A study on the toxigenesis by *Clostridium botulinum* in nitrate and nitrite-reduced dry fermented sausages

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Abstract

Nitrite has been traditionally used to control *Clostridium botulinum* in cured meat products. However, in the case of dry fermented sausages, environmental factors such as pH, a_w and the competitive microbiota may exert a more relevant role than nitrite in the inhibition of the growth and toxin production by *C. botulinum*. In this challenge test study, two varieties of Mediterranean dry sausages (*salchichón* and *fuet*) were inoculated with spores of *C. botulinum* Group I (proteolytic) and *C. botulinum* Group II (nonproteolytic). Sausages were prepared with 150mg/kg of $NaNO_3$ and 150 mg/kg of $NaNO_2$ (maximum ingoing amounts allowed by the European Union regulation), with a 25% and 50% reduction, and without nitrate/nitrite. The initial pH in both products was 5.6, and decreased to values below 5.0 in *salchichón* and to 5.2 in *fuet*. Lactic acid bacteria counts reached 8–9 log cfu/g after fermentation. The a_w decreased from initial values of 0.96 to about 0.88–0.90 at the end of ripening. Botulinum neurotoxin was not detected in any of the sausages, including those manufactured without nitrate and nitrite. Despite the environmental conditions were within the range for germination and growth of *C. botulinum* Group I during the first 8 days of the ripening process in *fuet* and 10–12 days in *salchichón,* acidity, a_w and incubation temperature combined to inhibit the production of toxin, independently of the concentration of curing agents. Although decreasing or even removing nitrate/nitrite from the formula did not compromise safety regarding *C. botulinum* in the conditions tested in this study, their antimicrobial role should not be underestimated in the case that other hurdles could fail or other ripening conditions were used, and also considering the effect of nitrite on other pathogens.