Botulism Control by Nitrite and Sorbate in Cured Meats: A Review

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**ABSTRACT**

Nitrite plays a major role in the botulinal safety of cured meat products. When used at appropriate levels, it retards *Clostridium botulinum* growth and delays production of its deadly neurotoxin. Even though the incidence of botulinal spores in meat is very low, factors such as the tonnage of cured meats consumed, the potential for mishandling such products, and the nature of the disease necessitate the use of nitrite or other equally effective compound(s) for extra safety. Residual nitrite and nitrosamine levels in cured meat products have been decreasing in recent years through control and research conducted by the meat industry and related institutions. Such levels are minimal compared to total nitrate and nitrite amounts ingested or formed in the human body. Sorbate, especially in combination with nitrite at concentrations adequate only for cured meat color and flavor development, is at least as effective as currently used nitrite levels in delaying *C. botulinum* growth and toxin production. The mechanism(s) through which nitrite and/or sorbate perform their actions upon *C. botulinum* remains to be found. A review of the pertinent research completed to date should be helpful in the search for the mechanism(s).

Nitrites, Nitrates and Nitrosamines recommended that alternate preservatives with the potential to replace nitrite in cured meats should be evaluated. One such preservative, potassium sorbate, has already been proposed for partial replacement of nitrite in bacon production. Assuming that no data disqualifying the formulation are submitted, product preserved in this way could be in regular manufacture within a year.

Considering the above, a comprehensive examination of botulism, cured meats, nitrite and sorbate and the relationships among these seemed appropriate and timely. Some aspects were examined only briefly, primarily to provide background information and knowledge to better understand the subject. Other aspects were more extensively reviewed and hopefully this will help us to understand and possibly explain the effects reported and the mechanisms involved.

**BOTULISM**

**Historical**

Botulism is a rare but very often fatal neuroparalytic disease affecting man and animals. It is caused by ingestion of a heat-labile protein neurotoxin elaborated by the vegetative cells of the microorganism *C. botulinum* (49,237,266). The disease can also be caused by wound infection (162,187). Botulism as a foodborne disease has been recognized for over 1000 years. Dollman (59) has summarized the historical documentation of botulism, and Dickson (55) gives an excellent review of the early history of the disease.

The incidence of the disease is world-wide. It can result from the consumption of a variety of foods, including canned meats, vegetables, fruits and fish, and it affects a large variety of animals, including fish, birds, and most mammals (266). Botulism was first recognized in Europe as a disease caused by the consumption of sausage products. The term “botulism” was derived from the Latin word *botulus*, meaning sausage. Some medical workers with classic Greek background called the disease “allantiasis,” from the Greek word *allantika* meaning sausage (237). This term is still being used for botulism in Greece. In recent years, plant rather than animal products have been implicated with the disease, and the historical derivation of the word botulism has lost its significance.
The organism

Van Ermengen (287) in 1897, was the first to isolate and describe in causative organism of botulism from an outbreak in Holland resulting from consumption of raw salted pork. He named the microorganism Bacillus botulinus and gave all the basic and essential facts about botulism: (a) it is an intoxication, not an infection; (b) a specific bacterium produces toxin in a food; (c) the toxin is ingested with the food and it is not inactivated by the regular digestive process; (d) it is relatively resistant to mild chemical agents and susceptible to heat; (e) it is inhibited by high salt concentrations; and (f) not all animal species are susceptible to the disease (237).

In Bergey’s Manual of Determinative Bacteriology, the causative organism of botulism belongs to the genus Clostridium, which together with the genus Bacillus, is classified in the family Bacillaceae (49). The genus Clostridium includes several pathogenic species which produce a variety of toxins. They are very strict anaerobes, catalase-negative, gram-positive rods, producing heat-resistant spores. The species C. botulinum, with the outstanding characteristic of elaborating toxins, is divided into seven types, A through G, according to the serological specificity of the toxins (237). Types A, B, E and F produce human botulism, while types C and D are implicated with animal botulism (172, 237, 266). Type G was isolated only recently from soil samples in Argentina (89), and no clinical cases have been reported.

Types A and B were the first to be isolated and identified (31, 32). Type C was first discovered in 1922, simultaneously in the United States and Australia (21, 227, 228). Type D was isolated from the carcass of a cow in South Africa, and it was designated as such by Meyer and Gunnison (164). Type E was encountered in the Ukraine and Germany in 1936 and 1937 (113), while Moller and Scheibel (169) were the first to recognize type F in Denmark. Another type, producing a toxin with serological characteristics of both A and F strains, was isolated by Gimenez and Ciccarelli (88) and it was designated as Af.

The different types of the species C. botulinum have been classified into four distinct groups, based on their cultural and serological characteristics (237, 266). The similarities among these groups are that they are all clostridia and produce toxin with similar pharmacological action. Group (I) includes the strains previously known as “ovolytic,” which are all the proteolytic type A strains and the proteolytic strains of types B and F. Group (II) includes all type E strains and the non-proteolytic strains of types B and F. Group (III) includes the type C strains and the strains of type D. Group (IV) includes the proteolytic but nonsaccharolytic type G strains.

The growth requirements of the organism include several amino acids, growth factors and inorganic salts. It can best grow in rich organic media, such as meat, and without competition it can even grow in relatively poor media such as vegetables, fruits, and moist straw (172, 237). The minimum pH for growth is 4.6 and the maximum 8.3 (172, 237, 266). The minimum temperature for growth and toxin production is 3.3 C for some type B and E strains and 10 C for type A strains, and the maximum is 55 C. The optimum temperature for growth of type B and F strains is 37 C and of type E strains 30 C (172, 209).

A water activity (aw) of 0.975 (5% NaCl) inhibits growth of type E strains, while types A and B can grow in up to 10% NaCl or up to 50% sugar concentrations and at an aw value of 0.935 (172, 266). Combinations of unfavorable pH, temperature and solute concentration are more restrictive to growth than is each variable alone (176). Although C. botulinum is a strict anaerobe, growth and toxin formation can take place in the presence of oxygen given a low enough oxidation-reduction potential, as in the interior of cooked sausages (172). In general, the vegetative cells have the heat resistance of mesophilic bacteria, being killed in a few seconds at temperatures above 60 C, while the heat resistance of the spores is high and varies with the type. The heat resistance of spores decreases with type, diminishing from A to E. Generally the nonproteolytic (Group II) strains are of lower heat resistance than the proteolytic (Group I) strains (69). Spores of type F strains have a heat resistance similar to that of type A and B spores. The Dj21 C value for type A spores is 0.232 min, which determines the botulinum cook or process of 12D equivalent to an F121 C or F0 value of 2.78 min (172, 266). The spores of some strains are among the most resistant bacterial spores encountered, surviving more than 30 years in a fluid medium (118).

The toxins

Vegetative cells of different C. botulinum types can synthesize during growth eight types of neurotoxins, based on their serological activity (237). The toxins are released mainly from the cells during autolysis or cell degeneration, after growth has reached a maximum (172, 266). They are simple proteins, composed of 21 amino acids with a molecular weight of 900,000, and appear to consist of an acid-labile autotoxic (Ea) and a non-toxicin (Eb) component. The Eb component protects the Ea component from the acidic gastric juice during ingestion (172, 237). A unique characteristic of the botulinum neurotoxins is their extreme toxicity. The estimated human lethal dose is about 5 x 10^{-8} g, or 1 g of toxin could kill more than 500 million people if properly diluted (266).

Purified toxins can be inactivated in a few minutes at 50 C at pH 6.9, while they are stable for months in phosphate buffer (0.5 M, pH 6.2-6.8) or in NaCl solution (0.1 M, pH 7.2). Higher temperatures are necessary to inactivate the toxins in food systems. Types A and B can be inactivated in 6 min at 80 C and 90 min at 65 C. More details about toxin stability are given by Riemann (199).

The activity of toxin produced by type E strains is increased by trypsin, while types A and B are believed to produce their own proteolytic enzymes (237, 266). After entering the circulatory system, the toxin molecules are
bound specifically to the neuromuscular junctions, where they act by blocking the presynaptic release of acetylcholine from cholinergic junctions, thus blocking normal voluntary muscle movement.

The disease and its symptoms

The symptoms of botulism are described in medical literature dating back to the 1700's. The usual incubation period is 18-36 h and the onset of symptoms may occur as soon as a few hours or as late as 8 days after the contaminated food has been ingested (35,266). The earlier the onset of symptoms, the higher the toxin concentration and the higher the probability of fatality.

Most common symptoms and percentage of their occurrence are: blurred vision, diplopia, photophobia (90.4%); dysphagia (76.0%); generalized weakness (57.7%); nausea and/or vomiting (55.8%); disphonia (54.8%); dizziness or vertigo (30.8%); abdominal pain, cramps, fullness (20.2%); and, diarrhea (15.4%). Other less common symptoms include: urinary retention or incontinence, sore throat, constipation and paresthesias (35). Most common signs of botulism and percentage of their occurrence are: respiratory impairment (73.1%); specific muscle weakness or paralysis (46.2%); eye muscle involvement, including prosis (44.2%); dry throat, mouth or tongue (21.2%) and dilated, fixed pupils (15.4%). Other less common signs are: ataxia, postural hypertension, nystagmus and somnolence (35). In general, botulinum toxin causes muscle paralysis, starting with the eyes and the face and progressing down to the chest and finally to the arms and legs. Eventually, when the toxin concentration is high, the diaphragm and chest muscles become paralyzed and death may occur due to asphyxiation. Disease therapy includes treatment of the symptoms associated with respiratory impairment and use of therapeutic antitoxin (266).

Incidence

In the United States, botulism began receiving increased attention during World War I when its occurrence appeared to increase. An explanation for that increase could be the rise in population in the area west of the Mississippi River, where C. botulinum type A is very common in the soil. Other reasons for the higher incidence of the disease recorded after World War I might very well be the increase in the amount of home canning as well as the better understanding and identification of the disease and its symptoms (237). From 1899 through 1973 a total of 688 outbreaks occurred in the United States (35). According to the Center for Disease Control (CDC), for the same periods (1899-1973) the number of cases was 1,784 with 978 reported deaths. For the first 50-year period the case fatality rate was above 60%, but since about 1950 it has shown a gradual decrease with a 23.5% rate for the period 1970-1973. This decline can probably be attributed to improvements in supportive care and intensive respiratory care (35,266). Of the 688 foodborne botulism outbreaks reported (1899-1973), 23.1% were caused by type A toxin, 6.3% by type B, 3.2% by type E, 0.1% by type F, and in 67.3% the type was not determined (35).

A distinctive geographical distribution of botulinal toxin types exists in the United States. Ninety percent of the recorded outbreaks of type A occurred in states west of the Mississippi River and 67% of the type B outbreaks were recorded in Eastern States. Type E outbreaks have been mostly reported from Alaska and the Great Lakes area (35). This regional distribution of outbreaks is in agreement with results of soil surveys showing a predominance of type A spores in samples from the West and of type B spores in samples from the Northeast and Central states (163). Burke (32) reported that nine of the 14 strains isolated on the Pacific coast were of type A and all nine strains isolated on the East coast were of type B. Spores of type E strains have been isolated from the marine life and sediments of the Pacific Northwest and the Great Lakes (24,69).

