USE OF STARTER CULTURES TO IMPROVE PORTUGUESE TRADITIONAL SAUSAGES

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Abstract – In Mediterranean countries, such as Portugal, traditional dry-fermented sausages are highly appreciated. They are often still being manufactured in small processing units, according to traditional procedures. The aims of the present study were to evaluate the effect of different starter cultures and their optimal concentration, to reduce the microbial load in end-products, with the purpose to improve the sausages' safety, without deteriorating sensory acceptability. pH, a_w, colour, texture and microbiological profile were assessed. On the other hand, a sensory panel evaluated the products. Based on the first results, *S. xylosus* and *L. sakei* were chosen to be inoculated together with a yeast strain. In the mixed starter culture experiment, a food safety issue arose probably related to the higher a_w value (0.91). The presence of *Salmonella* spp. detected in a few end-products sausages did not allow a full sensory evaluation in the mixed starter culture experiment. However, in the two preliminary experiments, the use of starter cultures did not depreciate the panellists' overall appreciation and products acceptability.

Key Words – *Staphyloccus, Lactobacillus*, yeasts.

I. INTRODUCTION

In the origin of sausage making is the need to preserve meat, a source of animal protein, which was a very important resource to certain rural populations until about 50 years ago. Nowadays, consumers are becoming more demanding and prefer homemade regional products of high sensorial quality. The specificity of these sausages is recognised through *Protected Designation of Origin* (PDO) and *Protected Geographical Indication* (PGI). Increasingly the food industry has attempted to react to the demands and expectations of the consumer, concerning food quality and safety, through the improvement of the sausage making technology at different levels throughout the process. The use of starter cultures (microorganisms that are part of the sausages house flora and which are intentionally added to the meat batters), a practice not currently used in the Portuguese traditional sausage making industry, primarily intends to ensure and improve the higio-sanitary, nutritional and sensorial quality and may result in further advantages to the technological process, as the higher degree of conformity and the increase in the sausages' shelf-life. Furthermore, starter cultures may add new sensory properties to old products, enable products' diversification, as well as to speed up and increase production, with the consequent economic impact, not only for the manufacturer, but also for the region, due to the valorisation of products and the increase in competitiveness in todays' exigent global market. Starter cultures are part of the native microbial flora of meat and meat products, but not in the required quantities to ensure their optimal performance. They significantly contribute to the quality and safety of products mainly through their bioprotector, (inhibition of foodborne pathogens growth) probiotic (production of substances essential to consumers' health) and fermentative action (production of secondary metabolites) on the substrates.

Nowadays, in the meat transforming industry, the microorganism mainly used as starters belong to four groups: lactic acid bacteria (LAB) from the genus *Lactobacillus*, *L. sakei*, *L. plantarum* and *L.*

curvatus, coagulase-negative staphylococci (CNS), such as *S. xylosus* and *S. equorum*, moulds of the genus *Penicillium* and *Debaromyces* spp. yeasts. The first two groups are used for inoculation of meat batters, whereas the last two are mostly used for superficial inoculation of sausages (1, 2).
The aims of the present study were:
-To evaluate the effect of different starter cultures and their optimal concentration;
-To reduce the microbial load in end-products, with the purpose to improve the sausages' safety, without deteriorating sensory acceptability.

