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Influence of reduced levels or suppression of sodium nitrite on the growth of psychrotrophic *Clostridium botulinum* group II type B in cooked ham and frankfurters (#322)

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Introduction

Sodium nitrite ($NaNO_2$) is commonly used in meat products manufacturing for its antimicrobial effect on *Clostridium botulinum*, responsible of botulism (a deadly paralytic disease) by production of neurotoxins. Its use can nevertheless be associated with nitrosamines production, which are considered as carcinogenic substances (EFSA, The EFSA Journal, 2013, 14: 1-31). The objective of the present study was to compare the growth and toxinogenesis of psychrotrophic *C. botulinum* Group II (non-proteolytic) type B in cooked ham and in frankfurters in function of the $NaNO_2$ incorporation rate (0, 30, 60 and 80 mg/kg) in order to evaluate the risk associated with this pathogen in two common cooked meat products.

Methods

A "cooked ham model" derived from Redondo-Solano *et al.* (Food Microbiol., 2013, 35: 108-115) was used in this experiment for cooked ham process simulation. A similar approach was used for developing a "frankfurter model". The basis of the formulation was ground pork issued from *biceps femoris* muscle for the cooked ham, and a preparation made with 55.6% of pork containing around 10% fat, 23.9% back fat and 29.5% ice for frankfurters. This basis was mixed with $NaNO_2$ (0, 30, 60 or 80 mg/kg), 1.35% or 1.80% of salt (except for the 80 mg/kg formulation), 500 mg/kg of sodium erythorbate and dextrose at 5 g/kg (ham) or 10 g/kg (frankfurters)). The meat mixtures were then inoculated or not (= negative control) with a cocktail of spores of three *C. botulinum* Group II (non-proteolytic) type B strains i.e. BL7, 300.05 and 815.12 (Pasteur Institute, Paris) at 3-4 log CFU/g and vacuum packed in portions of 50 g. Thermal treatment was applied on the cooked ham model in conditions simulating the core temperature of a 7 kg industrial cooked ham (core temperature of 67°C after 520 min) and a cooling treatment that required 820 min from 67°C to 4°C. Frankfurter model samples were cooked in conditions simulating industrial process (core temperature of 55°C after 20 min and 74°C after 50 min) and cooled from 74°C to 4°C within 340 min. The products were then stored 14 days at 4°C + 1h at 20°C + 33 days at 8°C for ham ; 14 days at 4°C + 1h at 20°C + 28 days at 8°C for frankfurters. Residual nitrite, nitrate and salt contents were determined according to ISO 2918:1975 (F), ISO 3091:1975 and ISO 1841-1:1996 (F), respectively. Enumeration of *C. botulinum* was performed by using culture-dependent method on tryptose sulfite agar. Water activity (a_w) and pH were measured according to NF ISO 21807:2005 and ISO 2917:1999. Toxin detection was performed by intraperitoneal injection of food extracts into two mice. Botulism symptoms were observed during 4 days. In case of positive samples, the confirmation of toxin presence was done by seroneutralisation with anti-toxins.

Results

The physicochemical parameters (a_w varying from 0.99 to 1 (ham); 0.95 to 0.99 (frankfurters); pH measured after cooking around 6 (ham) and 6.3 (frankfurters); salt content from 1.1 to 1.9% (ham), 1.2 to 1.8% (frankfurters); nitrate content from 1 to 10 mg/kg (ham), 1 to 12 mg/kg (frankfurters)) were in accordance with the values commonly observed in commercial cooked meat products. The residual $NaNO_2$ content was around 5-30-fold lower than the incorporation rate, this low level being partially explained by the use of sodium erythorbate at a high concentration. No growth and no toxinogenesis of *C. botulinum* was observed for both cooked meat models during the process and the storage whatever the levels of added nitrite (30, 60 or 80 mg/kg) and salt (1.35% or 1.80%) were. However, growth of *C. botulinum* until 5 log and toxinogenesis were observed when no nitrite was added.

Conclusion

From the present experiment, it can be concluded that, in the conditions applied during the present experiment, reduction of $NaNO_2$ incorporation at 30 mg/kg allowed to prevent the growth of *C. botulinum* during around 6 weeks. It can nevertheless not be totally excluded that neurotoxin can be produced in the case of longer storage. In contrast, removal of nitrite didn't prevent growth and toxin production in the two meat models tested. This observation is in accordance with previous results by Keto-Timonen *et al.* (J. Food Prot., 2012, 75: 1346-1349) who reported neurotoxin production in cooked ham without nitrite incorporation.

Notes