Of the 688 recorded outbreaks, 72% have been caused by home-processed foods, 9% by commercially processed foods and 19% by unknown foods (35,266). Vegetables, fish, fruit and condiments have been the most common vehicles of botulism intoxication, while meat and milk products have caused relatively few outbreaks (Table 1). Type E botulism was found to be important in 1963 when 22 cases were reported (216,217). Most type E outbreaks have been associated with consumption of fish or fish products. Types A and B are the major causes of botulism in heat processed foods, because the high heat resistance of their spores permits survival in underprocessed foods.

<table>
<thead>
<tr>
<th>Food Processing Type</th>
<th>Period</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home processed</td>
<td>382</td>
<td>113</td>
</tr>
<tr>
<td>Commercially processed</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>Unknown</td>
<td>47</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 1. Botulism outbreaks.

Methods to prevent botulism include: Destruction of the causative organism through sufficient heat processing (sterilization) or irradiation, mild thermal processing of the food combined with addition of appropriate food preservatives to retard growth of the heat-injured spores, product dehydration or solute addition to decrease the water activity to inhibitory levels, acidification, and refrigeration or freezing (49,266).
Besides the references mentioned, more information on all aspects of botulism can be found in the Proceedings of the First United States/Japan Conference on Toxic Microorganisms (115). Schantz and Sugiyama (223) reviewed several aspects of botulism such as history, epidemiology, diagnosis etc. They (223) also give more detailed information about the toxins and aspects associated with them. Hobbs (117) gives an excellent review of type E botulism in fish products, including a summary on botulism in general.

**SODIUM NITRITE IN CURED MEATS**

**Historical**

Use of nitrite in meat curing is lost through cons of time. Experience and scientific knowledge have indicated that when nitrite is added to meats, it performs the following functions: (a) it produces the characteristic cured meat color and affects flavor; (b) it has antioxidant activities which prevent the “warmed-over” flavor; (c) it retards *C. botulinum* growth and toxin production, which can occur if the product is mishandled and temperature-abused and (d) it may have, along with salt, a positive effect on product texture, which is not clearly defined. A short review follows of the early events that led to regular use of nitrite in meat curing, as well as the major highlights which have marked that use through the years.

Preservation of meat with salt preceded the intentional use of nitrate and nitrite by many centuries. Binkerd and Kolari (22) gave an excellent review of the history and use of nitrate and nitrite in meat curing, indicating that cooked meats and fish were preserved in sesame oil jars as early as 3000 BC in Mesopotamia. The Council for Agricultural Science and Technology (48) gave an extensive report on use of nitrite in meat curing, its effects on botulism control and human health and the possible consequences of unavailability of nitrite for meat preservation.

It is believed that meat preservation was first practiced in the saline deserts of Hither Asia and in coastal areas (22,220). During Homer’s time (900 BC) the preservation of meat with salt and smoke was well established, and later it was transferred to the Romans. In ancient Greece, salt was manufactured in “salt gardens” and used freely in the preservation of fish (134).

During the late Roman times it was noticed that red patches were formed on the surface of meat preserved with salt. Desert salts contain nitrate and borax impurities (220). The thought that the reddening effect noticed was due to nitrate impurities of the salt led to deliberate addition of nitrate to the meat to achieve color uniformity (49). The first form of nitrate to be used was saltpeter or nitre [Ca(NO₃)₂], which was formed by nitrifying bacteria and found on the walls of caves and stables (134). As time passed, use of nitrate became regular, curing techniques were developed, the importance of nitrite over that of nitrate was realized, nitrite’s effects and functions were determined, and curing of meat became a science. Finally, secondary effects of using nitrite were studied and these led to the current public interest and controversies.

In the National Provisioner Handbook of 1894, a pickle formula was given consisting of 7.66 lb. of salt, 2 lb. of sugar, and 0.33 lb. (3330 μg/g) of saltpeter (22). A saltpeter level of 5,000 μg/g for preserving and giving juiciness and flavor to bacon was common practice during that time. The transformation of meat curing from an art to a science started toward the end of the 19th century when chemists became employed by the meat industry. Development of curing methods such as dry cure, wet and pickle cure combinations, and pumping occurred during this time. Another achievement of this period was the recognition that nitrite was formed through microbial action on nitrate, and that nitrite instead of nitrate was responsible for cured meat color development (22). The first report of nitrite existence in cured meats was by Polenske (195) in 1891, who concluded that it was derived through bacterial reduction of nitrate. The effect of nitrite, and not nitrate, in producing cured meat color was reported by Lehman (153) and Kisskalt (144) in 1899. Haldane (108) in 1901 demonstrated nitrosohemoglobin formation by addition of nitrite to hemoglobin and transformation of the compound to nitrosohemochrome, which is the cured meat color. This pioneer scientific work was confirmed by Hoagland (116) in 1908, who also explained the microbial and enzymatic reduction of nitrate to nitrite, nitrous acid and nitric oxide.

On May 1, 1908 the Bureau of Animal Industry of the United States Department of Agriculture (USDA) permitted the addition of saltpeter to meat or meat food products (275). On January 19, 1923 the Bureau of Animal Industry (USDA) gave permission for experimentation on the direct use of nitrite in meat products. A series of experiments were undertaken by Kerr et al. (143) to determine the practicability, as well as the advantages and disadvantages, of the direct use of nitrite in meat curing. The aspect of human safety was given primary consideration. That extensive and pioneer work led to the following conclusions: (a) sodium or potassium nitrate could be successfully replaced by sodium nitrite in the curing of meat; (b) a quantity of 1/4 to 1 oz of sodium nitrite per 100 lb. of meat (156-625 μg/g) was sufficient for color fixation, depending on the meat and the curing process employed; (c) the levels of sodium nitrite necessary for meat curing were not higher than the nitrite levels found in meats cured with nitrates, and unconverted nitrate was avoided; (d) the curing period could be shortened by the direct use of nitrite; (e) the quality and wholesomeness of meats cured with sodium nitrite were not inferior compared to meats cured with nitrates and (f) as a result of these findings, the USDA authorized use of sodium nitrite in meat curing in Federally inspected plants in 1925. The authorization (October 19, 1925) among others, stated that “the finished product shall not contain sodium nitrite in
excess of 200 μg/g" (22).

In the 1920's, interest in the antibacterial effects of nitrite started to develop due to its preservative action in raw cured meat (74.154.256.257.258). Later, Tarr (260.261.262) reported that 0.02% (200 μg/g) nitrite at pH 6.0 inhibited several genera of bacteria such as Achromobacter, Aerobacter, Escherichia, Flavobacterium, Micrococcus, and Pseudomonas. Brooks et al. (28) in 1940 reported that nitrite reduced microbial growth on the surface of bacon.

Barnes and Magee (19) in 1954 discovered the hepatotoxic properties of nitrosamines, and Magee and Barnes (157) in 1956 discovered the carcinogenic properties of nitrosamines. A rare liver disease occurred in ruminants and mink in Norway during the 1957-1962 period. Studies to determine the cause of the disease showed that it was related to feeding animals a herring meal that was preserved with sodium nitrite. N-nitrosodi-methylamine was isolated and characterized as the toxic substance present in the feed (49). The suggestion was made that nitrosamines might also occur in human food if their precursors (amines and nitrite) are naturally present or added. Nitrosamines were found in nitrite-treated fish by Šen et al. (223) in 1970, and in various processed meats by Fazio et al. (76) in 1971. Since then, nitrosamines have been found in fried bacon and in some instances in severely fried country-style ham. Lijinsky and Epstein (155) in 1970 made one of the earliest public calls for reduction or elimination of nitrite from the human diet.

As a result of all these events, some questions were raised about the importance of nitrite and nitrate in relation to botulinal protection and nitrosamine formation in cured meat products. Late in 1969 a government-industry working group was organized to study the safety of nitrite and nitrate in foods (3). A combined effort was undertaken in 1972 by the USDA, the Food and Drug Administration (FDA) and the American Meat Institute (AMI) to study the roles of nitrite and nitrate in processed cured meats. Major interest was given to factors associated with C. botulinum toxicity and nitrosamine formation, and five cured meat product categories were examined. The cured meat products studied were: canned cured meats, smoked comminuted sausage, bacon, fermented sausage and dry cured primal cuts. The objectives of the studies, as listed by Bard (18), were: (a) to determine whether or not approved levels of sodium nitrite reduced the risk of C. botulinum toxin production, (b) to evaluate whether detectable levels of nitrosamines were formed when nitrite was used at approved levels, (c) to decide whether regulations governing use of sodium nitrite could be adjusted or modified in such a way that the benefits of such use would be retained while the risks would be minimized or eliminated and (d) to determine whether or not use of nitrate in conjunction with nitrite was of any benefit.

The experimental work was conducted by researchers of the USDA, FDA and the meat industry, and in some instances the Food Research Institute (FRI) of the University of Wisconsin was also involved. That extensive research concluded that; (a) sodium nitrite, when used at approved levels, reduced the risk of botulinal toxicity in cured meat products; (b) nitrosamine (nitrosopyrrolidine) was mostly found in crisp-fried bacon at parts per billion (ppb) levels; (c) lowering the nitrite and increasing the ascorbate or isoascorbate levels in bacon decreased nitrosopyrrolidine occurrence in the fried product and (d) sodium nitrate did not produce any apparent effects in controlling C. botulinum.

During the current decade the interest in nitrite and its effects on cured meat safety and human health has been increasing, and it has reached the point of being a controversy. Research on its role related to C. botulinum safety, nitrosamine formation, and possible reduction or total replacement has been intensified. In 1973, the Secretary of Agriculture established an Expert Panel on Nitrites, Nitrates, and Nitrosamines for a 2-year period, to examine the role of nitrite and nitrate in cured meats and their public health significance as related to botulism and nitrosamines. In July 1974 the Panel issued three recommendations (3): (a) use of nitrate be discontinued in all meat and poultry products, except dry-cured products and fermented sausages; (b) the nitrite level permitted for curing of meat should be limited to 156 μg/g in all cured meat products, except bacon and dry-cured meats for which more research was required and (c) the permitted residual nitrite level should be reduced from 200 to 100 μg/g in cooked sausage products, 125 μg/g in canned and pickle-cured products and 50 μg/g in canned, cured sterile products.

Data gathered during that period (1972-1974) indicated that nitrosopyrrolidine was rather consistently found in crisp-fried bacon. The importance of the matter as related to the Delaney clause led the Secretary of Agriculture to decide against incorporating the Panel recommendations into the regulations and to extend the charter of the Panel for an additional 2 years to further examine the issues and consider new scientific data. Later the Panel was enlarged, and its interests and expertise were broadened. Based on data from the industry and other institutions, the Panel issued its final report in September, 1977. The recommendations included lowering the ingoing nitrite levels in most products and eliminating them from all infant, junior, and sterile-processed products. The recommendations of the Panel appear in Table 2 taken from (3). In some instances the Panel did not make any recommendations due to insufficient data or requested more information before it could make its final recommendations.