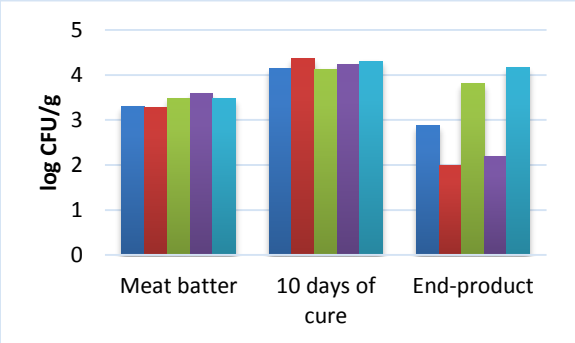
II. MATERIALS AND METHODS

Alentejano pig breed meat was used to prepare dry-fermented sausages known as "Paios". An inoculation experiment was designed in three distinct phases: (1) *Staphylococcus equorum* and *S. xylosus*, (2) *Lactobacillus curvatus* and *L. sakei* and (3) mixed culture (staphylococcus, lactobacillus and yeast). Two inoculum concentrations were used in the first two stages: 10^3 and 10^6. The strains and concentration to use in the final stage was selected based on the results obtained in the two previous phases: *S. xylosus*, *L. sakei* and a yeast strain at a concentration of 10^6 cfu/g meat batter each. Three independent batches with two replicates per treatment were produced in a local factory. Samples were collected at different stages throughout the ripening process: meat batters; mid-stage ripening (10 days after stuffing); and end-product (~38-40% weight loss).
pH and a_w were determined according to the ISO standards 2917 and 1442, respectively.
Microbiological counts of total mesophiles, total psycrotrophs, anaerobes, lactic acid bacteria, enterobacteria, yeasts and moulds and coagulase-negative staphylococci, were done according to the respective ISO standards, as well as detection of foodborne pathogens, such as *Salmonella* spp. and *L. monocytogenes*.
Colour was measured with a CR-400 colorimeter (Konica Minolta) and the chromatic coordinates L* a* b* of each sausage were determined using the CIELab System. All measurements were performed using the standard illuminant D65.
Texture profile analysis (TPA) experiments were conducted at room temperature (20 ºC ±1 ºC) according the analytical protocol disclosed in Laranjo et al. (3). A cylindrical flat-ended plunger (with a diameter of 1.13 cm and an area of 1 cm2) coupled to a Stable Micro System TA-Hdi (Stable Micro Systems, Godalming, England) was used in double compression cycle tests of 1 cm thick slices. Hardness, adhesiveness, springiness, cohesiveness, resilience and chewiness were assessed from force/time curves obtained for double 50% compressions at 1 mm/s speed, separated for 5s intervals each.
Product sensory evaluation took place in a special room. Samples were randomly disposed in white small dishes codified with a three-digit number. A group of 10 trained panellists including five men and five women (within 40 to 60 years old) were asked to rate the product according to colour intensity, off colour, aroma intensity, off aroma, hardness, succulence, flavour intensity, off flavour, salt perception and global evaluation. For each attribute a scale ranging from 0 to 100 was used. Regarding salt perception, 50% is the optimum.

III. RESULTS AND DISCUSSION

Sausages inoculated with staphylococci
As expected pH and a_w values decreased throughout the ripening process ($p<0.05$), however no significant differences were observed between different treatments.
Regarding microbiological analyses, *Salmonella* spp. was absent from end-products, contrary to *L. monocytogenes* which showed counts between 0 and 5 cfu/g. Significant differences were found in end-products between different inoculum concentrations concerning staphylococci counts (Fig.1).

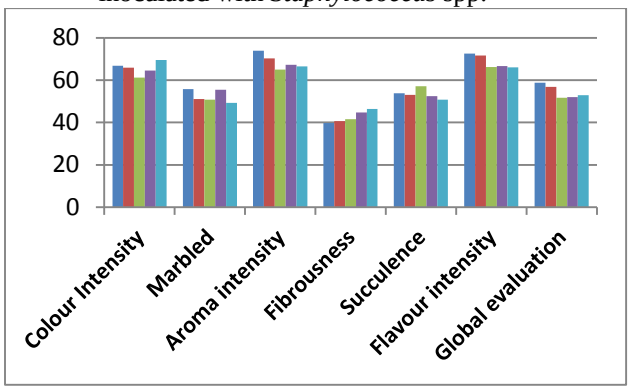
Figure 1. Coagulase-negative staphylococci by treatment along the ripening process.



Treatments in the following order: control, *S. equorum* 10^3, *S. equorum* 10^6, *S. xylosus* 10^3 and *S. xylosus* 10^6.

