USE OF NITRITE AND SORBATE FOR BOTULISM CONTROL

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INTRODUCTION

FOR MANY centuries nitrite, or its precursor, nitrate, has been used accidentally or intentionally in meat preservation. In the U.S.A., the direct use of nitrite in the production of cured meats was officially approved in 1925 (Binkerd and Kolari, 1975; Sofos et al., 1979c).

The functions of nitrite in meat relate to chemical and antimicrobial effects that influence the microbiological safety, organoleptic quality, and identity of the products. These functions have been established through scientific determinations and experience and include the production of the characteristic cured meat color; the positive effect on cured meat flavor; the prevention of "warmed-over" flavor; and the delay or inhibition of Clostridium botulinum toxin production in the event that the product is mishandled and temperature abused.

During the last decade, however, the use of nitrite in cured meats has been challenged and its removal from the list of ingredients has been requested by consumer groups and organizations. Reports implicating nitrite as a precursor of carcinogenic nitrosamines in some meat products, and especially crisp-fried bacon, as well as a controversial report implicating nitrite as a direct carcinogen of laboratory animals have been the stimuli for this challenge (Fiddler et al., 1974; Sen et al., 1974; Newberne, 1979). In the event that the use of nitrite in meat products was to be reduced, or completely banned, a suitable alternative should be sought and adequately tested in order to assure the continued production of processed meat products and the safety of the consumers (Sofos and Busta, 1980).

Our laboratory has been involved in research that evaluated sorbic acid and potassium sorbate as potential substitutes for nitrite in meat products. The nitrite function at which these compounds were aimed was the safety of the products from botulinal toxin. We also examined the effects of nitrite and sorbate in delaying toxin production in soy protein products that may be used, singly or in combination with meat, for human consumption. A report by Tompkin et al. (1974) which showed that sorbate delayed botulinal toxin production in an uncured sausage product was influential in selecting sorbate as the test substance. The Tompkin et al. report (1974) was in contrast to previous studies which indicated that sorbate in aqueous laboratory media was either ineffective or enhanced botulinal outgrowth (Emard and Vaughn, 1952; York and Vaughn, 1954). This communication summarizes our conclusions and presents a comprehensive comparison relating the incubation times required for observation of first toxic samples.

MATERIALS AND METHODS

THE EFFECTS of sodium nitrite (nitrite) and sorbic acid or potassium sorbate (sorbate) in delaying botulinal toxin production were examined in both comminuted products and bacon. In comminuted products a variety of substrates were examined, including mechanically deboned chicken meat (MDCM), beef, pork, and three types of soy protein products (textured soy protein-TSP, soy protein concentrate-SPC, and soy protein isolate-SPI). A comminuted mixture of beef and soy proteins was also studied. The comminuted products were examined using a model system that closely simulated commercial production conditions of frankfurter-type products, while the bacon study was commercial in design and magnitude (Sofos et al., 1979a; 1979d; 1979e; 1980b).

Sorbic acid was tested in the comminuted products, while the highly water soluble form of potassium sorbate was used in the brine-injected bacon. The treatments were formulated with varying nitrite (0, 20, 40, 80, 120, 156 ppm) and/or sorbate (0, 0.10, 0.15, 0.20% as sorbic acid) concentrations. The products were inoculated with heat-shocked (80°C, 15 min) C. botulinum spore suspensions consisting of mixtures of five type A (36A, 52A, 62A, 77A, 12885A) and five type B (41B, 53B, 213B, ATCC 7949, Lamanna B) strains. Target spore populations were 300/g in the comminuted products and 1,000/g in bacon. The samples were incubated at 27°C and tested for botulinal toxin at specified time intervals with the mouse bioassay. More details on basic product formulations, ingredients, and experimental procedures can be found in Sofos et al. (1979a; 1979b; 1979d; 1979e; 1980a; 1980b).

