITH ABNORMAL SENSORIAL CHARACTERISTICS DURING SHELF LIFE PERIOD IN MAP (MODIFIED ATMOSPHERE PACKAGE)

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Background

The portuguese smoked dry "sausage chouriço" is a very typical product and the characteristic flavour, nutritional value, long shelf life period and easy industrialisation make it a very well accepted and consumed product in Portugal. This product is intensively smoked at high temperature and can be considered a smoked sausage processed by heat. The originality of this product is the drastic processing and the intensively smoking by direct fire from hard wood (especially cork tree and holm oak)[1]. Besides the contributions of flavour and colour, smoke flavourings also contribute to the foregoing quality (shelf life) of processed meats. Smoke flavourings, such as aldehydes, aliphatic acids and phenolic compounds, are effective antibacterial and anti-fungal agents[2]. Meat transformation industries have the responsibility to produce safety products and to ensure the quality of the meat product during shelf life period defined. The shelf life of a product is the period of time between the end of the manufacturing process (in the case of packed goods the day of packing) and the moment when the product starts to lose its specific characteristics. Specific product characteristics are sensory characteristics (flavour, colour and consistency) and microbiology[3]. However, sometimes occurs that the product lose its marketability[4] (goods are no longer objectively fit for sale) and would not normally sold anymore besides the expiration date does not being reached. In these cases it is normal practice by the commercialisation points to return the product to the processing industry and to receive the corresponding credit. Besides the factors which have influence on the shelf life, like plant and personnel hygiene, the meat freshness, the type of package and packaging technology and the storage conditions[3] is important to evaluate which microbial groups are present when the meat product lost its specific characteristics in order to response and help to solve the economic losses felted by the processing industry.

Objectives

The aim of this work was to study the microbiological composition of two types of Portuguese smoked dry sausages with abnormal sensorial characteristics during shelf life period which were returned to the processing industry.

Methods

The experiments were conducted at a processing meat plant and it was studied two types of Portuguese smoked dry sausage, which contained 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 "Alentejano", 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 "Ribatejano". The technological process of "chouriço" consisted in a mixture of pork meat and fat minced (± 20 mm) with the formulation ingredients. This paste is matured for one day at 5ºC. The stuffing is filled into pork gut in *Ribatejano* sausage type and into dehydrated bovine gut for *Alentejano* sausage type, subject to the drying effect of smoke and temperature. Thermal processing is divided in two phases. First phase is in industrial cooked/smoker chambers with temperature between 50 and 60ºC for 4 hours. Second fase is in a traditional smokery for 3 days. Final product is kept one day in a stabilisation room at 17-19ºC with RH of 75% until packaging. The product was packed separately in modified atmosphere with 45% of CO_2 and 55% of N_2. The material package is composed by plastic polymers laminate of several very thin polymers. It was used 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 recommended is 4 months at room temperature (20 to 25ºC). Samples to be analysed were taken randomly from those products which were returned to the industry from the market points. Sausage samples were not peeled to accomplish the microbial analyses. Counting and search of microorganisms, aiming at the sanitary and technological aspects included: Total count of Mould and Yeast (CRBA, Oxoid CM549 with chlorophenicol, Oxoid SR78E – 5 days at 25ºC); Total count of mesophylic anaerobic bacteria (after 80ºC for 10 minutes the enumeration was performed in COLID, Portuguese Standard Methods 2262/86 – 5 days at 30ºC); Search of *Clostridium perfringens*, sulphite reducing Clostridia and coagulase-positive staphylococci - Portuguese Standard Methods 2261/86, 2262/86 and 2260/86; Search of *Enterococcus* (ADB, Merck 1.01590 - 37ºC for 24 hours and confirmation in VAB, Difco 0606-01-7 - 37ºC for 48 hours); Search of coliforms and *E. coli* (COLID medium, BioMerieux - 48 hours at 37ºC). Viable counts per gram were transformed to logarithms (base 10) and results reports the mean value of three analyses. For search results the presence of the organism is presented as positive per grams (g) of sample and the absence as not detectable (ND).