Sensory analysis evidenced the role of *S. xylosus* (10^6) in enhancing colour intensity (Fig. 2) as already observed with L* a* b* (data not shown).

Figure 2. Sensorial analysis of end-products inoculated with *Staphylococcus* spp.



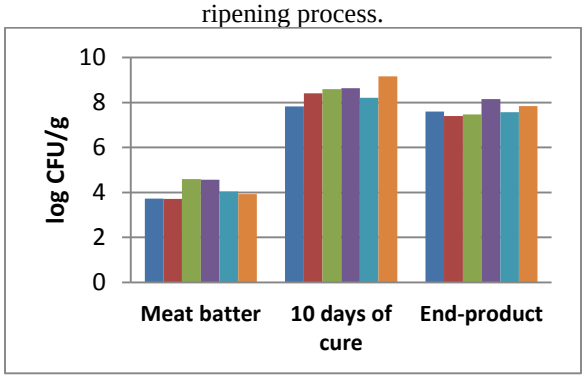
Treatments in the following order: control, *S. equorum* 10^3, *S. equorum* 10^6, *S. xylosus* 10^3 and *S. xylosus* 10^6.

Sausages inoculated with lactic acid bacteria

As reported for staphylococci inoculated sausages, pH and a_w values decreased throughout ripening ($p<0.05$), without significant differences between different treatments.

Salmonella spp. was present until 10 days of cure, but absent from end-products in the treatments inoculated with lactobacilli. Similarly, *L. monocytogenes* counts decreased along the ripening in the inoculated products. No differences were observed in LAB counts between treatments ($p>0.05$) (Fig. 3).

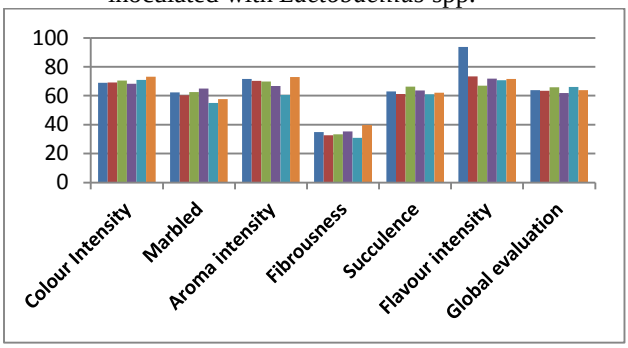
Figure 3. Lactic acid bacteria by treatment along the ripening process.



Treatments in the following order: control, control with dextrose, *L. curvatus* 10^3, *L. curvatus* 10^6, *L. sakei* 10^3 and *sakei* 10^6.

No significant differences between treatments were observed in the panellists' global evaluation ($p>0.05$) (Fig. 4), which means that inoculation with *Lactobacillus* spp. does not depreciate the products' sensory evaluation.

Figure 4. Sensorial analysis of end-products inoculated with *Lactobacillus* spp.



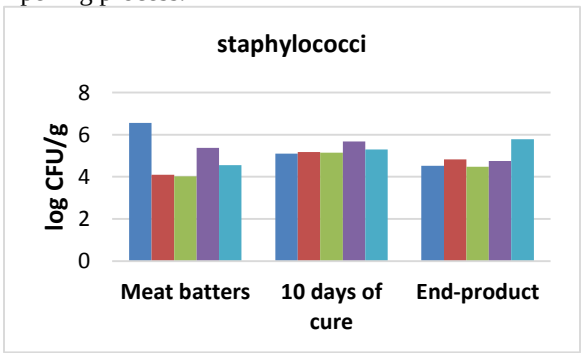
Treatments in the following order: control, control with dextrose, *L. curvatus* 10^3, *L. curvatus* 10^6, *L. sakei* 10^3 and *sakei* 10^6.