RESULTS AND DISCUSSION

FIGURES 1, 2, 3, and 4 present average times (in days) of incubation before botulinal toxin was detected in at least one sample of the respective treatments during temperature abuse at 27°C. These numbers (days to first toxic sample) represent the average of up to nine replicates per treatment.

In MDCM, where a larger number of treatments was examined, the results (Figure 1) permit a more detailed comparison of nitrite and sorbate antibotulinal effects at various concentrations. Nitrite and sorbic acid concentrations up to 80 ppm and 0.15%, respectively, proved less than adequate in delaying toxin production when tested singly in MDCM. Some delay in toxin production was observed when sorbic acid was tested at 0.20% and nitrite at 156 ppm. Addition of 20 ppm of nitrite to sorbic acid containing formulations did not produce any significant delay in toxin production. However, a combination of 40 or 80 ppm of nitrite with 0.20% sorbic acid significantly delayed toxin production for up to 17 days. These effects increased significantly when sorbic acid (0.20%) was combined with a concentration of nitrite of 156 ppm. This increased nitrite concentration (156 ppm) showed an effect even with a sorbic acid concentration of 0.10-0.15%, which was ineffective with lower levels of nitrite. Generally, experimental nitrite concentrations lower than 156 ppm or

NITRITE (ppm)	SORBIC ACID (%)	
0	0	____4
20	0	____4
40	0	____4
80	0	____4
156	0	______6
0	0.10	___3
0	0.15	___3
0	0.20	_______7
20	0.10	____4
20	0.20	_______7
40	0.10	___3
40	0.15	____4
40	0.20	_________________17
80	0.20	_________________17
156	0.10	________8
156	0.15	____________12
156	0.20	______________________________31

FIGURE 1. Days to detection of first toxic sample(s) in temperature-abused (27°C) mechanically deboned chicken meat (MDCM) frankfurter-type products inoculated with C. botulinum spores and formulated with nitrite and/or sorbic acid.

sorbic acid levels of less than 0.20% were less than highly effective in delaying botulinal toxin production in MDCM frankfurter-type products. Combinations of 0.20% sorbic acid with nitrite levels as low as 40 ppm, on the other hand, were more effective than sorbic acid or nitrite used singly.

These effects of nitrite and/or sorbic acid in comminuted beef and pork products (Figure 2) were greater than in MDCM (Figure 1). The results indicate that MDCM was more susceptible to botulinal toxin production in the presence of nitrite and/or sorbic acid than beef and pork. This is a significant observation, because it demonstrates the need for the development of antibotulinal inhibitors to be used in comminuted products made of MDCM. These differences among meats may be useful in determining the mechanism(s) through which nitrite and/or sorbic acid exert their antibotulinal properties.

Generally, it appears that botulinal toxin is produced in meats (Figures 1 and 2) more readily than in soy proteins (Figure 3). It is also evident that nitrite and/or sorbic acid are more potent inhibitors of botulinal toxin production in soy proteins than in meats. However, there appear to be some differences among the

a - Beef
b - Pork
c - Beef-soy

NITRITE (ppm)	SORBIC ACID (%)		
0	0	a	____4
		b	____4
		c	_____5
80	0	a	_____6
		b	________8
		c	____4
156	0	a	_____________13
		c	______________________________31
0	0.2	a	________8
		b	________8
		c	________8
80	0.2	a	____45
		b	____45
		c	____52

FIGURE 2. Days to detection of first toxic sample(s) in temperature-abused (27°C) beef, pork, and beef-soy protein frankfurter-type products inoculated with C. botulinum spores and formulated with nitrite and/or sorbic acid.

types of soy protein products tested. Nitrite (156 ppm) was ineffective in SPI and sorbic acid (0.20%) was ineffective in TSP (Figure 3). Results reported elsewhere (Sofos et al., 1979d; 1979e) indicated that residual nitrite depletion was more rapid in SPI than TSP and SPC. Considering the recent evidence that residual nitrite is a key factor in antibotulinal activity of nitrite-formulated products (Tompkin, 1978; Tompkin et al., 1978), it could be postulated that nitrite was ineffective in SPI due to its more rapid depletion. The reason for the ineffectiveness of sorbic acid in TSP is not apparent.