The Panel also recommended the evaluation of alternate preservatives with the potential to replace or reduce nitrite in cured meats, and if the testing were adequate and positive the USDA should approve such alternates. On April 27, 1978 a petition was filed with the USDA to allow the addition of potassium sorbate to bacon in conjunction with 40 μg of nitrite/g. The petition
stated that it allowed a reduction of nitrite in bacon along with a reduced potential for nitrosamine formation on frying, that the bacon produced in this manner was essentially of the same color and flavor as bacon presently available, that based on experimental data the antibotulinal protection in such bacon was at least equivalent to present commercial products and that mold inhibition during aerobic storage was improved (6). On May 15, 1978 the USDA, after considering the above petition, proposed that bacon in the future be produced with 40 μg of nitrite/g of product 0.26% (wt/wt) potassium sorbate (277). This proposal is to become effective within 1 year unless data are submitted to show inadequate botulinal protection, or nitrosamine formation at levels detectable by presently available techniques.

A review of nitrite and its effects in cured meat products follows. Some aspects are only briefly examined, while others, related to botulism, are more extensively reviewed. Finally a summary of sorbate, its effects on foods in general, and in cured meats in particular with regard to botulism and nitrite is given.

*Nitrite effects on color, flavor, rancidity*

As previously stated, the effect of nitrate and subsequently nitrite on the cured meat color development was noticed many years ago. Haldane’s work (108) demonstrated the mechanism of cured meat color formation through nitrite and hemoglobin. Kerr et al. (143) reported that as little as 20 μg of residual nitrite/g was sufficient for an acceptable color and flavor in hams.

In recent years, several reports show the effect of nitrite on the color of cured meat products. An unpleasant gray color in unsmoked frankfurters prepared without nitrite in the cure was reported by Wasserman and Talley (293). Hustad et al. (123) reported that cured meat color was absent from nitrite-free wiener, while no color differences were noticed in wiener formulated with 50, 100, 150 μg of nitrite/g. In a bacon study (114), product formulated without nitrite received the lowest color ratings, while bacon produced with 15 μg of nitrite/g was rated slightly higher. All bacon treatments made with 30-170 μg of nitrite/g were bright pink and retained their color during storage. Improved color and organoleptic properties were reported by Kemp et al. (142) in packaged slices of dry-cured hams containing nitrate and/or nitrite. The effect of nitrate and nitrite on the color and flavor of country-style hams was studied by Eakes et al. (67). They reported that curing with nitrate and/or nitrite gave a more acceptable color than did curing with sucrose and salt only. Eakes and Blumer (66) found that 70 μg of nitrate and/or nitrite/g gave an acceptable and adequate color to pork loins and country-style hams.

It has been shown that nitrite concentrations considerably lower than those used in practice, will provide the characteristic cured meat color (159). The main portion of the total nitrite (156 μg/g) added to cured meat products is used for control of C. botulinum, and only a small fraction (about 25 μg/g) is needed for development of the characteristic color and flavor of the products. Hustad et al. (123) reported that as little as 25-50 μg of sodium nitrite/g was adequate to give the typical color and flavor in wieners. Theoretically, only 3 μg of sodium nitrite/g is needed to give the characteristic cured meat color (220). This nitrite concentration allows for only 50% conversion of myoglobin to nitric oxide myoglobin. To attain a stable color, and since nitrite is also used in other reactions, at least 25 μg/g is necessary, assuming that the distribution is adequate. However, under commercial conditions, a level of up to 75 μg/g could be needed (220). Incomplete color formation was attributed by Kerr et al. (143) to insufficient nitrite penetration into the meat, and to an unusually low hemoglobin concentration. Ingram (125) states that for 30 years it has been known that as little as 5 μg/g of nitrite/g is adequate to give a satisfactory cured color for a limited time, and that concentrations up to 20 μg/g are necessary to provide adequate color stability. However, detailed experimentation to confirm the above is lacking.

Brooks et al. (28) in 1940 repeated Haldane’s (108) conclusions that cured meat color is related to nitrosohemoglobin formation through the reaction of nitrite with hemoglobin found in muscle tissue and blood. The role of nitrite in formation of cured meat color has been well established, and the chemistry of the

### TABLE 2. Sodium nitrite, sodium nitrate, sodium ascorbate/isoascorbate usage and residual sodium nitrite in various cured meat products (3).

<table>
<thead>
<tr>
<th>Canned, cured meats</th>
<th>Perishable</th>
<th>Shelf-stable</th>
<th>Comm. sterile</th>
<th>Bacon</th>
<th>Cooked sausage</th>
<th>Other pickle cured</th>
<th>Dry cured cuts</th>
<th>Fermented sausage</th>
<th>Infant, junior, toddler foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingoing sodium nitrite (target level)</td>
<td>156 ppm</td>
<td>156 ppm</td>
<td>50 ppm</td>
<td>120 ppm</td>
<td>100 ppm</td>
<td>156 ppm</td>
<td>100 ppm</td>
<td>156 ppm</td>
<td>100 ppm</td>
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<tr>
<td>Ingoing sodium nitrate (target level)</td>
<td>0 ppm</td>
<td>0 ppm</td>
<td>0 ppm</td>
<td>0 ppm</td>
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<td>0 ppm</td>
<td>0 ppm</td>
<td>300 ppm</td>
<td>0 ppm</td>
</tr>
<tr>
<td>Ingoing sodium ascorbate or isoascorbate (target level)</td>
<td>550 ppm</td>
<td>550 ppm</td>
<td>550 ppm</td>
<td>550 ppm</td>
<td>550 ppm</td>
<td>550 ppm</td>
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<td>0 ppm</td>
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</tr>
<tr>
<td>Minimum residual sodium nitrite at time of manufacture</td>
<td>125 ppm</td>
<td>125 ppm</td>
<td>80 ppm</td>
<td>125 ppm</td>
<td>125 ppm</td>
<td>125 ppm</td>
<td>125 ppm</td>
<td>0 ppm</td>
<td>0 ppm</td>
</tr>
</tbody>
</table>

*USDA will set maximum residual sodium nitrite levels at appropriate time after manufacture if it is deemed necessary for protection of public health.*

1. In the meat portion of any infant, junior or toddler food product.

2. In the proposed rulemaking, USDA will present acceptable ranges around target level.

3. The manufacturer may present data to USDA showing need for more sodium nitrate and safety of requested levels from nitrosamine formation in product.

4. USDA to set level in proposal, but less than 50 ppm.

5. USDA to set level in proposal, but less than 100 ppm.

6. Insufficient data available at this time to make any recommendation.

7. Insufficient evidence presented to the Panel, although indications are that its role as a blocking agent would be similar to that in bacon.
curing process has been reviewed (11,13,17,61,84,168, 259). Fox (84) gave a good report on the chemistry of meat pigments and indicated that nitrite was responsible for development of cured meat color through its reduction to nitric oxide and reaction with the meat pigment myoglobin. The compound formed through the reaction of nitric oxide and myoglobin is called nitrosomyoglobin or nitric oxide myoglobin and is relatively unstable. The protein moiety of the compound is denatured by heat during thermal processing and a much more stable compound, the cured meat pigment nitrosohemochrome, is formed. However, it is unstable in the presence of light.

Brooks et al. (28) were the first to describe the relationship of nitrite to cured meat flavor. Bailey and Swain (14) reviewed the very limited studies and reported on the interaction of nitrite and meat constituents that influence flavor. Ingram (125) reported that the nature of the effects of nitrite on cured meat flavor was still unknown. He (125) also reported that about 50 μg of nitrite/g was necessary for flavor development, but satisfactory quantitative evidence for this is lacking. Most studies reported on the effect of nitrite on cured meat flavor are either on processing and sensory evaluation of different products, or on chemical analyses of the reactions between nitrite and the meat components. Wasserman (288) gave a review of these studies, but the information available was insufficient for a complete discussion of the role of nitrite on cured meat flavor development. Cho and Bratzler (39) on pork longissimus dorsi muscle and Wasserman and Talley (293) on frankfurters, performed the first rigorous tests on the role of nitrite in cured meat flavor formation, indicating that nitrite-containing products showed a more intense cured flavor.

Considerably less than 200 μg of nitrite/g is required to provide the characteristic cured meat flavor (171). Hustad et al. (123) reported that the flavor of wieners made with nitrite was judged significantly higher than the flavor of wieners formulated without nitrite. The acceptance and stability of bacon were affected by added nitrite level (114). Bacon made without nitrite had lower initial flavor scores and the flavor was lost more rapidly than bacon formulated with nitrite. Kueper and Trelease (147) reported that nitrite dramatically improved the flavor and appearance of fermented sausage. An increase in bacon and a decrease in “porky” flavor were reported by Mottram and Rhodes (171) with increasing nitrite levels. The effects of nitrate and nitrite levels on thuringer sausage were studied by Dethmers et al. (52). Addition of 50 μg of nitrate/g was necessary for the development of typical thuringer flavor and appearance. A higher nitrite concentration (100 μg/g or more) gave higher flavor and appearance ratings. However, Wasserman and Kimoto (291) reported that the flavor of laboratory-produced bacon without nitrite was rated comparable to that of a popular national brand.

Information on rancidity retardation by nitrite in cured meats is given by Cross and Ziegler (50) and Watts (294). Herring (114) found that off-flavors were higher and increased more rapidly in bacon without or with only 15 μg of nitrite/g, while the rates of off-flavor development and the decrease in acceptance scores were lowest in bacon produced with 170 μg of nitrite/g.

**BOTULISM, NITRITE, CURED MEATS**

The most important nitrite effect in cured meat products is its inhibitory action against *C. botulinum* growth and toxin production. Extensive research has been conducted in relation to the role of nitrite in controlling growth of spore-forming anaerobic bacteria. To retard bacterial growth, nitrite concentrations higher than those needed for color and flavor development are necessary. The botulinal safety of commercially processed cured meat products through the years has been attributed to the action of nitrite, as well as other factors such as low spore incidence, heat process, pH, refrigeration etc.

**Nitrite levels**

An excellent review of the interaction between nitrite levels and clostridia was presented to the USDA Expert Panel on Nitrates, Nitrites and Nitrosamines by Foster and Duncan (87) in 1974. Generally the effect of nitrite in controlling *C. botulinum* growth and toxin formation increases with increasing nitrite concentrations. This has been known since the early stages of nitrite use when it was observed that higher initial nitrite concentrations extended the shelf-life of the products.

Greenberg (98) and Johnston et al. (137) indicated that the initial nitrite concentration was more critical in controlling botulinal growth than the residual nitrite concentration. This has recently been challenged and it will be discussed in more detail later in the text.

Grever (103) reported that to guarantee complete prevention of clostridial growth, 100 or 200 μg of nitrite/g should be added, depending on the heat treatment, and the pH should be lower than 6.2. This, however, can be questioned since several other factors can be involved and change the conditions. Christiansen et al. (40,41) determined that increased levels of formulated nitrite decreased the probability of botulinal toxin formation in canned, comminuted cured meat and in bacon inoculated via pickle or after slicing. Similar results were reported with wieners (25,123). Bowen et al. (25) found that nitrite levels above 50 μg/g inhibited toxin formation. Hustad et al. (123) reported that without nitrite, toxicity was detected after 14 days at 27°C, whereas with 50 μg of nitrite/g one toxic sample was detected after 56 days, and with higher nitrite concentrations no toxic samples were found.

