

INFLUENCE OF SOME COMBINED PRESERVATIVE FACTORS ON THE SHELF LIFE OF “MARANHOS”

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Key Words: meat products, sodium nitrite, ascorbic acid, sodium metabisulphite, potassium sorbate.

Introduction

“Maranhos” (Figure 1) is a goat meat sausage traditionally homemade in Beira Baixa region in the center of Portugal, where it is a very popular dish. It is a product with low price raw materials, easily perishable, with a reasonable composition, reasonable energetic value and low salt content (Salavessa & Barreto, 2003).

The ingredients hygienic quality is generally low therefore the raw product is heavily contaminated with microorganisms. Sometimes pathogens can be present. The shelf life of the raw product is very short and, therefore, it can be considered a dangerous product because it may be a source of cross contamination to other foods. The heat processing of the product improves its hygienic standards and eliminates vegetative forms of pathogens but not all spore forms. However, it cannot be overestimated because there is a risk of recontamination during assembly that may present a food safety hazard. The shelf life of this heat-processed product depends on the control of many factors. The growth of microbiological flora can be controlled by product formulation, packaging systems and chill storage conditions, so modifications based on technology and combined preservative factors should be researched in order to develop a hurdle technology able to prevent microbiological and chemical spoilage as well as the risk of food borne diseases to the consumer (Salavessa & Barreto, 2004).

Sodium nitrite (E250) is frequently used as antimicrobial and color preservative as well as flavor enhancer. Ascorbic acid (E300) enhances the action of nitrites on meat pigments, stabilizes the color of meat, and inhibits growth of microorganisms and formation of N-nitrosamines. Potassium sorbate (E202) acts like an antimicrobial preservative especially on Fungi broad spectrum. Sodium metabisulphite (E223) is generally used as an antimicrobial, antioxidant and bleaching agent (Smith & Hong-Shum, 2003).

Objectives

The combined preservative action of sodium nitrite (E250), ascorbic acid (E300), potassium sorbate (E202), and sodium metabisulphite (E223) has been tested in order to get a shelf life extension of “Maranhos”. The potential synergic effect of using these preservatives together against food borne pathogens and spoilage flora is evaluated. We

hope to improve the shelf life of this product, which is very important in order to expand the selling market.

Methodology

Two different batches of “Maranhos” were prepared and the experience was repeated four different times. “Maranhos” were prepared from raw ground adult goat meat mixed with rice and seasoned with salt, peppermint (*Mentha sp.*) and white wine. All the ingredients were then stuffed into natural casings, small bags especially made from the goat gastric compartments. Batch L1 was prepared according to the traditional recipe, 1% NaCl added, boiled for 75 minutes and then cooled in current water and vacuum packaged according the technological procedures used by a local manufacturer. In batch L2, salt was added with nitrite (1.5%) and ascorbic acid (1.5%). After the boiling process, the product was cooled inside a water solution containing sodium metabisulphite (1%) and potassium sorbate (2,5%). All the batches were brought to the laboratory under cool conditions and stored at 4°C, random samples were collected and analyzed at 0, 7, 14, 21 and 28 days of storage.

pH was measured with a pH-meter HI9023-HANNA INSTRUMENTS and Water activity (aw) was measured with the ROTRONIC HYGROSKOP DT, with the measure cell WA-14TH at 25°C of constant temperature as described by Martins & Patarata (1993).

For the microbiological analysis, 25 g sample of each sausage was aseptically transferred to a sterile plastic bag and pummeled in a stomacher LAB BLENDER-400 with 225 ml of buffered peptone water (DIFCO). Decimal dilutions of suspension were prepared using triptone salt solution (SCHARLAU) and plated in duplicate on different growth media. The following media and incubation conditions were used: total aerobic in Plate Count Agar (SCHARLAU) at 30°C for 2 days; total psychrophiles counts in Plate Count agar (SCHARLAU) at 7°C for 10 days; total anaerobes count in Anaerobic Agar acc. to Brewer (MERCK) inside an anaerobic jar at 7°C for 10 days; total thermophiles counts in Plate Count agar (SCHARLAU) at 42°C for 2 days; moulds and yeasts counts in Cook Rose Bengal agar with chlorophenicol (OXOID) at 25°C for 5 days; Enterobacteriaceae counts in Violet Red Bile agar (OXOID) at 37°C for 2 days; lactic acid bacteria counts on Man Rogosa Sharpe agar (OXOID) at 30°C for 3 days; *E. coli* detection with Kovacs reagent in Brilliant Green broth (SCHARLAU) and Peptone water (DIFCO) at 45°C for 2 days; Clostridium sulphite reducers spores detection in Sulfadiazine Polimyxine Sulphite agar (MERCK) at 45°C for 2 days; *S. aureus* detection by the coagulase test after isolation of suspicious colonies in Baird Parker agar (OXOID) and then Brain Heart Infusion (DIFCO) at 37°C for 1 day; *Salmonella* spp. identification by biochemical test API 20E (BIOMÉRIEUX) after isolation of suspicious colonies; and *Listeria monocytogenes* identification by biochemical test API Listeria (BIOMÉRIEUX) after isolation of suspicious colonies.

Means of results were compared using paired-samples T test to verify the significance of differences between treatments, L1 and L2, with SPSS 12.0.1 statistical package for Windows.