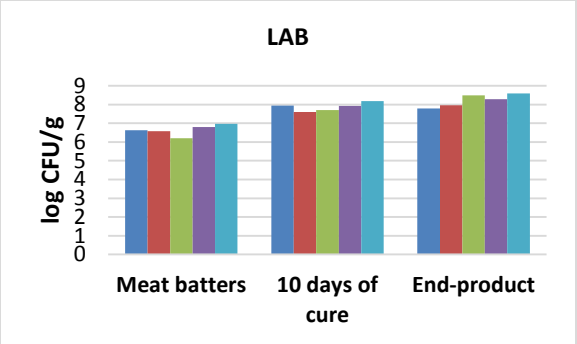
Sausages inoculated with mixed starter cultures

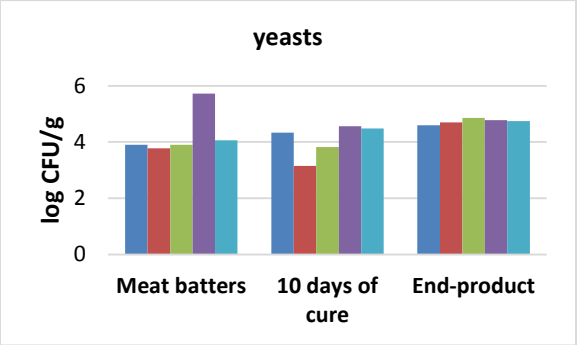
a_w values did not significantly decrease throughout ripening (~0.91), which led to the detection of *Salmonella* spp. a few end-product sausages. In the meat batters, in the treatment with *L. sakei* alone, no salmonellas were detected, however this effect seems to be lost when *L. sakei* is combined with *S. xylosus* or *S. xylosus* and the yeast. Furthermore, *L. sakei* seems to lose its effectiveness against *Salmonella* spp. throughout the ripening process, which might probably be explained by lack of competitiveness compared to other lactobacilli or by insufficient inoculum concentration.

The presence of staphylococci in end-products is favoured by the use of a mixed starter culture, the same tendency being observed for LAB, although not so marked (Fig. 5). On the contrary, yeasts counts do not differ between treatments in the end-products (Fig. 5).

Figure 5. Microbial counts by treatment along the ripening process.



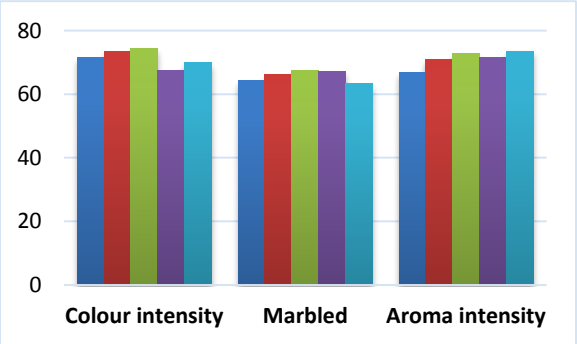




Treatments in the following order in all three graphs: control with dextrose, *S. xylosus*, *L. sakei*, *S. xylosus**L. sakei*, *S. xylosus**L. sakei**yeast strain.

Due to the presence of *Salmonella* spp. in a few end-products, a full sensory analysis was not carried out. Colour and aroma analyses were performed and no significant differences were observed between treatments (Fig. 6).

Figure 6. Sensorial analysis of end-products inoculated with mixed starter cultures.



Treatments in the following order: control with dextrose, *S. xylosus*, *L. sakei*, *S. xylosus**L. sakei*, *S. xylosus**L. sakei**yeast strain.

TPA results generally agree with the results of sensory analyses (data not shown).

VI. CONCLUSION

Microorganisms selected to be used as starter cultures must have a high growth rate to outcompete the autochthonous technological microflora. Based on the results obtained in the first two experiments, *S. xylosus* and *L. sakei* were chosen to be inoculated together with a yeast strain. The use of starter cultures did not depreciate their sensory evaluation by panellists. Sausages inoculated with mixed starter cultures were not tasted by panellists due to the unexpected presence of *Salmonella* spp. in a few end-products. We hypothesise that the meat used in this final experiment might have been contaminated due to inadequate hygiene rules either at the slaughterhouse or during transportation.

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