**pH effects**

The average pH of most cured meat products falls within the range of 5.6 to 6.6 (150). Dozier (60) in 1924 examined the pH growth range of 37 strains of vegetative inocula and 19 strains of spore inocula in phosphate buffered double strength veal infusion-2% Difco
peptone. The results revealed that the pH growth range for the vegetative inocula was 4.89 to 8.89 and for the spore inocula was 4.6 to 5.0. (128,266). Townsend et al. (274) studied the effect of pH on growth of \textit{C. botulinum} in a variety of foods and found that pH 4.7 was the lowest level at which germination and growth could be expected. It was also determined that some food products were less favorable for germination than others, and that type A strains were equally or more tolerant to acid than type B strains as far as toxin production and growth were concerned.

Nitrite has been shown to be more effective with decreasing pH in controlling \textit{C. botulinum} growth. Grindley (104) was the first to observe this nitrite effect under acidic conditions and suggested that this phenomenon could be associated with the presence of nitrous acid. Tarr (260,261,262) confirmed Grindley's (104) conclusions, showing that the preservative action of nitrite in fish was increased by acidification. He also showed that the inhibitory effect against several species of bacteria increased markedly at pH levels below 6.0. The data indicated that at pH 6.0, 0.02% (200 μg/g) sodium nitrite entirely inhibited or strongly retarded bacterial growth, while at pH 7.0 very little or no inhibition was observed. That the nitrite effect in controlling bacterial growth increases at lower pH levels has also been shown by Castellani and Niven (34), Silliker et al. (236), and Roberts and Ingram (210). A ten-fold increase in the inhibitory effect of nitrite from pH 7.0 to 6.0 was reported by Roberts and Ingram (210). This ten-fold change with drop of one unit in pH was also demonstrated by Castellani and Niven (34) with \textit{Staphylococcus aureus}; Eddy and Ingram (68) with \textit{Bacillus} species; and Perigo et al. (184) with \textit{Clostridium sporogenes} strain Putrefactive Anaerobe 3679 (PA 3679). Shank et al. (234) reported that undissociated nitrous acid was the molecular species active for inhibition of \textit{C. botulinum} spores, and consequently maximum protection was afforded at lower pH levels. They (234) also showed that as the pH approached 5.5, the nitrite effect started to diminish as it reacted with constituents of the medium.

In one study of actual meat systems undertaken by the USDA, FDA and AMI, Christiansen et al. (42) investigated the effects of nitrite, dextrose and starter culture on growth of \textit{C. botulinum} in a summer-style sausage. The data showed that dextrose in the formulation helped decrease the pH during storage and that a level of 50 μg of nitrite/g was sufficient to inhibit growth and toxin production. When the product was formulated without dextrose, the pH remained at its initial level of 5.63 and 150 μg of nitrite/g was insufficient to prevent toxin production. In formulations without nitrite, fewer of the samples containing starter culture became toxic compared to those containing only dextrose even though the pH was the same 1 week after storage. Since the pH of meat cannot be easily lowered due to its high buffering capacity (49), it is fortunate that the pH of cured meat products is low enough (5.6-6.6) for nitrite to be effective in retarding \textit{C. botulinum} growth.

**Spore incidence in meats and inoculation levels**

Raw material contamination and the inoculum size used in botulism studies can greatly influence the rate of \textit{C. botulinum} growth and toxin production. The inoculum levels employed can override the effects of the inhibiting agents used, or they can interact with them and accordingly affect the safety of the products. The natural spore inocula found in raw meats are also of importance because knowledge of their size can help decide what inoculation levels would be logical in studies. Knowledge of the levels of natural contamination would also be helpful in judging the practical significance of botulism studies and how they can relate to real life.

Generally the anaerobic spore contamination of raw meats is considered to be low and the botulinal contamination even lower. Ayres (12) determined the number of putrefactive anaerobic spores in canner-grade beef to be in the range of 0.007 to 0.06 per gram, with a maximum of 1.4 spores per gram. In freshly ground trimmings, the maximum anaerobic spore load was found to be 42, while the aerobic spores ranged from 1 to 19,000 per gram. In another study, Steinkraus and Ayres (245) found the usual number of putrefactive anaerobic spores in fresh pork trimmings to be less than 0.18 per gram. In cured pork trimmings and cured pork luncheon meat the average spore load was less than 1 per gram, while the maximum spore count found in any sample tested was 51 per gram. A variety of meat products was surveyed by Greenberg et al. (101) in the United States and Canada. Of the 2,358 samples tested, only one botulism-positive was detected. The incidence determined by the above studies is significantly lower than what subsequent studies have shown, and use of a selective medium in the isolation procedure could be responsible for that (150). Pivnick et al. (190) stated that it is probable that some cured meats are safe because of the scarcity of \textit{C. botulinum} in the raw products. Insalata et al. (129) examined 100 samples of vacuum-packaged meat and some cheese products and found only one type B-positive frankfurter sample. Of 372 meats surveyed by Abrahamson and Riemann (1), only six cooked ham samples were positive. When the sample size was increased (75 g), 19 of 26 samples showed toxin production.

United States meats and meat products surveys for \textit{C. botulinum} incidence were discussed by Roberts and Smart (214) and indicated only an occasional incidence of one cell per 0.6-3 kg of pork. A United Kingdom study of experimentally-produced bacon from commercial bacon pigs showed that 36 of the 297 samples tested contained \textit{C. botulinum} types A and B (214). The sample weight ranged from 25 to 100 g. The above study was followed by a more systematic one (215) which demonstrated that 30 of the 684 bacon samples tested...
Botulinum control in cured meats

considered when evaluating spore incidence studies on location and food product under consideration. They concluded that, although the data available were higher. Lechowich et al. (150) indicated that the average incidence was one increased sample size, the determined incidence will be affected only the extent of spoilage or toxicity at 15°C. It is expected that with improved methodology and increased sample size, the determined incidence will be higher. Lechowich et al. (150) indicated that the occurrence of spores is a function of geographical location and food product under consideration. They (150) also stated that some important factors to be considered when evaluating spore incidence studies include sample size, isolation and growth media and incubation temperatures. The above authors concluded that, although the data available were relatively inadequate and the procedures and sample sizes varied from one study to the next, an approximation could be made that the average incidence was one C. botulinum spore per 1-7 lb. of product. This appears to be a low incidence, but as Lechowich et al. (150) stated, the potential for botulism is quite apparent when the tonnage of products manufactured and consumed is considered.

Inoculation studies have demonstrated that increases in the spore inoculum level can override the inhibitory effect of nitrite and allow toxin production (25,40,41,123, 190,193,244). Some reports indicate that the commonly employed heat processes (F0 = 0.05-0.6 min) and curing salts concentrations (78-156 μg of nitrite/g/2.0-2.5% sodium chloride) would be inadequate in controlling botulism toxicity if the spore inoculum level was in the range of 100 to 10,000 per gram of meat. Ingram (125) stated that systems safe with 1-10 spores/g failed when challenged with inoculum sizes 100 to 1000 times greater. Christiansen et al. (40) working with comminuted canned cured meat, found that the nitrite concentration necessary for complete botulinal inhibition was dependent on the spore inoculum level. With 90 spores/g, toxic samples were detected with 150 but not with 200 μg of nitrite. With 5,000 spores/g, toxin was detected with 400 but not with 500 μg of nitrite/g at 27°C.

Vacuum-packaged bacon with 100 spores/g showed no toxic samples during 32-day incubation at 20°C. With the same nitrite concentrations (100 and 200 μg/g) and 10,000 spores/g, three toxic samples were detected. Bacon samples inoculated via pickle and showing an average of 52 spores/g after processing were protected with 170 or 340 μg/g but not with 120 μg of nitrite/g or less (41). With increased spore concentration (4,300 spores/g) toxic samples were detected at all nitrite concentrations used and inoculation methods tested (via pickle or after slicing). However, there are instances that the spore level effect is limited or minor. Such instances could be due to other factors highly promoting growth. Roberts et al. (212) in a meat slurry system at pH 6.0 reported that the spore inoculum level (103, 104 or 105) affected only the extent of spoilage or toxicity at 15°C.

Heat processing and storage temperatures

The safety of all hermetically sealed, shelf-stable low-acid foods depends on a thermal process designed to destroy at least 109 heat-resistant spores of C. botulinum. This is necessary to accomplish the classical 12D concept, (D value:time necessary to reduce the microbial population by 90%). To obtain this destruction in phosphate buffer at pH 7.0, a thermal treatment of 2.78 min at 250 F (121°C) is necessary (F0 = 2.78 min). In food systems, this process is doubled due to economic spoilage of low acid foods from putrefactive anaerobes. Most cured meat products would be organoleptically unacceptable after such a thermal process (F0 = 2.78 min) or even at a process of F0 = 1.0 min. Processes of F0 = 0.05-0.4 min are usually employed by most manufacturers. Such a process is 10-100 times lower than that necessary for low acid foods such as cured meats (150,190). Cured meat products receiving an adequate heat treatment are classified as commercially sterile and include deviled ham, corned beef spread, potted meat, Vienna sausage, canned corned beef, and corned beef hash (150).

The low thermal processes indicated above as being employed for most cured meat products have been proven to be adequate due to the supplementary effects of nitrite, salt and the very low incidence of anaerobic bacteria in raw meat (198,235). Thermal processes even less than F0 = 0.01 min have been described to give shelf-stable cured meat products with nitrite concentrations much higher than those commonly employed or permitted in the United States and Canada (190). The current practices employed by the meat industry are based on excellent bacteriological studies related to the thermal processing of canned meat performed by Stumbo et al. (246,247,248), Gross et al. (105,106), Vinton et al. (285,286), Schack et al. (222) and Pivnick et al. (190,193). Summaries of the safe thermal processing of canned cured meats with regard to bacterial spores were given by Duncan (62) and Riemann (198). The above authors reaffirm the fact that the stability of cured meats is a complex interaction between the heat treatment and the curing agents. Pivnick et al. (190) reported on the interaction of heat treatment and spore inoculum size. In a ground pork system inoculated with C. botulinum type A and B spores, a thermal process of F0 = 0.15 min did not prevent toxin production with 104 spores/g. An F0 = 0.30 min prevented toxinogenesis with 104 spores/g but it did not with 105 spores/g inoculum size, which was inhibited by a thermal process of F0 = 0.60 min. Viable spores were recovered after 18 months even though the meat was unspoiled and non-toxic.

It has been reported that the sensitivity of heat-damaged spores to inhibition by curing salts is higher than is the sensitivity of unheated spores (208,210). Ingram and Roberts (127) demonstrated that C. botulinum spores which were subjected to a sublethal heat process at 95°C were inhibited more by nitrite.
heated in the recovery medium for 15 min at 115 C than by unheated nitrite. Ashworth et al. (9), working with a pasteurized meat system, reported that spores heated in the meat for up to 4 h at 80 C were inhibited by a nitrite level similar to that found to inhibit unheated spores inoculated into the meat after heating. The above observations were the reasons Jarvis et al. (132) suggested that, although spores heated (e.g. 90 C) are sensitized to the inhibitory action of salt and nitrite, heated spores at pasteurization temperatures (70-80 C) may not be sensitized to the inhibitory action of the curing salts. The same researchers (132) found lower sensitivity of spores to heated and unheated nitrite in a meat system, compared with sensitivity in culture media. This would suggest caution in drawing conclusions from data obtained in laboratory media in relation to potential effects in real meat systems.