Results & Discussion

The evolution of pH, aw and the microbiological results during the storage time are shown in Table 1. It is possible to observe that the pH values drops more quickly in batch L1 than in L2, indicating a bigger incipient spoilage rate in L1, this difference was observed to be very significant at 28 days of storage. In relation to aw no significant differences were observed between batches, presenting values of 0.948 in L1 and 0.946 in L2 in day 0, remaining practically constant along the storage time. Due to the product characteristics and especially by the observation of the pH and aw values, both batches results as easily perishable meat products.

In this kind of meat product after the cooking step, usually it seems that only sporulating forms of bacteria can resist, generally came from some vegetable ingredients that can harbour sporulating forms of *Bacillus* that are used in products composition. The presence of microbial flora in product 24 hours after packaging is commonly due to the post-cooking contamination of the product that happens during the cooling and packaging steps (Borek et al., 2002).

In the aerobic plate counts very significant differences ($p < 0.01$) were observed since storage day 7, when L1 products already presents 6.68 log cfu/g. The combined preservatives tested in L2 had a positive effect decreasing the initial microbial load of the packed product, it was also verified an inhibition of spoilage flora, presenting only 1.89 log cfu/g at the end off the trail, without changes on pH parameter. It was possible to observe that psychrophiles and anaerobes total counts were always quite similar to the aerobic plate counts. These presences were higer in batch L1 being significantly ($p < 0.01$) different from batch L2. L1 started with 3.60 log cfu/g and 3.08 log cfu/g for psychrophiles and anaerobes counts respectively and finished the trail with 8.09 log cfu/g and 7.87 log cfu/g. In Moulds, no significant differences were observed between L1 and L2 without any increase in their counts during all the storage time. This is effect of the vacuum package action on moulds growth, once these kinds of microorganism are strictly aerobe. In relation to yeast counts, we observed very significant ($p < 0.01$) differences between batches L1 and L2 in days 0, 7 and 14. L1 reveled always a higher level of this kind of Fungi, which plays an important role on the shelf life of this meat product. In L2 it was noted the known inhibitory action of potassium sorbate on the growth of the Fungi population during all storage time. No growth of Enterobacteriaceae was verified in L2, the counts were significantly different ($p < 0.01$) from L1 with values of 1.56 log cfu/g and 7.23 log cfu/g respectively on day 0 and 28. Similar effect was observed in relation to lactic acid bacteria, an important dominating group of the spoilage population. The potential antimicrobial effect of sodium metabisulphite on the microbiological safety of the product is noted by no *Staphylococcus aureus* coagulase positive presence in 1g of L2 product as well as no detection of *E. coli* or *Clostridium* sulphite reducers' spoores (absent in 1 g), *Salmonella* spp. and *Listeria monocytogenes* (absent in 25 g), something that didn't happen in L1 where sometimes were detected the presence of *Listeria monocytogenes* and *Staphylococcus aureus* coagulase positive.

Conclusions

According to the results found in this work, the combined effect of tested additives in L2 have a positive effect on extension of microbiological shelf life of “Maranhos”, that can go over 30 days. Besides, this combination does not seem to decrease the sensory attributes of the product, however further work must be done in order to prove that the use of these combined substances does not take risk to the consumer as well to the typical sensory properties of the final product.

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Tables and Figures

Table 1 – pH, a_w and microbiological counts (log cfu/g).

		Days				
		0	7	14	21	28
pH	L ₁	6.31	6.28	6.16	5.94	5.85**
	L ₂	6.24	6.26	6.24	6.13	6.11**
a_w	L ₁	0.948	0.950	0.948	0.944	0.943
	L ₂	0.946	0.945	0.945	0.947	0.944
<i>Aerobic</i>	L ₁	3.73	6.68**	7.59**	8.20**	8.22**
	L ₂	1.76	2.02**	1.72**	1.44**	1.89**
<i>Termophiles</i>	L ₁	1.87	3.08**	4.55	4.89*	4.51*
	L ₂	1.01	1.48**	0.76	1.09*	0.82*
<i>Psicrophiles</i>	L ₁	3.60	6.94**	7.64**	8.27**	8.09**
	L ₂	1.00	0.54**	0.75**	1.15**	1.62**
<i>Anaerobes</i>	L ₁	3.08	6.81**	7.65**	8.27**	7.87**
	L ₂	0.64	0.41**	0.25**	0.25**	0.65**
<i>Moulds</i>	L ₁	0.50	0.50	0.62	0.65	0.25
	L ₂	1.05	0.54	0.33	0.37	0,00
<i>Yeasts</i>	L ₁	1.95**	2.97**	3.47*	3.73	3.90
	L ₂	0.50**	0.00**	0.25*	1.79	0.58
<i>Enterobactereacea</i>	L ₁	1.56	4.69**	6.31**	6.96**	7.23**
	L ₂	0.00	0.00**	0.00**	0.00**	0.00**
<i>Lactic acid bacteria</i>	L ₁	0.99	4.21**	6.37**	6.90**	7.27**
	L ₂	0.00	0.00**	0.25**	0.25**	0.00**

Significant * $p < 0.05$, very significant ** $p < 0.01$



Figure 1 – “Maranhos”.