Results and Discussion

The variation of the microbial population of Portuguese sausage type *Ribatejano* is shown in Fig. 1 and for Portuguese sausage type *Alentejano* is presented in Fig. 2. From all samples with abnormal sensorial characteristics analysed the predominant flora was mesophylic anaerobic bacteria and in second place mould. These results can be explained considering that lactic acid bacteria constitute the predominant anaerobic flora in the final product (data not shown) and remains during shelf life period in anaerobic conditions. The maturation promotes the growth of lactic acid bacteria but the presence of moulds and yeast can indicate package rupture or film permeability to the oxygen, which should be considered by the industry packaging technology. Results of search analyses of microorganisms are listed in Table 1. and 2. for *Alentejano* and for *Ribatejano* sausage type, respectively. Mesophylic Sporeformer Bacteria and *Enterococcus* were observed on all samples. 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[6]. The role of enterococci in Portuguese smoked sausages should be evaluated. However, prevalence of staphylococci coagulase-positive was founded in eleven different samples with values higher than those allowed[5]. Is crucial to respect the hygiene and good manufacture practices in what concerns meat hygiene, temperature and time of maturation phase, thermal process and smoke phase. The presence of Coliforms and *Escherichia coli* can be explained by the handling of the paste and stuffing into natural guts and constitutes a critical point of the technological procedure. *Clostridium perfringens* and sulphite reducing clostridia results were in accordance to the bacteriological standard for portuguese food[5].

Conclusion

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 packaging technology of these types of Portuguese products. It is also necessary to study in detail the maturation process in order to establish the connection between the microflora in this phase and the one present in the final product. Finally is vital to identify and characterise the microorganisms which will remain in the final product regarding the shelf-life period and the stability and safety of the product during storage stage.

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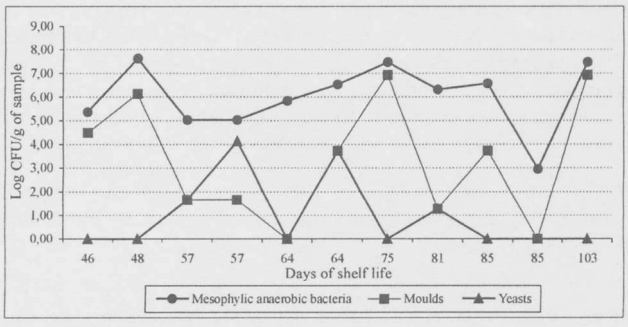

Figure 1. Total counts in *chouriço* type *Ribatejano*.

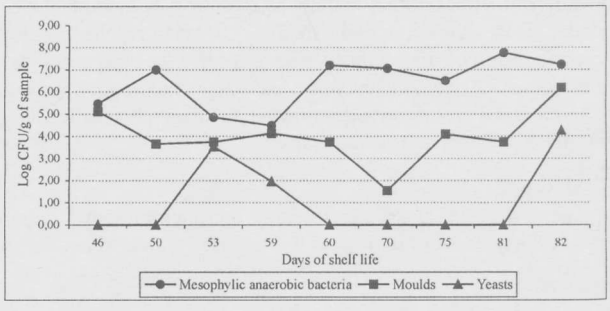

Figure 2. Total counts in *chouriço* type *Alentejano*.

Table 1. Search of microorganisms in *Alentejano* type sausage. ND, not detectable; (*), positive results per grams of sample.

Days of shelf life	Mesophylic Sporeformers (*)	Enterococcus (*)	Sulphite Reducing Clostridia (*)	Coliforms and *E. coli* (*)	Clostridium *Perfringens* (*)	Coagulase-positive Staphylococci (*)
46	0.01	0.00001	1	0.1	ND	0.1
50	0.1	0.000001	0.1	ND	1	ND
53	0.01	0.0001	1	ND	ND	ND
59	0.01	0.0001	1	ND	ND	ND
60	0.001	0.000001	ND	ND	1	ND
70	0.01	0.00001	1	ND	1	0.1
75	0.01	0.000001	ND	ND	ND	0.1
81	0.1	0.0001	1	ND	ND	1
82	0.1	0.000001	ND	ND	ND	1

Table 2. Search of microorganisms in *Ribatejano* type sausage. ND, not detectable; (*), positive results per grams of sample.

Days of shelf life	Mesophylic Sporeformers (*)	Enterococcus (*)	Sulphite Reducing Clostridia (*)	Coliforms and *E. coli* (*)	Clostridium *Perfringens* (*)	Coagulase-positive Staphylococci (*)
46	0.001	0.000001	1	ND	ND	1
48	0.01	0.000001	ND	1(*E.coli*)	ND	0.01
57	0.001	0.0001	ND	ND	ND	0.01
57	0.001	0.0001	ND	ND	ND	0.01
64	0.01	0.0001	ND	ND	ND	ND
64	0.01	0.001	1	ND	1	ND
75	0.001	0.00001	1	0.1	ND	ND
81	0.01	0.000001	ND	ND	1	1
85	0.01	0.000001	ND	ND	ND	ND
85	0.01	1	ND	ND	ND	1
103	0.001	0.00001	1	0.1	ND	ND