Tompkin et al. (271), studying the effect of final internal processing temperatures within the range of 63 to 74 C, reported no alternations of the degree of botulinal inhibition in inoculated, perishable canned, comminuted, cured pork abused at 27 C. Erdman and Idziak (73) reported a botulism outbreak due to an “underprocessed” liver paste product.

The minimum temperature to prevent outgrowth of types A and B C. botulinum spores in cured meats would normally be less than 10 C (177). Christiansen et al. (40,41), working with canned comminuted cured meat and bacon, reported that no toxic samples were detected at 7 C, while 27 C permitted growth and toxin production. More toxic bacon samples were found at 30 than at 20 C with either 100 or 10,000 spores/g (44). Roberts et al. (212) studying a pH 6.0 meat slurry system over a 6-month storage, found more inhibition at 15 C than at 17.5 C, while little inhibition was observed at 20, 22.5 and 25 C even with 300 µg of nitrite/g. The salt-in-water concentration of the treatments was 1.8 or 3.5%.

The average optimum temperature for C. botulinum outgrowth is considered to be around 32 C, while the average room temperature is 22 C. Considering the above, the most common temperature chosen for C. botulinum studies in actual food or model systems has been 27 C. Temperatures around this value (27 C), while approximating real life product abuse temperatures, at the same time permit reasonably rapid outgrowth of thermally injured spores.

Salt and nitrate

As previously stated, salt was the first additive used for preservation of meat and that accidentally led to use of nitrate for the cured meat color development. Through the years it has been shown that nitrate serves only as a source of nitrite and that its direct effect on botulinal growth and toxin production is minor or negligible. Silliker et al. (236) reported that nitrate played no role in retarding putrid spoilage at all levels studied. On the contrary, it actively stimulated aerobic Bacillus spores. Duncan and Foster (64) reported that sodium nitrate had no apparent effect on germination and outgrowth of PA 3679h spores at concentrations up to 2%. No practical antibotulinal activity by nitrate was found in the model system studied by Greenberg (98). In winemakers and comminuted canned cured meat, Hustad et al. (123) and Christiansen et al. (40) found the nitrate effect on botulism inhibition insufficient to be of practical importance. The USDA Expert Panel on Nitrites, Nitrates and Nitrosamines has recommended elimination of nitrate from all cured meat products except fermented sausage and dry-cured products. A 2-year period should be allowed for gathering of data to determine any need for nitrate in these two types of cured meat products (3). A need of 50-100 µg of nitrate/g in irradiation-sterilized (radappertized) ham was reported by Wierbicki and Heiligman (296). This nitrate was needed to assure color and flavor stability of product formulated with 25 µg of nitrite/g and preserved by irradiation. The same conclusions were reached by Wierbicki et al. (297), reporting that small amounts of nitrate had to be added to prevent fading of cured meat color and possibly to supplement the nitrite to scavenge electrons produced in meat by irradiation.

Salt (NaCl) has been shown to be one of the active ingredients in controlling C. botulinum growth in cured meats (30). The effect of salt is mostly associated and interrelated with other factors such as nitrite, pH, heat treatment, meat type, and spore level. More information on the interaction of the above factors will be given later, while a brief summary of the single effect of NaCl follows. Type A and B spores and vegetative inocula of C. botulinum can grow in media containing up to 10% NaCl, while type E grows only in NaCl concentrations less than 5-6% (172). Gould (93) reported that up to 8% NaCl had no effect on germination of six Bacillus species heat-shocked spores. However, concentrations ranging from 4 to 7% inhibited outgrowth, whereas concentrations of 10-15% progressively reduced and finally prevented germination. Gould (93) also noticed that the effect was similar at pH 6 and 7. Similar work was done with spores of PA 3679h strain by Duncan and Foster (64). It was found that salt concentrations above 6% prevented complete germination. In the presence of 3 to 6% NaCl most of the spores germinated and produced vegetative cells, but cell division was often blocked.

Pivnick et al. (190), working with canned, cured, shelf-stable luncheon meat inoculated with C. botulinum, concluded that higher salt concentrations were necessary to prevent botulinal outgrowth in the absence of nitrite than when nitrite was present. Brine levels of 5.8 to 6.1% were needed to prevent toxin formation with 0 or 75 µg of nitrite/g but less than 4.9% brine with 150 µg of nitrite/g. They concluded that, since the usual salt levels are close to 2.3%, salt alone is not a practical inhibitor of C. botulinum growth and toxin production, unless its concentration is in excess of 7%. Growther et al. (107) reported that “mildly salted” pork (containing about 4% salt in water) without nitrite or nitrate readily
supported *C. botulinum* growth, while "medium-salted" pork and bacon (containing about 5.5% salt in water) did not readily support growth of *C. botulinum*.

**Effects on other bacteria and spoilage**

Any inhibitory nitrite effect on other pathogenic microorganisms besides *C. botulinum* would be of great value, while inhibition of normal spoilage flora might be a disadvantage. Prevention of product spoilage for a longer time than botulinic toxin inhibition would extend the normal state of appearance of the products, and it might prevent the consumer from discarding toxic products.

Brooks et al. (28) reported reduced microbial growth on the surface of nitrite-treated bacon, while Tarr (260,261,262) listed several bacterial genera as being inhibited by 200 μg of nitrite/g at pH 6.0. The list of bacteria reported by Tarr included *Achromobacter*, *Aerobacter*, *Escherichia*, *Flavobacterium*, *Micrococcus* and *Pseudomonas*.

It is certain that the nitrite effect is not the same on all bacterial species. Research on the subject has shown that some bacteria are more resistant to nitrite than is *C. botulinum*. This has been reported for salmonellae (34), lactobacilli (34,243), *Clostridium perfringens* (103) and bacilli (103). However, when nitrite levels were increased, resistance was decreased or eliminated. Reduced survival of *C. perfringens* spores in meat containing nitrite was reported by Gough and Alford (92). The effect of nitrite on growth of salmonellae, *Staphylococcus* sp. and natural spoilage flora in frankfurters was evaluated by Bayne and Michener (20). Growth of the above organisms was at most only slightly faster in nitrite-free frankfurters at 20 C than in frankfurters with nitrite. Nitrite significantly reduced recovery of *C. perfringens* spores in cured ground pork (221). *C. perfringens* inhibition by nitrite has also been reported by Riha and Solberg (200,201,202). Nitrite concentration effect on total bacterial growth in vacuum-packaged bacon has shown that 15-60 μg of nitrite/g had very little effect on the lag phase while 120-170 μg of nitrite/g delayed bacterial growth for 4 to 5 weeks. In non-vacuum-packaged bacon, the nitrite level showed very little effect on bacterial growth (114).

Inhibitory effects of nitrite under anaerobic conditions were greater than under aerobic conditions on *Staphylococcus aureus* growth (29,34,151). The same greater nitrite effect under anaerobic conditions in retarding *S. aureus* growth was demonstrated by Barber and Deibel (16) in dry sausages. Labots (417) confirmed the above results by showing that growth of *S. aureus* in the surface layer of brine fermented sausages was lower and more affected by nitrite than growth in the surface layer of air-fermented sausages. That nitrite is more inhibitory under anaerobic than aerobic conditions has also been reported by Eddy and Ingram (68).

Product spoilage and gas production by putrefactive and gas producing organisms has often been used as an indication of *C. botulinum* growth (204,268,269,270,271).

A survey of the related literature indicates that botulinal toxin formation is not always associated with gas production and spoilage. Townsend et al. (274), working with several food products, reported toxin production at the next lower tested pH level below the lowest at which gas was formed, indicating that gas production was not a good index of botulinal growth and toxin formation in food products. Greenberg et al. (102) and Pivnick and Bird (191) have shown that cured meat products inoculated with *C. botulinum* became toxic before they were organoleptically spoiled. In canned comminuted cured meat, it was reported by Christiansen et al. (40) that not all toxic cans were swollen and not all swollen cans were toxic, but all toxic cans were putrid and proteolyzed. Collins-Thompson et al. (44) found one-third of all toxic bacon samples tested to have an acceptable odor and appearance. Roberts et al. (212), in their meat slurry system, detected toxin formation without overt spoilage, especially at 15 C.

**Cured meat products-botulism safety**

Commercially processed foods and cured meat products have shown a very good record of botulinal safety when compared to home canned foods and especially vegetables (Table 1). Greenberg (98) reported that in a 25-year period only nine botulism deaths occurred due to commercially processed foods compared to 700 from home-canned foods. In that period there was one death caused by underprocessed and canned liver paste, one by underprocessed canned vichyssoise, one by canned chicken vegetable soup, and two due to canned and vacuum-packaged unrefrigerated fish. Generally, commercially cured meat products in the United States have been proven botulism-safe. This is in contrast to the average of four outbreaks a year in France from home-cured ham (224). A variety of factors and conditions, and especially their interactions, have been reported as being responsible for such a safety record. Factors such as heat process, input and residual nitrite, NaCl, product pH, storage temperature, spore number and interrelated factors were reported by Spencer (244) as affecting and being responsible for the safety of canned cured meat products.

