

Fate of Clostridium botulinum in fermented sausages processed with or without nitrite

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Experiments were carried out to assess whether or not the omission of nitrite from fermented sausages increases the risk of toxin formation by Clostridium botulinum in the product. Sausage mixtures ("Salami" and "Teewurst") were prepared with the addition of 0, 50 and 115 mg sodium nitrite per kg, respectively, and inoculated with approx. 10^4 spores per gram of various (proteolytic and non-proteolytic) C. botulinum types. The sausages were ripened with or without concomitant drying at 15, 20 and 25° C. Chemical and microbiological analysis of the products in various stages of ripening revealed that C. botulinum was unable to develop in fermented sausages, irrespective of the amount of nitrite added and the ripening conditions employed. In the early stages of ripening, many spores germinated but were subsequently rapidly inactivated, as were vegetative C. botulinum cells introduced into the sausage mixture.

Although no starter culture were added and very few lactobacilli were detected in the fresh sausage mixture, Lactobacillus counts increased rapidly to $> 10^8$ /g, and sausages of normal pH and water activity were obtained. Numbers of Enterobacteriaceae were somewhat higher in products manufactured with little or no nitrite.

The results show that the addition of nitrite to "European-style" fermented sausages (i. e. not heated in any stage of manufacture) is not necessary for control of C. botulinum. However, our experience and data from other laboratories indicate that nitrite contributes to the control of Salmonella in the early stages of sausage fermentation and inhibits the formation of rancidity.

Introduction

Because of the hazard of nitrosamine formation, it is desirable to add only as much nitrite to meat products as necessary. Since nitrite has been shown to be inhibitory to Clostridium botulinum in model systems and several meat products (see HAUSCHILD, 1982, for a recent review), it has been stated that nitrite addition to meat products reduces the risk of botulism. In contrast to large raw hams and home-canned meats, fermented sausages apparently do not represent a botulism hazard in Europe. The aim of our investigation was to clarify to which extent nitrite contributes to the inhibition of C. botulinum in fermented sausage types common in Germany and other countries in Continental Europe.

Materials and Methods

Sausage mixtures were prepared from lean pork (40 %), lean beef (30 %) and pork back fat (30 %). To "Salami" mixtures, 2.8 % curing salt (containing various amounts of nitrite and ascorbate), 0.3 % glucose, 0.3 % sucrose and 0.35 % spice mixture were added. "Teewurst" (a fermented, but not substantially dried, spreadable raw sausage made from finely ground meat and fat) mixtures contained 2.4 % curing salt (with various levels of nitrite and ascorbate), 0.3 % "Kristallpur" (a starch hydrolysate containing ca. 25 % each of mono- and disaccharides) and 0.32 % spice mixture. A part of each sausage batch was homogenously inoculated with ca. 5×10^3 spores each of proteolytic and non-proteolytic strains of C. botulinum. The strains used and the preparation of spores have been described (LÜCKE et al., 1982). Another part of the mixtures served as control. Material from batches of "Teewurst" was also inoculated with vegetative C. botulinum cells instead of spores. For the vegetative inoculum, equal amounts of washed suspensions from overnight cultures in Reinforced Clostridium Medium were pooled and added to the sausage mixture to obtain ca. 10^3 cells/g each of proteolytic and non-proteolytic C. botulinum strains.

The "Salami" mixtures were stuffed into vapour permeable casings (NATURIN R2) of 90 mm diameter while for "Teewurst", cellophane casings (KALLE; 45 mm diameter) with low vapour permeability were used. The sausages were ripened at 15 - 25° C (see Results) for up to five weeks. The relative humidity in the ripening chamber was adjusted to 5 - 10 % below the equilibrium relative humidity (water activity x 100) in the core of the sausages. For safety reasons, the sausages were not smoked.

To prevent excessive mould growth on their surface, the salamis were shortly dipped into a 20 % solution of potassium sorbate after two days.

At various time intervals, one or two inoculated sausages and one control sausage from each batch were analyzed. Botulinal toxin was assayed with the mouse test (see LÜCKE et al., 1982). Viable C. botulinum spores and vegetative cells were enumerated in Cooked Meat Medium (LÜCKE et al., 1982) by a five tube Most Probable Number technique. Growth of C. botulinum in the tubes was confirmed by microscopy and toxin test, where necessary. Total aerobic mesophilic bacteria, Enterobacteriaceae, Lactobacillaceae and Micrococcaceae were quantified using Standard I-nutrient agar, DHL agar acc. to SAKAZAKI, MRS agar and KRANEP agar, respectively (see MERCK Handbook, 1980). The pH was determined with a combination electrode, and water activity was measured in the core and the outer layer of the sausage by means of calibrated LUFFT a_w meters (RÖDEL et al., 1979).

Results

1. "Salami" (Figs. 1 and 2): For each experiment, three batches were prepared: one without nitrite, one with 40 mg NaNO_2 and 550 mg sodium ascorbate per kg, and one with 115 mg NaNO_2 /kg. In the first experiment, the sausages were ripened at 15° C, in the second at 25° C for seven days and subsequently at 20° C. Although the freshly prepared "Salami" mixtures often contained less than 100 presumptive Lactobacillaceae (colonies on MRS agar) per gram, the Lactobacillus count reached 10^8 /g within only seven days at 15° C and three days at 25° C respectively. The pH of the salamis dropped from 5.8 - 5.6 to ca. 5.0 within the same period, and subsequently, water loss proceeded normally both in inoculated and control sausages. Botulinum toxin was not detected in any sample, irrespective of nitrite addition and ripening temperature, and the clostridial count decreased about one log cycle during ripening. Inactivation proceeded most rapidly during acid production and at the beginning of the drying period. Apparently, the conditions prevailing in this phase of ripening still allowed spore germination, but no multiplication. The composition of the microflora of the sausages was not markedly altered by the addition of nitrite, apart from a slight decrease in the Enterobacteriaceae count (Fig. 4). The rancid aroma of the sausages prepared without nitrite is most likely due to autoxidation reactions.

