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Antibotulinal Efficacy of Sulfur Dioxide in Meat

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The addition of sodium metabisulfite as a source of sulfur dioxide delayed botulinal outgrowth in perishable canned comminuted pork when it was temperature abused at 27°C. The degree of inhibition was directly related to the level of sulfur dioxide. Levels greater than 100 μg of sulfur dioxide per g were necessary to achieve significant inhibition when a target level of 100 botulinal spores per g was used. Sodium nitrite partially reduced the efficacy of the sulfur dioxide. Sulfur dioxide offers a new option for the control of botulinal outgrowth in cured or noncured meat and poultry products.

A variety of bacteriological media are used for enumerating clostridia in foods. These media commonly incorporate sodium sulfite (1, 5, 6, 12, 13), sodium metabisulfite (8, 17, 26), or sodium thiosulfate (6) to permit black-colony formation. The optimum level of sulfite to use in such media is somewhat controversial (5). Some workers have reported that too high a level of sulfite will inhibit certain clostridia; however, the experience of Takacs did not confirm this (18). Sulfite-containing media have been used in surveys of the incidence of *Clostridium botulinum* in meats (7, 16).

Sodium sulfite and sodium metabisulfite also are added as antimicrobial agents to fresh pork and beef sausages in the United Kingdom and Australia (2, 11). The legal limit in the United Kingdom is 450 μg/g, calculated as sulfur dioxide. The antibotulinal efficacy of sulfur dioxide in meat is not known. Unpublished data by B. C. Hobbs have indicated inhibition of *Clostridium perfringens* in British fresh sausage (4). Today, it is more common to refrigerate these unique sausages, but, traditionally, they were sold non-refrigerated; yet the British-style sausage has had a favorable record of microbiological safety (11).

Early abusive practices have led to a lack of interest in research on sulfur dioxide in meat in the United States. Also, sausages of the type sold in the United Kingdom are not made in this country. Because sulfur dioxide helps to preserve the desirable color of fresh meat, some butchers and packers used to add sulfites to help retain the fresh appearance of the meat. Laws were passed long ago to forbid this practice. Even today, the thought of adding sulfites to meat conjures bad images within regulatory agencies of this country.

A test system has been described to evaluate additives which alone, or in combination with a minimum level of nitrite, provide the same degree of protection as the level of nitrite currently permitted (20). This system has been used to measure the effect of a variety of additives (10, 19, 21-25). It has been used herein to measure the antibotulinal efficacy of sulfur dioxide as derived from sodium metabisulfite.

MATERIALS AND METHODS

Inoculum. The *C. botulinum* inoculum consisted of a mixture of five type A and five type B strains prepared as described previously (22). The mixed-spore suspension was heated at 80°C for 15 min and then added to the meat during formulation to obtain a target level of 100 spores per g of product.

Formulation and processing. Except as otherwise indicated, perishable canned comminuted pork was formulated, inoculated, processed, and chilled as described previously (3). Sodium metabisulfite (Allied Chemical Corp., New York, N.Y.) was added as the source of sulfur dioxide on the basis of the weight of meat in the formulation. Sodium metabisulfite yields about 67% sulfur dioxide (11). Thus, for example, 148 μg of sodium metabisulfite per g was added to obtain 100 μg of sulfur dioxide per g. Sodium nitrite and sodium isoascorbate (Pfizer, Inc., New York, N.Y.), the disodium calcium salt of ethylenediaminetetraacetic acid (CIBA-Geigy, Greensboro, N.C.), and ferric chloride ($FeCl_3 \cdot 6H_2O$; J. T. Baker Chemical Co., Phillipsburg, N.J.) were added on the basis of the weight of meat in the formulation.

Holding conditions. Twenty-five cans of inoculated product per test variable were held at 27°C for up to 110 days. Cans were removed from incubation as they swelled.

Microbiological analyses. Spore levels were determined as previously described (3). The geometric means for six samples from the first test and nine samples from each of the second and third tests were 49, 27, and 310 spores per g, respectively, after processing and chilling. Toxin assay consisted of removing half the product (about 40 g) from each can and, when it was not proteolyzed, dicing the product into cubes of about 4 mm. The product was mixed with an equal weight of gelatin phosphate buffer and then held for 1 or 3 days at 4°C for toxin extraction. On the next

day, each sample was tested by injecting 0.5 ml of the supernatant into each of three Swiss strain white mice (18 to 20 g). The mice were examined over a 4-day period. Death with typical symptoms of two of the three mice was considered to be evidence of toxin. Past experience with inoculated packs of this nature has shown that toxicity was caused by botulinal toxin (3, 10, 20). The first five cans to swell within 110 days from each variable were tested for toxicity. In this series of three tests, only 5 of the 114 samples tested were not toxic.

