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Behavior of *Clostridium sporogenes*, *Listeria monocytogenes* and *Salmonella* in *salpicao* – A cured pork loin made with wine marinated meat. (#609)

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Introduction

Salpicão is a dry-cured sausage in traditional Portugal. It is made from whole pieces of pork loin marinated in a brine composed by wine, garlic, bay leaf and salt. The industrial manufacture of *Salpicão* includes in its formulation of sodium or potassium nitrite and nitrate, to contribute to the formation and stability of the color and to guarantee the control of *Clostridium botulinum*. Recently, the World Health Organization (WHO) released a report by the International Agency for Research on Cancer (IARC), informing that meat products may increase the risk of colon cancer, among other reasons due to the presence of nitroso-compounds. The difficulty in finding options for the use of nitrite and the limited knowledge available about the biological safety and the sensorial characteristics of the products in which nitrite is eliminated or reduced leads to the need to study alternatives. The objective of this work was to evaluate the consequences of removing nitrite from *salpicão* on the behavior of *Clostridium sporogenes* (used as a surrogate for *Cl. botulinum*), *Listeria monocytogenes* and *Salmonella*.

Methods

Pork loins were cut transversely in portions of ±300g each and were exposed to UV illumination for 15 minutes on each side and freeze until use. The meat portions were thawed for 48 h at 4 ° C. On the day of inoculation, a 24 h culture of each microorganism to be inoculated (*Cl. sporogenes* DSM 767; *L. monocytogenes* ATCC 35152; Salmonella ATCC 49214. The last two included also two wild strains isolated from meat products)

The culture(s) of each microorganism was diluted in 1 L of 0.85% NaCl in order to obtain a concentration of about 10E6 viable microorganisms per ml. The contamination was carried out in three different blocks, separated in time, with the respective microorganism, *Cl. sporogenes*, *L. monocytogenes* or *Salmonella* spp. The meat portions were dipped into the suspension of each microorganism for about 1 minute and shaken manually. Then the pieces of meat were removed from the suspension and placed on steel net trays of about 2 cm mesh. The base brine consisted of 50% red wine from the region and 50% water, 2% salt, 1% fresh chopped garlic and 0.5% dried bay leaf. In the control assay, this brine was used without any other ingredient. In the brine in which the nitrite effect was studied, 150 mg / L of sodium nitrite was added. After 5 days marinating the pieces of loin were filled in a collagen casing. The smoking process was carried out in a chamber with an electric smoke generator, using beechwood chips. In this process, the temperature did not exceed 35ºC. The curing/drying phase was performed in a climatic chamber at 15 ± 2 ° C for 30 days. Samples were taken (in triplicate) for analysis after contamination, at filling, and 8, 21 and 30 days after smoking.

Counts of *Cl. sporogenes* were made after pasteurization of initial dilution in medium PA3679. *L. monocytogenes* and *Salmonella* were counted in Compass media (Biokar). The activity of water was measured in a rotronic Hygroscop DT,WA-40.

Results were compared by ANOVA using the software XLStat (Addinsoft)

Results

The counts of *Clostridium sporogenes, L. monocytogenes* and *Salmonella* spp. In salpicão prepared without and with sodium nitrite are presented in table 1. The use of the additive showed an improvement ($p<0.05$)in the control of *Cl. sporogenes*. That effect only detectable at the filling stage. *L. monocytogenes* was not inhibited by the presence of nitrite, when compared with the control. The experiment inoculated with salmonella was not influenced in any processing phase. After 8 days of storage (21 and 30 days) the counts were below the detection limit; At 30 days, nor *L. monocytogens* nor *Salmonella* was detected in 10g after enrichment. At 30 days the products had a mean aw of 0.89.

Table 1. Counts of Clostridium sporogenes, L. monocytogenes, and Salmonella spp. In salpicão prepared without and with sodium nitrite. Results are expressed in mean±SD of log CFU/g

Microorganism Production phase	Control	Nitrite	p
Clostridium sporogenes			
Meat after contamination	2.73±0.23		
Filling (5d in brine)	2.60±0.31	1.84±0.13	0,018
8 d after smoking	0.07±0.12	0.08±0.14	0,916
Listeria monocytogenes			
Meat after contamination	4.43±0.13		
Filling (5d in brine)	3.49±0.14	3.89±0.03	0,009
8 d after smoking	2.18±0.58	2.75±1.41	0,552
Salmonella spp.			
Meat after contamination	4.39±0.03		
Filling (5d in brine)	4.41±0.30	3.97±0.37	0,178
8 d after smoking	0.89±0.78	1.21±1.22	0,716

Notes

Conclusion

The results of the present study allow contributing to the validation of the safety of traditional products manufactured in small manufacturing units without the use of chemical additives that open interesting perspectives for the possibility to the industry to withdraw or decrease the nitrite dosage in the manufacture since it is provided the appropriate drying conditions.

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Notes