Substitution of half of the lean meat with hydrated soy proteins (Sofos et al., 1979d; 1979e) did not increase the potential for botulinal toxin production in the product (Figure 2). From the results in Figures 2 and 3 it is evident that meat products fortified with proteins are expected to be of at least the same, if not higher, botulinal safety as products formulated entirely with meat. The use of nitrite and/or sorbic acid as inhibitors of botulinal toxin production in soy-fortified meat products is equal to, if not better than, their use

NITRITE (ppm)	SORBIC ACID (%)		Days	
		a	10	a - TSP
0	0	b	10	b - SPC
		c	12	c - SPI
		a	10	
80	0	b	10	
		c	10	
		a	35	
156	0	b	24	
		c	13	
		a	10	
0	0.2	b	17	
		c	17	
		a	>60	
80	0.2	b	>60	
		c	>60	

FIGURE 3. Days to detection of first toxic sample(s) in temperature-abused (27°C) soy protein products, inoculated with C. botulinum spores and formulated with nitrite and/or sorbic acid. TSP, textured soy protein; SPC, soy protein concentrate; SPI, soy protein isolate.

in all-meat products.

Botulinal toxin production occurred in bacon (Figure 4) later than in the other meat products examined (Figures 1 and 2). The natural differences in these products (composition, processing) are probably responsible for this difference. Combinations of nitrite and potassium sorbate were effective in delaying toxin production in bacon. A combination of 80 ppm nitrite and 0.26% potassium sorbate showed no toxic samples (out of 250 tested) in an abuse (27°C) period of 60 days. From the antibotulinal standpoint, it appears feasible that nitrite levels used in bacon may be lowered if potassium sorbate is included in the formulation (Sofos et al., 1980b). Such a decrease in nitrite also decreases potential nitrosamine formation in the fried product (Robach et al., 1980). Sensory evaluation of this bacon showed that the reduction in nitrite and inclusion of potassium sorbate did not cause any adverse effects on organoleptic qualities and color (Paquette et al., 1980). However, recent unpublished USDA studies implicated bacon from similar experiments as causing aller-

NITRITE (ppm)	POTASSIUM SORBATE (%)	Days
0	0	10
120	0	16
0	0.26	13
40	0.26	14
80	0.26	>60

FIGURE 4. Days to detection of first toxic samples(s) in temperature-abused (27°) bacon inoculated with C. botulinum spores and formulated with nitrite and/or potassium sorbate.

gic reactions to some individuals. This unanticipated and unverified allegation has caused a delay in any action on the use of sorbate to partially replace nitrite in bacon production. The USDA is now working with the FDA and awaiting appropriate actions to resolve the issue.

Results reported elsewhere (Sofos et al., 1979b; 1979e) demonstrated that sorbate retarded botulinal spore germination (loss of heat resistance) in meat products. It was also shown (Sofos et al., 1979a), that in the presence of sorbate, residual nitrite depletion was slower during storage of the products. These effects may prove very significant in determining the mechanism(s) of action of nitrite and/or sorbate. Furthermore, results presented here demonstrate that combinations of nitrite and sorbate exert effects of a magnitude much larger than the sum of the effects of each compound tested individually. This synergistic activity of nitrite and sorbate needs further research to be well defined and clearly determined.

Our work has also shown that for sorbate to be effective, the pH of the product should be lower than 6.20 (Sofos et al., 1980a). Average pH values of treatments reported here were in the range of 5.60-6.20. This pH effect, of course, is in agreement with well established knowledge that the antimicrobial activity of organic acid food preservatives increases as the pH approaches their dissociation constant (pKa) which, for sorbate, is 4.75.

In conclusion, it is evident that ingoing nitrite concentrations in meat products not only may be significantly reduced, but the antibotulinal activity can also be increased when sorbate is introduced into the formulation.

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