2. "Teewurst" (Fig. 3): To maximize the theoretical risk of development of C. botulinum in a fermented sausage, "Teewurst" mixtures with higher initial water activity (a_w 0.965) and reduced

amounts of fermentable carbohydrates were prepared and ripened in such a way that the a_w value remained above 0.94 and the pH above 5.2 throughout fermentation and storage. The amounts of nitrite added were 0, 50 (with 550 mg sodium ascorbate), 94 and 120 mg/kg, respectively; fermentation was at 25° C for seven days, storage at 20° C. As in salamis, there was no growth and toxin formation of C. botulinum at any level of nitrite added. Vegetative C. botulinum cells introduced into the mixture died rapidly, but preformed toxin was not destroyed. In view of the known stability of the toxin against attack of proteases at low pH values, this is not surprising but should not cause concern because unprocessed meat is highly unlikely to contain botulinum toxin unless severely spoiled. Nitrite had only little influence on the composition of the microflora of the sausages (see Fig. 4): Lactobacillus spp. dominated after only three days of ripening although only about 5×10^3 of them were present per gram of fresh sausage mixture. However, as in "Salami", some nitrite (50 mg/kg, + 550 mg/kg sodium ascorbate) was necessary to yield a sensorically acceptable product.

Discussion

We found C. botulinum unable to grow and form toxin in any of our experimentally prepared raw sausages. The probability, calculated by us as described by HAUSCHILD (1982), of toxin formation by a single spore present in the raw material was below 10^{-8} for both "Salami" and "Teewurst". In view of the physicochemical properties of the raw sausages, this is not surprising because, even in the absence of nitrite, a combination of pH 5.8 and a_w 0.965 is highly unfavourable to the growth of C. botulinum (BAIRD-PARKER and FREAME, 1967; ROBERTS and INGRAM, 1973), and the conditions become even more inhibitory during ripening. From the experiments of CHRISTIANSEN et al. (1975) it can be concluded that C. botulinum only has a chance to develop in a "fermented" sausage if acid production is inhibited (e.g. by heat inactivation of the lactobacilli or by lack of fermentable carbohydrates) and the water activity remains high. The latter authors showed that under such circumstances nitrite addition indeed reduced the risk of toxin formation. Our results illustrate the strong selection pressure in favour of a Lactobacillus flora in unheated sausage mixtures. If, for any reason, no lactic acid fermentation takes place during manufacture of "European-style" raw sausage, the product deviates so much from standard that it will not be sold and eaten.

Somewhat conflicting results were reported by INCZE and DELÉNYI (1979). They did observe

transient formation of botulinum toxin in some of their experimentally inoculated Hungarian salamis. They had inoculated the stuffed sausages by means of a syringe and may thus have created niches with high spore content and possibly slightly more favourable conditions. Apart from their observation, there does not seem to be any practical or experimental evidence for the ability of clostridia to grow in fermented sausages (see CORETTI, 1971; PUOLANNE, 1977; BALDINI et al., 1979). Furthermore, SKJELKVÅLE et al. (1974) and GERICK and GOSSLING (1981) did not find any significant influence of the amount of nitrite added on the microflora and ripening parameters of fermented sausage. PUOLANNE (1977) reported that the activity of the lactic acid bacteria was slightly influenced by nitrite but stated that the amount of nitrite and nitrate added to fermented sausage was of minor importance for the prevention of food poisoning. However, there is evidence that the addition of nitrite contributes to the control of salmonellae during the early stages of ripening of fermented sausages, especially at high ripening temperatures (LEISTNER et al., 1973, 1982). The addition of about 50 mg sodium nitrite per kg is also necessary for inhibition of rancidity and accordingly for the development of the desired colour and flavour of these products (WIRTH, 1973). If added in moderate amounts, most of the nitrite and nitrate is reduced to ammonia during sausage fermentation and do not constitute a hazard any more. Therefore, the total omission of nitrite from fermented sausages is not advocated.

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Legends to figures:

- Fig. 1: pH value, water activity and log clostridial count (\pm standard deviation; n = 6) in "Salami" ripening at 15° C. Data from sausages processed with various amounts of nitrite have been pooled for calculation of the mean values given.
- Fig. 2: pH value, water activity and log clostridial count (\pm standard deviation; n = 6) in "Salami" ripening at 25° and 20° C. Data from sausages processed with various nitrite levels have been pooled for calculation of the mean values given.
- Fig. 3: pH value, water activity and log clostridial count (\pm standard deviation; n = 8) in "Teewurst" ripening at 25° and 20° C. Data from sausages processed with various nitrite levels have been pooled for calculation of the mean values given.
- Fig. 4: Fate of Enterobacteriaceae during sausage fermentation (log viable count \pm standard deviation; n = 4).
- A: "Salami", 15° C ripening temperature. Sampling after 0, 3, 7 and 23 days.
 - B: "Salami", 25°/20° C ripening temperature. Sampling after 0, 2, 5 and 14 days.
 - C: "Teewurst", 25°/20° C ripening temperature. Sampling after 0, 2, and 28 days.
- 1. no nitrite added;
 - 2. 40 - 50 mg NaNO₂ + 550 mg sodium ascorbate added per kg;
 - 3. 115 - 120 mg NaNO₂ added per kg.



