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THE EFFECT OF SODIUM NITRITE ON GERMINATION AND GROWTH OF SPORES FROM CLOSTRIDIUM BOTULINUM TYPE B IN A MEAT PRODUCT

by

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SHORTENED VERSION OF THE TITLE:

THE EFFECT OF SODIUM NITRITE ON CLOSTRIDIUM BOTULINUM

Summary

In the present work the effect of various concentrations of sodium nitrite on germination and growth of spores from Clostridium botulinum type B in a meat product was investigated. It was not possible to demonstrate any preservative action of sodium nitrite at concentrations under 200 ppm, that is the maximum level used in meat products. In samples containing 480 ppm sodium nitrite and incubated at 37°C botulinum toxin was found i 4 out of 8 samples.

Early investigations (Lewis & Moran 1928, Tanner & Evans 1934) indicated that fairly large amounts of sodium nitrite had to be used in order to obtain an inhibitory effect on bacteria. Lewis & Moran (1928) reported that 0,2 % sodium nitrite inhibited the growth of Clostridium putrefaciens, while Tanner & Evans (1934) recommended the use of 0,5 % to inhibit growth. However, these investigations were preformed without taking into account the acidity of the growth media used. In other investigations (Castellani & Niven jr. 1955, Ingram 1962, Gould 1964, Roberts & Ingram 1966) the effect of pH was demonstrated and generally showed that reducing the pH gave an increased inhibitory action of sodium nitrite. Henry & al (1954) stated that the pH - optimum for the inhibitory action of sodium nitrite was 5,6. Different food constituents such as ascorbic acid (Henry & al 1954) and glucose (Castellani & Niven 1955) seem to increase the inhibitory action. The inhibitory action of sodium nitrite is also increased under anaerobic conditions (Eddy & Ingram 1956).

The initial bacterial load of a food product influences the antimicrobial effect of nitrite (Silliker, Greenberg & Schack 1958, Bulman & Ayres 1952). Roberts & Ingram (1966) investigated the inhibitory action of sodium nitrite on the germination and growth of spores from Clostridium sporogenes at various pH and with heat treatment. At pH = 7,5 and heating at 80°C for 20 minutes, 1200 ppm sodium nitrite stopped germination and growth of more than 100 spores, while 100 ppm was sufficient if the pH was adjusted to 6,5 with the same heat treatment. These investigations have since been confirmed by Duncan & Foster (1968 a,b,c).

Perigo, Whiting & Bashford (1967) demonstrated an increased inhibitory action of sodium nitrite if nitrite was heated together with protein in a growth medium. Adding sodium nitrite after heat treatment of the growth medium did not give the same inhibitory action on vegetative cells of Clostridium sporogenes. Perigo & Roberts (1968) found the same results when investigating different types of Clostridium botulinum. While 80 - 120 ppm sodium nitrite had an inhibitory effect on Clostridium botulinum type B 751 A, when the

nitrite was added after heat treatment of the growth medium, only 7,5 - 10 ppm was sufficient to give inhibition when the nitrite was added before heating to 121°C for 20 minutes. Other investigators (Simonsen 1968, Roberts 1971) have demonstrated the same results when using laboratory media. However, when including different amounts of meat in the culture substrates the same inhibitory action after heat treatment could not be demonstrated intil the concentration of sodium nitrite in the growth substrate reached 600-800 ppm (Roberts 1971). These seems to be some disagreement in this matter, as several investigators (Silliker & al 1958, Riemann 1963, Bulman & Ayres 1952, Steinke & Foster 1951, Stumbo & al 1954, Koelensmid & van Rhee 1968) have demonstrated an inhibitory action of sodium nitrite in meat products using 200 ppm or less.

The aim of the present work was to investigate the effect of various concentrations of sodium nitrite on germination and growth of spores from Clostridium botulinum type B in a meat product.

Materials and methods

Spores from Clostridium botulinum type B strain Beans were used. Growth medium for spore production was 5 per cent trypticase (BBL), 0,5 % peptone (Difco) and 0,1 % sodiumthioglycollate (Wagenaar & Dack 1956). The pH was adjusted with hydrochloric acid to 7,0. To 2 l growth medium, 50 ml inoculate from a 12 hour culture of Clostridium botulinum type B strain Beans in Robertson's meat broth, was added.

Incubation was performed in Mc Intosh-Fildes anaerobic jars for one week at 37 °C and the following week at 22°C. The spores were harvested in a centrifuge (Sorwall RC 2 B) at 10.000 rpm for 25 minutes. The spores were washed 6 times with sterile deionized water. The spore suspension was diluted in 30 per cent glycerol and spore counts were carried out in a phase contrast microscope with a Petroff- Hauser counting chamber. The spores were added to the meat substrate to give 17 and 1700 spores per gram, respectively. Two per cent sodium chloride was added to and the meat substrate which contained about 30 per cent fat had a pH of 5,8.