It has been stated several times that the microbial and botulinal stability of cured meat and fish products cannot be ascribed singly to any inhibitory parameter. It depends on the interacting effects of a number of factors, including salt concentration, water activity (a_w), heat treatment, injury of microorganisms, pH, Eh, product composition, nitrite concentration, microbial contamination, and packaging and storage conditions (15,81,193,198,235,244,298). Silliker et al. (236) surveyed the literature and concluded that no reasonable amount of any single curing ingredient or combination would completely prevent growth of *C. botulinum* and other spore-forming bacteria. From their results they (236) showed that the shelf-stability of canned comminuted cured meat resulted from the joint effect of nitrite, salt,
thermal injury of the spores and a low indigenous spore load. Ingram (125,126) summarized that in unheated systems the effect of nitrite in inhibiting botulinial growth depends on several significant factors including salt, pH, incubation temperature and number of microbial cells. Heated systems are a more complex situation, with the major inhibitory effect due to salt and nitrite, while the supplementary effect of heating to the order of $F_0 = 0.1-1.0$ min is of critical importance. The active ingredients of a curing mixture found by Bullman and Ayres (30) were salt and nitrite. Riemann (198) described an experiment where factors such as salt, nitrate, nitrite, pH, heat process ($F_0$) and spore level were tested. After 6 months at 30°C all factors, except nitrate were found to have a significant effect on spore development. Of the interactions, $F_0 \times$ NaCl, $F_0 \times$ NaN0₂, NaCl $\times$ NaN0₃ $\times$ NaN0₂, NaCl $\times$ pH, and NaCl $\times$ NaN0₂ showed significance, while nitrite and pH showed no interaction. Nitrate and pH alone showed no effect. Silliker et al. (236) indicated that some of the commonly employed thermal processes and curing salt concentrations would be inadequate if large numbers of anaerobic spores were present. The role of curing salts in preservation of canned cured meat products was studied by Duncan and Foster (63), and they found that their stability was a combined effect of heat treatment and curing salt mixtures. The significance of nitrite and pH was also discussed. Pivnick et al. (190) inoculated ground pork with C. botulinum A and B spores and thermally processed the product. Toxin production was affected by spore number, salt levels, nitrite concentration, and thermal processing. Meat inoculated with one spore per gram became toxic when nitrite and salt were omitted. Meat inoculated with $10^6$ spores per gram remained non-toxic when protected with 6.1% salt in water and no nitrite or 4.6% salt in water and 300 μg of nitrite/g. The amount of heat processing necessary to prevent toxin production in meat containing 146 μg of nitrite/g and 5.5% brine depended on the concentration of spores. The inhibitory effects of pH, NaCl and NaN0₂ combinations at 35°C in a laboratory medium on C. botulinum type A, B, E and F vegetative cells were studied by Roberts and Ingram (211). The results indicated that growth inhibition was the result of the interaction of all three factors. The authors (211) also discussed the importance of such studies. Roberts (205) used the data of Roberts and Ingram (211) to measure the antimicrobial properties of cured meat systems to develop a model for predicting growth or inhibition in such systems. Roberts et al. (212) in their pasteurized pork slurry system showed that C. botulinum types A and B developed more slowly with higher nitrite concentrations and lower incubation temperatures. The relative effects of pH, salt and nitrite on the growth of PA 3679 spores inoculated into ground cured pork were studied by Nordin et al. (175). It was found that outgrowth increased with pH and decreased with nitrite and salt concentrations. The effect of 0-400 μg of nitrite/g was similar to that of 0-4% NaCl. The pH effect in the range of 5 to 7 was greater than that of either salt or nitrite concentration. Certainly there exist combinations of factors such as salt and nitrite concentrations, pH, heat treatment, and incubation temperature that act synergistically to preclude the growth of the low number of spores found in meat products.

### Nitrite mechanism

The microbiological role and effects of nitrite in the safety of cured meats have been extensively reviewed by Ingram (125,126) and Roberts (206). Although nitrite has been used in meat curing for many years and its effect in delaying C. botulinum toxin production is well established, the exact mechanism(s) through which nitrite performs that action is still unknown.

Several possible mechanisms have been proposed through the years but final conclusions have not been reached. Early reports (34,104,133,260,261,262) found nitrite to be more effective at lower pH (<6.0) values and suggested that undisassociated nitrous acid ($HNO_2$) might be the active form. Although admitting that the role of nitrite in maintaining stability was not fully understood, Johnston et al. (137) discussed four possible roles of nitrite which were later repeated and reviewed by Ingram (125,126). According to Johnston et al. (137) the role of nitrite might be: (a) to enhance destruction of spores by heat, (b) to increase spore germination during thermal processing with subsequent destruction of the germinated spores by heat, (c) to prevent germination of outgrowth of the spores and (d) to react with some type of meat component(s) to form a more inhibitory compound(s). The enhanced destruction of spores by heat was first mentioned by Jensen and Hess (135), suggesting that bacterial spores were more sensitive to heat in the presence of curing ingredients. Roberts and Ingram (210) examined the ability of aerobic (Bacillus) and anaerobic (Clostridium) spores to grow in media containing different concentrations of curing salts (NaCl, KNO₃, NaN0₂) after various degrees of heating. The data indicated that heating at realistic temperatures and in the presence of acceptable nitrite and salt concentrations had no effect on subsequent development of the spores. The nitrite effect was found to be pH-dependent, increasing ten-fold from pH 7.0 to pH 6.0. In another study, Duncan and Foster (63) studied the effects of salt, sodium nitrate and nitrite on the growth of PA 3679h strain. Heated spores were found less tolerant to all three curing agents than unheated spores. Spores heated in the presence of, but grown in media free of the curing salts, were found to be protected against heat injury by nitrate and sodium chloride, but not by nitrite. When the spores were both heated and grown in the presence of the curing agents, nitrate and salt increased their heat resistance at low concentrations (0.5 to 1.0%) and decreased it at concentrations of 2 to 4%. Sodium nitrite was found highly inhibitory, especially at pH 6.0. Ingram (125,126) stated that the nitrite effect was not
clear and the data were confusing. This increased sensitivity of spores reported by Duncan and Foster (63) when heat treated in the presence of nitrite was not confirmed by Ingram and Roberts (127) working with *C. botulinum* type A at pH 6.0. Ingram (125,126) stated that the possibility of enhanced spore destruction during heating in the presence of nitrite was now largely eliminated.

Although some of the spores are killed during thermal processing, some of them survive and can produce toxin. Siliker et al. (236), Riemann (198), and Christiansen et al. (40,41,43) found spores after thermal processing in the presence of curing salts, and Siliker et al. (236) calculated that 20% of the inoculated spores survive in cured meats. Pivnick and Chang (192) found *C. botulinum* spores surviving a heat treatment of *F*₀ = 0.4 min in a raw meat juice with 4.5% NaCl and 150 µg of sodium nitrite/g. It is generally well established in the literature that in either the presence or absence of nitrite, enough spores can survive thermal processing for future growth if conditions allow.

Germination and growth of spores of six *Bacillus* species in the presence of nitrite were studied by Gould (93). All species were reported to germinate in the presence of <0.03% sodium nitrite (300 µg/g) at pH 6.0, and their development stopped immediately after germination and before lysis or rupture of the spore coats. Increased nitrite concentrations (0.075-0.25%) inhibited germination, and the effects were 3-5 times greater at pH 6.0 than at 7.0. Duncan and Foster (64) examined germination and outgrowth of PA 3679h spores in the presence of nitrite in microcultures. As much as 4% nitrite failed to prevent germination (complete loss of refractility) and swelling of the spores. Lower nitrite concentrations, 0.06% at pH 6.0 or 0.8-1.0% at pH 7.0, allowed emergence and elongation but prevented cell division, and the cells lysed. With nitrite levels above 0.06% (pH 6.0) or 1% (pH 7.0), the spores lost refractility and swelled, but vegetative cells did not emerge. In a subsequent report, Duncan and Foster (65) concluded that sodium nitrite stimulated germination of PA 3679h spores, indicating that the process was accelerated by using increased nitrite concentrations, a low pH and a high incubation temperature. A delay in germination (36-48 h) occurred with 0.01-0.02% nitrite at 37 °C, while with 3.45% nitrite at 45 °C and pH 6.0, most of the spores germinated within 1 h. With increasing pH the germination rate decreased, and at pH 8.0 it was negligible. A temperature of 60 °C gave the highest germination rate, and hydroxylamine completely inhibited the nitrite-induced germination, while L-alanine-induced germination was inhibited by nitrite. Duncan (62) suggested that nitrite, instead of stimulating spore germination in cured meats, may actually inhibit germination when present at the low concentrations. In a more recent study with a canned, cured meat product, Christiansen et al. (43) examined the fate of *C. botulinum* spores in the presence of 50 or 156 µg of added sodium nitrite/g. Spore germination (loss of heat resistance) occurred readily at both nitrite levels. Working with the same product, Tompkin et al. (270) also found spore germination at 27 °C with 156 µg of nitrite/g. At lower temperatures in one trial, spore germination did not take place at 4.4 and 10 C with 156 µg of nitrite/g, whereas in a second trial with the same nitrite concentration, germination was recorded at 10 C. They suggested that this discrepancy might be due to variations between *C. botulinum* strains used to inoculate the meat. Baird-Parker and Baillie (15) reported that *C. botulinum* strains varied markedly in their resistance to sodium nitrite, and were differentiated into two groups: one including the most resistant strains growing in at least 150-200 µg/g at 25 °C and a second including the heat sensitive, nonproteolytic, psychrotrophic types inhibited by 100-150 µg of nitrite/g. Generally, a close look at work related to *C. botulinum* spore germination and nitrite favors the conclusion that nitrite does not affect spore germination.

The possibility of an inhibitory nitrite effect on outgrowth of heat-damaged spores is more probable and logical. Roberts and Ingram (210), Roberts et al. (208), Duncan and Foster (63), Pivnick and Thacker (194), and Ingram and Roberts (127) have shown growth inhibition due to nitrite or salt. Interaction of nitrite, salt, pH, spore level and incubation temperature have also been implicated. Since it is almost certain that spore germination is not inhibited by nitrite in cured meat products and that botulinum toxicity delay due to nitrite is well established, the theory of outgrowth retardation by nitrite seems worthy.

Besides the possible inhibitory effect of nitrite in conjunction with other factors on the outgrowth of botulinal cells, another mechanism is the possible formation of a more inhibitory substance(s) from the reaction of nitrite with components of the system (125,126,137). It was observed by Castellani and Niven (34) and Gough and Alford (92) that sterilization of nitrite in the presence of glucose reduced the nitrite concentrations necessary to inhibit growth. Castellani and Niven (34) attributed the effect to production of more anaerobic conditions. In 1967, Perigo et al. (184) undertook extensive research to examine the inhibitory effect of sodium nitrite on growth of *C. sporogenes* (PA 3679 strain 8053) vegetative cells in a laboratory medium. When nitrite was added as a filter-sterilized solution to the medium, a ten-fold increase in its activity was calculated as the pH decreased from 6.8 to 5.8. Heating of nitrite in the basic medium for 20 min at temperatures above 90 °C resulted in an increase in the inhibitory effect of the substrate, and much smaller nitrite concentrations were effective. The effect was observed in the temperature range 95-125 °C, but a ten-fold greater concentration was found at 100-110 °C at pH 6.0. In the summary, Perigo et al. (184) postulated that sufficient heating of nitrite in the medium produced some unknown inhibitory substance which differed from inorganic nitrite in three important respects. First, its...
activity was less pH-dependent, compared to inorganic nitrite. Second, its response was less variable. Third, it was an extremely potent inhibitor, formed even with only 3.5 µg of nitrite/g heated for 20 min at 109 C. The final statement of the authors (184) was that “it would be naive to suppose that the mechanism described above could wholly account for the remarkable stability of sublethally processed cured meats, but it may well play an important complementary role to the spore sensitivity mechanism described by Roberts and Ingram (210).” In a subsequent study, Perigo and Roberts (185) tested thirty clostridial strains including fourteen strains of *C. botulinum* types A, B, E and F and eight strains of *Clostridium welchii*. The enhancement of the inhibitory effect of nitrite after heating in laboratory media was extended and confirmed. The inhibitory substance supposedly formed by autoclaving nitrite with laboratory media, was designated as “Perigo Factor” (PF) by Johnston et al. (137), and it is also known as “Perigo Inhibitor” (PI) or “Perigo Type Inhibitor” (PTI) for meat products (37). Roberts and García (207) screened a range of *Bacillus* species and fecal streptococci and two strains of *Salmonella typhimurium* for their resistance to the Perigo Inhibitor. At pH 6.0 it inhibited nine of the 14 strains of *Bacillus* sp.; *B. circulans, B. polymyxa, B. macerans, B. pantothenticus* and *Bacillus* F were resistant. *Streptococcus durans* was inhibited, while *Streptococcus faecalis, Streptococcus faecalis var. zymogenes* and the salmonellae strains were not.