RESULTS

Figures 1, 2, and 3 show that sulfur dioxide delays botulinal outgrowth in perishable canned comminuted pork when it is temperature abused at 27°C. The degree of inhibition is proportional to the amount of sulfur dioxide in the product.

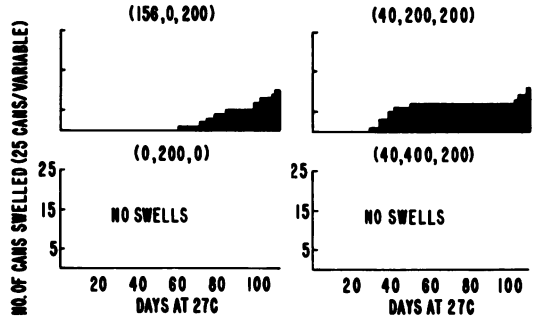

FIG. 1. *Effect of sulfur dioxide on C. botulinum (49 spores per g) in perishable canned pork held at 27°C. The values within parentheses indicate the levels (micrograms per gram) of added sodium nitrite, sulfur dioxide, and sodium isoascorbate, respectively.*

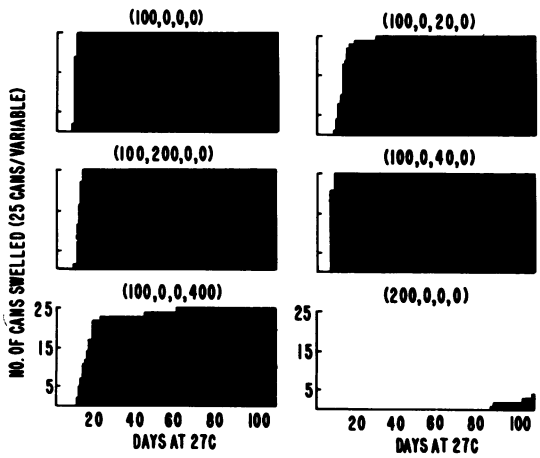

FIG. 2. *Effect of sulfur dioxide on C. botulinum (27 spores per g) in perishable canned pork held at 27°C. The values within parentheses indicate the levels (micrograms per gram) of added sulfur dioxide, sodium isoascorbate, iron, and ethylenediaminetetraacetic acid, respectively.*

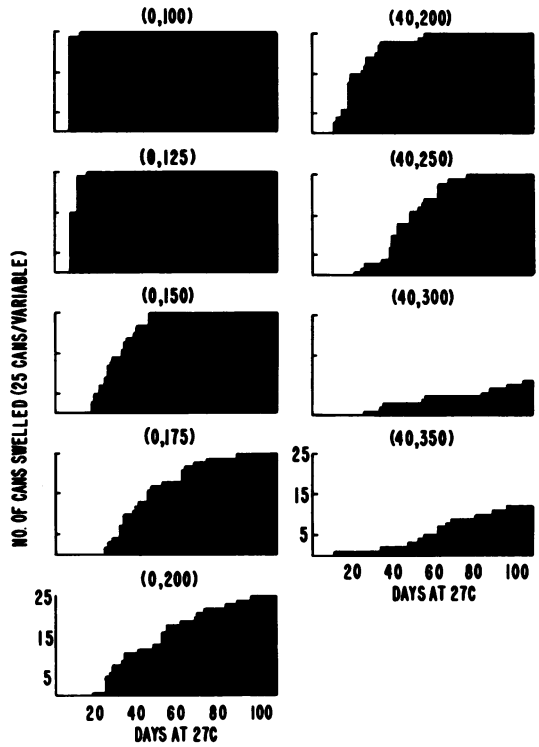

FIG. 3. *Effect of sulfur dioxide on Clostridium botulinum (310 spores per g) in perishable canned pork held at 27°C. The values within parentheses indicate the levels (micrograms per gram) of added sodium nitrite and sulfur dioxide, respectively.*

Levels of sulfur dioxide greater than 100 μg/g were necessary to achieve significant inhibition under the conditions of these tests. Less inhibition occurred in the third test (Fig. 3) as compared with the first two tests (Fig. 1 and 2) in the variables with 200 μg of sulfur dioxide alone. This might be attributable to the higher spore level in the third test.

