Effect of wine on the behavior of *Listeria monocytogenes* in a dry-cured sausage made with reduced nitrite

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Objectives: To evaluate the effect of wine and the main wine organic acids and ethanol on the behaviour of Listeria monocytogenes during the processing of a dry-cured sausage made with reduced addition of nitrite

Materials and Methods: A mixture of four *L. monocytogenes* strains (ATCC, plus three strains isolated from meat products) was used for inoculation. The level of inoculation was set at 4 log cfu/g. We produced three batches of the dry-cured sausage - *chouriço*

- each one with three formulations. All the formulations included 1.5% salt, 0.5% dry garlic and 0.01% bay and 60 mg/kg of sodium nitrite. The control batch was added with 7.5% of water, and the other two were added with red wine (pH 3.5, 12%ethanol) or a solution made with the main organic acids usually found in wine and ethanol (hereafter referred to as "artificial wine" - 12% etha- nol, tartaric acid 0.26%, acetic acid 0.09%, lactic acid 0.09%, citric acid 0.05%); the final pH was adjusted to 3.5 with tartaric acid. The inoculated meat rested at 4ºC for 16h to assure the adherence of the bacteria to the meat. The ingredients were mixed with the meat; the mixture was filled into collagen casings, smoked with beechwood scraps smoke, and dried for 21 days at 15ºC with 85% of relative humidity. We collected three sausages (one from each batch) for analysis after the mixture preparation (4h), after the smoking, and at 7, 14, and 21 days of drying. Once the inoculation levels were low and close to the counting methods' detection limit, an initial dilution of 1:5 was prepared in peptone water. When necessary, decimal dilutions were prepared. The count was made by seeding 0.1 ml of the dilution(s) CHROMagar Listeria after 48h incubation at 30ºC. When low counts were expected, 0.5 ml of the first dilution inoculation was spread on two Petri dishes (0.25 ml each) and slightly dried in the laminar flow for 3 min to avoid biofilm formation. LAB were enumerated in MRS (30ºC, 48h) and Enterobacteriaceae in VRBG (37ºC, 24h). The pH was measured following homogenization of 10 g samples with 100 mL of deionized water in a lab mixer for 30 s. Water activity was measured with a Hygroscope DT apparatus with aWA40 probe.

Results and Discussion: After the inoculation and before adding the ingredients, the contaminated meat presented 4.34±0.14 log cfu/ g of L. monocytogenes. Four hours after adding the other ingredients, the count was reduced by nearly one log (3.20±0.10 log cfu/ g) without any detected effect ($p=0.385$) of the wine or artificial wine. The same pattern of no differences ($p=0.170$) was observed after the smoking (2.93±0.40 log cfu/g). After the seventh drying day, the chouriço made with wine presented lower counts ($p<0.05$) than those prepared with artificial wine or water. At the last sampling time (21 days), the counts of samples prepared with wine were 2.07±0.32 log cfu/g and ca. 0.6 units higher in the other formulations. Enterobacteriaceae counts were reduced concomitantly with the aw and were generally undetectable in the finished product. This microbial group was not affected by using wine. LAB count reached around 8 log cfu/g on the seventh drying day and maintained these counts until the end of the process. No dif- ferences ($p>0.05$) were observed at any sampling time. The aw of the finished product was 0.89, and the pH was 5.6. No differenc- es were detected between different formulations. These results suggest that the wine has a moderate effect on the survival of *L. monocytogenes*. The reduction of aw combined with the growth and microbial dominance of LAB were probably the main factors affecting the pathogen's survival. However, the differences between samples prepared with wine and artificial wine suggest that the inhibitory effect is not linked only to the ethanol and organic acids but probably associated with other wine compounds, namely phenolics, that have a reputed antimicrobial activity. Considering the reduced level of nitrite addition, 60 mg/kg, as previewed in the derogation of Denmark, the results of the present work indicate that, taking into consideration the behaviour of L. monocyto- genes, that reduction is possible without compromising the safety of the product once no growth of the pathogen occurs and it ex- periences a reduction of about two logarithmic units.

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