Roberts and Smart (213) examined whether nitrite heated in a laboratory medium was an equally effective inhibitor to clostridial spores as it had been shown for vegetative cells (184,185). Spores of *C. botulinum* types A and F and *C. sporogenes* were tested, and the effectiveness of heated nitrite was confirmed. It was also observed that the inhibitory activity of heated nitrite medium was not stable indefinitely. Growth sometimes took place on reincubation with vegetative cells, and some spores remained viable over a 3-month period. A study was undertaken by Huhtanen (119) to develop a more rapid assay for Perigo-type Inhibitors, since the original procedure developed by Perigo et al. (184) required 10 days of incubation. A medium consisting of 0.5% yeast extract or tryptone, 0.2% glucose, 0.12% KH2PO4 and 0.1% cysteine-HCl or sodium thiglycollate was used and vegetative cells for *C. botulinum* type A were tested. It was found that yeast extract or tryptone, together with a reducing agent such as cysteine, sodium thiglycollate, or glucose autoclaved with nitrite at 15 psi for 15 min produced a Perigo Inhibitor. Tryptone was more active than yeast extract, and of the reducing agents tested, cysteine was more effective than thioglycollate which in turn was better than glucose. Enhancement of inhibition against *C. perfringens* by autoclaving nitrite in the medium has been shown by Riha and Solberg (200,201,202).

The possibility and evidence of the probable formation of a potent microbial inhibitor by heating nitrite in laboratory media gave rise to suggestions that a similar compound might be formed by heating nitrite in meat. Not long after the first report on the subject by Perigo et al. (184), a study was undertaken by Johnston et al. (137) to examine whether nitrite could interact with meat to form a compound which could prevent botulinum spore growth in meat products. Culture media as well as meat suspensions were heated with various concentrations of nitrite up to 200 µg/g. It was found that heating the medium at 110 C for 20 min with as little as 20 µg of nitrite/g produced *C. botulinum* inhibition. When meat was blended with medium and nitrite and heated, the inhibition at high nitrite inputs (> 150 µg/g) was attributed to the residual nitrite which was greater than 100 µg/g. The inhibitory substance in meat was found to be dialysable, while the Perigo Inhibitor in the medium was not. Addition of as little as 1% meat to culture medium interfered with development of the inhibitor, and 20% or more meat prevented it. Addition of non-fat meat solids to the culture medium with the inhibitor already present neutralized its activity, while fat and water-soluble meat extracts did not. Johnston et al. (137) concluded that the inhibitor produced in media was of little or no consequence in explaining the role of nitrite in the safety of commercially produced cured meats. The inhibitory activity of sodium nitrite on the *C. botulinum* outgrowth was also studied by Johnston and Loynes (136) in various bacteriological media and meat suspensions. Nitrite was heated at 110 C for 20 min in the media or meat suspension at pH 6.2. Perigo Factor was detected in the original Perigo medium (184) and reinforced clodstralid medium when heated with 20 µg of nitrite/g, but not in liver veal medium and Wynne fluid medium even in the presence of 100 µg of nitrite/g. In 50% meat suspensions, 500, 150 and 50 µg of nitrite/g were required for inhibition when underprocessed, normal-processed and overprocessed, respectively. The inhibitory activity was dialysable in meat suspensions, liver veal medium and Wynne fluid medium but not in Perigo and reinforced clodstralid medium. Addition of reducing agents to meat suspensions decreased the redox potential and increased inhibition without inducing the formation of the Perigo Factor. Jarvis et al. (132) reported that growth and toxin formation in a meat system were inhibited by 300-400 µg of unheated or by 200 µg of heated nitrite/g. In a culture medium only 20 µg of heated nitrite/g were necessary to inhibit a similar inoculum. Ashworth and Spencer (10) added nitrite directly to minced pork, heated it at 115 C for 20 min and subsequently challenged the system with a *C. sporogenes* inoculum. This system was more inhibitory than a similar one with the nitrite added after heating. The effect was observed at nitrite concentrations 150-300 µg/g and attempts to magnify it failed. Ashworth and Spencer (10) concluded that no evidence had been obtained which would implicate a Perigo effect in the safety of canned cured meats under practical conditions.
In another attempt to determine whether a Perigo-type factor was formed in meat, Chang et al. (38) autoclaved canned commercially formulated luncheon meat with various nitrite concentrations and stored them until all nitrite was depleted. The cans were then inoculated with a C. botulinum spore inoculum heated to F0 = 0.4 min in a meat homogenate formulated with 4.0% NaCl and 150 μg/g of nitrite/g. An inhibitory effect was detected with increasing initial nitrite concentrations and lower spore inoculum. However, the effect was small since with only 10 spores/can and an initial nitrite concentration of up to 200 μg/g one out of 16 cans showed growth. The model meat system of Ashworth and Spencer (10) was used by Ashworth et al. (9) to study the production of any antimicrobial effects with sodium nitrite heated under simulated commercial pasteurization conditions. Lower residual nitrite levels were found inhibitory to C. sporogenes when nitrite was heated with the meat at 115 C for 20 min, or 80 C for 4 h, or from 20 to 70 C in 4 h, compared to nitrite added unheated to the meat. Average residual nitrite levels of 64 to 149 μg/g in nitrite-heated systems and 91 to 234 μg/g in nitrite-unheated systems were necessary to inhibit growth. Chang and Akhtar (37) homogenized and adjusted to the same nitrite levels canned luncheon meat which had been formulated with various nitrite concentrations, heat-processed and stored for different periods. The homogenate was then inoculated with C. botulinum strain 13983B spores. They concluded that inhibition increased with increasing nitrite concentrations before processing and could not be accounted for by residual nitrite. Attempts by van Roon (282) to extract a Perigo Factor from luncheon meat failed. Grever (103) has suggested that the Perigo Inhibitor is destroyed by fat and adsorbed by muscle fibers. It has been stated by Ingram (125) that "the outcome of a substantial amount of work on the Perigo effect seems disappointing" and by the same author in 1976 (126) that "it seems that observations made in culture media may have little relevance to meat; for which reason it is better, for the time being, not to use the term Perigo Factor or Inhibitor in connection with meat." The necessity of heating to above 90 C and that the number of spores used makes little difference to inhibitory concentrations, are reasons for doubting the applicability of Perigo-type Inhibitors to meat systems.

Points of general agreement in Perigo-type work listed by Ingram (125) are: that a reducing agent (thioglycolate, ascorbic acid or cysteine) is necessary; that a protein hydrolysate, preferably casein, is necessary and that iron might be involved. Wasserman and Huhtanen (290) investigated the possibility that the Perigo Inhibitor might be certain volatile nitrosocompounds. After confirming the findings of Perigo et al. (184) they (290) tested against C. botulinum the inhibitory activity of various nitrosamines found in the medium or implicated in meat products. None of the nitrosocompounds examined inhibited C. botulinum.

Moran et al. (170) undertook a study to characterize chemically the nitrite inhibitor found by Riha and Solberg (200,201,202). It was found that cysteine and ferrous ions were reacting in some manner with nitrite under autoclaving to cause inhibition. The inhibition was lost when the inhibitor was added to cooked meat medium and it was not formed when the necessary components were autoclaved with the same medium. It was speculated that the inhibitor observed might be different than that in cured meats. Continuing their efforts, Moran et al. (170) investigated several compounds (S-nitrosocysteine, Roussin black and red salts, cysteine-Fe-NO complex) that could conceivably be produced by interaction of cysteine, ferrous ions and nitrite. S-nitrosocysteine inhibited the test organisms, but the amount formed in the test system was inadequate to explain the total inhibition observed. It should be mentioned that Incze et al. (124) found S-nitrosocysteine more inhibitory than nitrite in a beef bouillon-based medium. Roussin red salt was unstable in the test system, whereas Roussin black salt was inhibitory and could form in amounts sufficient to explain the inhibition recorded. Cysteine-Fe-NO complex was detected but the levels found were non-inhibitory. Moran et al. (170) concluded that the inhibition found may have been due to the combined effects of sublethal concentrations of each compound tested. Van Roon (282) suggested the possibility that iron-nitrosyl coordination complexes could be formed in canned cured meat products during heating. Roussin black salt and nitrosyl-cysteyl-ferrate were studied and found to inhibit growth of clostridial spores. Attempts to detect Roussin black salt in luncheon meat failed. Inhibition of a variety of microorganisms by Roussin black salt was also reported by Ashworth et al. (8), while Dobry-Duclaux (57,58) found low concentrations of the compound to inhibit the enzyme alcohol dehydrogenase.

Huhtanen and Wasserman (121) reported that inhibition of C. botulinum by nitrite was potentiated by the addition of Fe(II) or Fe(III) to the culture medium, and that the effect was more pronounced when the nitrite was added after autoclaving. It was suggested (121) that a potent anticontrolidial inhibitor could be produced without autoclaving nitrite in the medium, that iron was the limiting factor and that sulphydryl groups were probably necessary for its formation. Black iron nitrosyl-sulfide (INS) was tested by Huhtanen et al. (120) and showed a minimum inhibitory concentration of 0.16 mg/l compared to 80 mg/l for nitrite against C. botulinum in a tryptone-yeast extract medium. Addition of meat to the medium prevented inhibition, while autoclaving INS with the medium inactivated it, indicating that INS was different from the Perigo Factor. Van Roon and Olsmann (283) found S-nitrosocysteine and nitrite inhibitory to clostridia in canned cured pasteurized beef and ham products, while a cysteyl-nitric oxide ferrate complex was ineffective. Inhibition of [14C]uracil incorporation into ribonucleic acid of Bacillus cereus...
during outgrowth was caused by a Perigo-type Inhibitor, nitrosothiols of thioglycollate and beta-mercaptoethanol (1/0). Inhibition occurred before and after germination and during outgrowth.

In spite of all the research conducted around Perigo-type inhibitors, Fe, and related compounds, no definite conclusions have been reached, and the value of such research in relation to real cured meat systems is debatable. Discussing the mechanism of action of nitrite, Ingram (126) stated the assumption that nitrite blocks energy metabolism by reacting with the amino groups of dehydrogenases. Ingram (126) also stated that work by Dainty and Meredith (51), while confirming that nitrite can inhibit various respiratory enzymes, suggested that it is "likely that the growth inhibitory effect of nitrite is because it generally inhibits the uptake of energy sources."

The inhibitory activity of reaction products of nitrite with degradation compounds of carbohydrates was investigated by Mirna and Coretti (165). Growth of Micrococcus sp. and S. aureus was inhibited by S-hydroxymethylfurfural at a concentration of 500 mg/l; Enterobacter liquefaciens showed inhibition at 2,000 mg/l only; and Escherichia coli was not inhibited by the above mass fractions of S-hydroxymethylfurfural. Of the other compounds examined, only 3,4-dihydroxyphenylaniline was inhibitory against three of the bacterial strains. Van Roon and Olsman (283) related clostridial inhibition to an increase in protein-bound nitrite during storage, and Lechowich et al. (150) stated that protein-bound nitrite contributes to the stability of cured meats.