Adding a low level of sodium nitrite (40 μg/g) provided a cure color for the product. However, the added nitrite reduced the efficacy of the sulfur dioxide (Fig. 1 and 3).

The mode of inhibition of *C. botulinum* by sulfur dioxide differs from that of sodium nitrite. The addition of ferric chloride, sodium isoascorbate, or ethylenediaminetetraacetic acid did not alter the pattern of inhibition in the presence of sulfur dioxide (Fig. 2). Since the rate of swelling was so rapid in product formulated with 100 μg of sulfur dioxide per g, two additional variables were prepared with 200 μg of sulfur dioxide per g, one of which had 40 μg of added iron per g. The added iron did not cause the loss of inhibition that occurs with sodium nitrite.

Omitted from Fig. 1 were data from two variables with 400 and 600 μg of sulfur dioxide per g, alone. Also omitted were two variables with 40 μg of sodium nitrite per g and 600 μg of sulfur dioxide per g, one of which had 200 μg of sodium isoascorbate per g and the other of which did not. None of the cans in these four variables showed swells during the 110 days of incubation at 27°C.

Omitted from Fig. 2 were two variables with 25 and 50 μg of sulfur dioxide per g, alone. Each was tested on two separate occasions. All cans from each variable swelled within 9 days at 27°C.

Product formulated with sulfur dioxide was beige in color after processing. The texture was normal with 100 μg of sulfur dioxide per g but less firm with 125 μg of sulfur dioxide per g. Product formulated with 150 to 200 μg of sulfur dioxide per g, alone, or 200 to 350 μg of sulfur dioxide per g with 40 μg of sodium nitrite per g had a softer texture and showed reduced binding of the meat. Swelled cans of product formulated with sulfur dioxide and ferric chloride (Fig. 2) had a gray appearance with black deposits throughout, due to botulinal outgrowth and sulfide production.

DISCUSSION

Sulfur dioxide is an approved additive for preserving a variety of foods. It is used for microbial inhibition, to prevent browning, as an antioxidant, and as a reducing agent (15).

The present research shows that sulfur dioxide has antibotulinal properties within the 450-μg/g level permitted in the United Kingdom and Australia. Its effectiveness in cured meats is reduced by the presence of nitrite. That sulfur dioxide and nitrite are not compatible is well known. In the United Kingdom, solutions of sodium metabisulfite are used to remove traces of nitrite from equipment (11). Also, a recent patent (M. H. Coleman, R. S. Hannan, and D. R. D. Osborne, U.S. patent 3,878,307, April 1975) describes the use of sulfur dioxide to cause more rapid depletion of residual nitrite in cured meats, such as bacon. Thus, the ratio of nitrite to sulfur dioxide must be carefully considered to achieve an acceptably cured product without decreasing antimicrobial protection. Sulfur dioxide destroys residual nitrite, thereby reducing the potential for nitrosamine formation; yet, in sufficient concentration, sulfur dioxide still provides antimicrobial protection. No other additive has been reported to have this unique combination of properties, except the addition or formation of acid (9, 14).

Sulfur dioxide may not be suitable for use in some cured meats. For example, it could not be incorporated into a curing solution containing nitrite. Meats (e.g., bacon) which are injected with or immersed in solutions of nitrite would require a two-step process such as that described in the above-mentioned patent of Coleman et al. In the case of comminuted meats, substances such as sulfite or metabisulfite could be added either mixed with the dry ingredients or, as in this research, in solution immediately after the addition of nitrite.

Sulfur dioxide could be used in meat or poultry products that are not cured and for which botulinal inhibition is clearly needed for safety. Also, it offers a new option for use with replacements for nitrite which impart the desirable flavor and appearance to cured meats.

Whether the meat is cured or noncured, the use of sulfur dioxide would require research to determine its limitations with regard to its effect on binding, appearance, and flavor in different products. Another aspect which must be considered is that sulfur dioxide has a tendency to destroy thiamine. The extent to which this may be a problem is debatable (11) and would depend upon the extent to which it may be approved for meats. From a toxicological viewpoint, sulfur dioxide is comparable to sodium nitrite. The maximum acceptable daily intake for humans has been estimated to be 0.7 and 0.2 mg/kg for sulfur dioxide and sodium nitrite, respectively (27, 28).

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