Table 1 shows the concentrations of sodium nitrite added, and nitrite remaining after heat treatment. The control did not contain sodium nitrite. Determination of nitrite was performed according to the method described by the Association of Official Agriculturial Chemists (1965) .

Table 1. Concentrations of sodium nitrite added to the meat substrate and nitrite remaining after different heat treatment.

	Sodium nitrite measured (in ppm)					
Amount of sodium nitrite added to the meat substrate	Control (0)	30	60	120	240	480
Amount of sodium nitrite after 110°C for 10 minutes	0	10	15	41	97	147
Amount of sodium nitrite after 80°C for 20 minutes	0	10	19	40	104	160

The meat substrates were kept in 73 x 119 mm cans with and without vacuum. The samples were divided into three groups. One group was not given any heat treatment, the second group was heated at 80 °C for 20 minutes while the third group was heated at 110°C for 10 minutes. The samples were then incubated at 4°C, 22°C and 37°C and the cans were kept until bulging occurred. Cans without bulges were investigated for botulinum toxin after 75 days of incubation. In the toxicity tests the dilutent was 0,2 per cent gelatin in 0,06 M sodium phosphate buffer pH= 6,2. The mice were kept for 96 hours and observed for characteristic symptoms.

Results

The cans incubated at 4°C did not bulge or produce toxin. Table 2 and 3 show the time span in days before bulging of the cans and/or toxin production took place in the samples incubated at 22°C and 37°C, respectively.

Table 2. Interval before detection of bulging cans and toxin production in a meat substrate containing different amounts of sodium nitrite and two levels of spores from Clostridium Botulinum type B strain Beans. Incubation temperature 22°C.

Heat treatment	No heat treatment				80°C for 20 minutes						110°C for 10 minutes					
Atmpsphere in can	No vacuum				No vacuum				Vacuum		No vacuum				Vacuum	
Spore load per gram substrate	17		1700		17		1700		1700		17		1700		1700	
Sodium nitrite (ppm) / Bulging or toxin production	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.
0	25	+	25	+	11	+	11	+	19	+	17	+	11	+	÷	+
30	33	+	17	+	11	+	11	+	17	+	21	+	11	+	÷	+
60	40	+	33	+	11	+	11	+	19	+	48	+	11	+	÷	+
120	37	+	33	+	19	+	11	+	19	+	18	+	18	+	÷	+
240	÷	÷	÷	÷	19	+	19	+	17	+	48	+	25	+	÷	+
480	÷	÷	÷	÷	÷	÷	19	+	÷	÷	÷	÷	÷	÷	÷	÷

Table 3. *Interval before detection of bulging cans and toxin production in a meat substrate containing different amounts of sodium nitrite and two levels of spores from Clostridium Botulinum type B strain Beans. Incubation temperature 37°C.*

Heat treatment	No heat treatment				80°C for 20 minutes						110°C for 10 minutes					
Atmosphere in can	No vacuum				No vacuum				Vacuum		No vacuum				Vacuum	
Spore load per gram substrate	17		1700		17		1700		1700		17		1700		1700	
Sodium nitrite (ppm) \ Bulging or toxin production	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.	bulging days	toxin prod.
0	8	+	6	+	5	+	4	+	3	+	5	+	5	+	+	+
30	7	+	6	+	5	+	4	+	3	+	6	+	6	+	12	+
60	11	+	7	+	4	+	4	+	7	+	5	+	5	+	+	+
120	11	+	11	+	5	+	5	+	6	+	6	+	5	+	+	+
240	11	+	11	+	6	+	5	+	11	+	6	+	7	+	+	+
480	+	+	+	+	6	+	6	+	+	+	17	+	6	+	+	+

Discussion

The maximum level of sodium nitrite allowed in meat products is usually 200 ppm. In the present investigation it was not possible to demonstrate any preservative action of sodium nitrtie at the concentrations usually used in meat products. Botulinum toxin was demonstrated in most samples containing 240 ppm when incubated at 22°C and 37°C. In samples containing 480 ppm sodium nitrite and incubated at 37°C botulinum toxin was found in 4 out of 8 samples. However, incubation at 22°C, gave only one toxic can at the 480 ppm level. Formation of botulinum toxin took place at a faster rate in the meat substrates given heat treatment. This was probably due to heat activation of the spores with faster germination and growth as a result.

The results obtained are in close agreement with the results published by Simonsen (1968) and Roberts (1971), but in disagreement with several other investigations where an inhibitory effect of sodium nitrite levels under 200 ppm was demonstrated. Perigo, Whiting & Bashford (1967) found an increased inhibitory action of sodium nitrite when heating nitrite together with protein in the growth medium. This effect was not demonstrated when heating nitrite together with meat proteins.

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