A major issue in explaining the inhibitory activity of nitrite is the relative importance of the initial nitrite input versus that of the residual nitrite found in the product after processing and during storage. Greenberg (98) concluded that the level of nitrite at the time of product manufacture, rather than the residual nitrite concentration, was the key to botulinal protection. Greenberg (98) also stated that "it would appear that the nitrite either reacts with the spores, or that in the reaction with the meat component a substance(s) is formed which inhibits germination and/or outgrowth of the spores." Bowen and Deibel (26) and Christiansen et al. (40) concluded also that the initial nitrite level rather than the residual was the important factor in determining inhibition of botulinal toxin production, and Christiansen et al. (40) made a statement similar to that by Greenberg (98). From papers presented at the Second International Symposium on Nitrite in Meat Products (264) it was also concluded that the safety of cured meat products during storage was mostly determined by the concentration of nitrite added to the products before processing. Ingram (126) stated that "we do not know whether the best index of the inhibitory effect of nitrite is the initial or residual concentration, or some fraction of the amount of nitrite which disappeared." Ashworth and Spencer (10) and Chang and Akhtar (37) reported that the inhibition found in the systems tested (minced pork and luncheon meat) could not be accounted for by residual nitrite levels alone. On the other hand Johnston et al. (137) suggested a high residual nitrite level as being responsible for the inhibition observed in meat suspensions.

Recently, a series of reports has appeared implicating residual nitrite or an intermediate, isoascorbate, and iron in the mechanism of botulinal safety of cured meats. Christiansen et al. (43) followed spore germination, cell survival and residual nitrite depletion during 27-C storage of a perishable canned cured pork product prepared with 50 or 156 mg of nitrite/g and 102 or 104 botulinal spores/g. The data indicated a race between death of the germinated cells and nitrite disappearance, and the time at which outgrowth occurred was dependent upon residual nitrite levels and surviving botulinal cells.

It was stated (43) that "the data suggest that safety of these products is dependent upon sufficient residual nitrite until the viable cell level has decreased to a point at which growth can no longer be initiated." In another study with the same product, Tompkin et al. (270) examined the effect of prior refrigeration on botulinal outgrowth at abuse temperatures. It was demonstrated that botulinal protection decreased with storage at 10 C before incubation at 27 C. The decrease in inhibition was related to the decline in residual nitrite. However, the data that were presented (270) were inadequate to prove that residual nitrite, per se, was the key to inhibition, and it was stated, (270), that residual nitrite could be indirectly related to botulinal inhibition serving as a reservoir for a reactive intermediate such as nitric acid. The possibility also exists that some inhibitory substance could be formed during processing involving nitrite and could be dissipated during storage simultaneously with residual nitrite. In a subsequent report, Tompkin et al. (271) examined the effect of adding hemoglobin or using different meat sources, on the inhibitory activity of nitrite against C. botulinum outgrowth. Addition of 1% hemoglobin to product formulated with 50 or 156 mg of nitrite/g and replacing pork ham, beef round, veal or turkey meat with beef or pork hearts decreased or eliminated the inhibitory effect of nitrite. It was postulated (271) that the responses obtained were due to two different phenomena. Hemoglobin addition decreased residual nitrite which in turn decreased botulinal inhibition. A high level of available iron in heart meats also brought about a loss in botulinal inhibition. Tompkin et al. (271) offered the hypothesis that nitric oxide reacted with iron in the vegetative cells and blocked some essential metabolic step such as ferredoxin or some other iron-dependent enzyme. They (271) explained that the conclusion reached by Christiansen et al. (40), i.e., the nitrite level added at the time of formulation was the important factor in controlling botulinal outgrowth, was due to the pattern of botulinal swells observed which appeared to be unrelated to the levels of residual nitrite.
Continuing their efforts, Tompkin et al. (269) reported that addition of 0.02% isoascorbate markedly enhanced the antibotulinal effect of nitrite. A combination of 0.02% isoascorbate - 50 μg of nitrite/g gave protection similar to that obtained with 156 μg of nitrite/g, while isoascorbate alone was ineffective. Bowen et al. (25) found that sodium ascorbate in wieners did not decrease or potentiate the effectiveness of sodium nitrite to inhibit botulinal growth. Bowen and Deibel (26) found that ascorbate levels in the range of 0.1 to 0.2% in bacon decreased the ability of nitrite to inhibit botulinal outgrowth while lower ascorbate levels correlated with higher nitrite effectiveness. On the other hand, Growther et al. (107) reported that 0.2% sodium ascorbate did not reduce nitrite protection against C. botulinum. Increased inhibition due to nitrite by addition of reducing agents such as ascorbate was also reported by Johnston and Loynes (136) and Ashworth and Spencer (10). Expanding their efforts on isoascorbate Tompkin et al. (272) reported that isoascorbate enhanced nitrite inhibition due to its sequestering and not its antioxidant or reducing properties, and the same effect was produced with ascorbate, cysteine and EDTA. It was postulated (272) that moderate (0.02%) isoascorbate levels enhance nitrite inhibition of C. botulinum by sequestering a metal ion in the cured meat. Tompkin et al. (273) in a final report presented data showing that iron was critical to the inhibition of C. botulinum in perishable canned cured meat. The data indicated that nitric oxide reacted with some essential iron-containing compound within the germinated cell and prevented outgrowth, which would probably be related to injury of the electron transport system. It was concluded (273) that the degree of botulinal inhibition depends on the following factors: the residual nitrite level and viable botulinal cell counts at the time of temperature abuse, the iron available for repair of nitric oxide-damaged cells and/or the level of iron available outside the cell to react with nitric oxide and thus prevent injury. A summary of all this work was presented by Tompkin (265) in the 1978 Reciprocal Meat Conference.

Of all the possible mechanisms presented through the years and the vast amount of work reported, no single mechanism seems to explain entirely the nitrite effect on the safety of cured meat products and to apply in culture media and all the types of meat products. It is not safe to always conclude that what applies to culture media can be assumed as taking place in real meat systems, or that all the cured meat products are the same. On the other hand, results from work with meat systems such as inoculated packs are a problem to interpret since it is difficult, if not impossible, to know what changes are occurring inside the pack. A reasonable conclusion would be that most of the results reported are true and important for the conditions and systems tested in each particular case. The effectiveness of nitrite in delaying botulinal toxicity is probably due to one or more mechanisms, or one or more factors involved in each particular product or system considered. The remarkable safety of cured meat products experienced through the years can be accounted for by action of one or more nitrite-inhibition mechanisms involved. Low incidence of botulinal spores in raw meats, heat process involved during product manufacture, effect of salt and other ingredients and proper handling and storage of the products.

Nitrite depletion

Recently residual nitrite has been directly or indirectly linked with the safety of cured meat products from botulinal toxicity (43,270,271). Also, nitrite is believed to react with secondary and tertiary amines and to form nitrosamines. In light of the above, an understanding of the fate of nitrite in cured meat products, and the factors influencing the rate of nitrite depletion, is essential.

As soon as nitrite is added to meat, its depletion starts. The depletion is continuous and its rate depends on product formulation, pH, time and temperature-relationships during processing and subsequent storage (85,174). Nordin (174), working with canned ham, determined a relationship between the initial and final nitrite concentration, pH, temperature and length of storage. At 30°C the half-life of nitrite at pH 6.0 was 5 days. The rate of nitrite disappearance was followed in bacon by Herring (114) and in canned pork by Sebranek et al. (226). No simple relationship between nitrite level and time was found. This was probably the result of uncontrolled variables such as pH.

A large amount of the added nitrite is lost during processing. Fiddler et al. (80) found 10-33% nitrite retention in commercially manufactured wieners after processing, while in another study (123) an average of 33% of the added nitrite remained in the wieners after manufacture. A survey of the literature showed a large variation in nitrite disappearance during and after processing (Table 3). Differences can be expected from one meat lot to the next, among laboratories, due to formulation differences, products examined, and storage temperatures. Nitrite depletion was shown to be directly proportional to the meat concentration (180). Higher nitrite losses were observed during processing when 1% hemoglobin was added to canned comminuted pork (271). In canned comminuted pork a large nitrite depletion was observed during formulation while cooking had little effect (40). However, nitrite disappearance in wieners during cooking was higher than during processing (123).

The temperature at which the product is stored influences the rate of nitrite depletion. The higher the storage temperature, the faster the rate of nitrite disappearance. This has been shown in products such as wieners (123), thuringer sausage (52), comminuted pork (40) and bacon (41).

Several reports have looked at nitrite depletion and found it to be rapid, but all have failed to account for most of the nitrite depleted (9,114,174,190). It is well
known that nitrosomyoglobin is formed from nitrite and myoglobin during curing. Formation of several other compounds when nitrite is added to meat has been proposed: nitrosothiol compounds (146); nitrosated amino acids, such as nitrosoprolines (148); nitrotyrosine (145,300); and some gaseous derivatives such as nitric oxide (NO), nitrous oxide (N₂O) and nitrogen (N₂) (299). Fate of nitrite in whole meat and meat fractions was studied by Emi-Miwa et al. (72). Of the nitrite added, 66 to about 90% was traced as nitrite, nitrate, nitrosothiol, nitrosomyoglobin and gaseous nitrogen compounds. The remaining nitrite was unidentified and depended on both the curing period and the amount of ascorbate added. Ascorbate can act as a reducing compound and enhance the rate of nitrite depletion, and high concentrations have been recommended to prevent nitrosamine formation (3).

The specific interactions between nitrite and the sulfhydryl groups of meat protein have been studied in a model system consisting of myosin and nitrite (146). The rate of the reaction between nitrite and sulfhydryls was slow under pH and concentration conditions similar to those expected in meat. The findings led to the assumption that the direct reaction between nitrite and sulfhydryls in the myosin fraction can account for only a small proportion of the total nitrite lost during curing. A decreased loss of nitrite was recorded when the sulfhydryl groups in a chopped beef product were blocked (78).

Olsman and Krol (180) reported that in the pH range prevailing in meat products, only a small fraction of the nitrite was present as nitrous acid (HNO₂), which was considered as a leak through which the reservoir of nitrite was emptied. At pH values 6.0 or higher the nitrite loss followed first order kinetics, whereas at lower pH values the reaction was between first and second order. Low molecular weight muscle cell components have been reported as active in nitrite reduction (263), while the ability of unsaturated fatty acids to combine with nitric oxide has been demonstrated (86) with no products being identified.

The effects of back and belly fat on nitrite disappearance were studied by Goutefongea et al. (94) with fat cured with ¹⁵N-labelled nitrite. Ninety percent of the added nitrite was recovered as free nitrite. Connective tissue and lipid fraction extracted from the cured fat contained 6.9% and 2.5% nitrite, respectively. When fatty acids and glycerides were treated with ¹⁵N-labelled nitrite, the amount of label incorporated was considerably higher in more unsaturated compounds. Woolford et al. (300) showed results suggesting that one of the major pathways for nitrite depletion in cured meat products might be through reaction with nonheme proteins, while Olsman and Krol (180) indicated that the concentration of protein-bound nitrite increases with decreasing pH.

Olsman (179), working with model meat emulsions of pH 5.35 to 5.80, reported that more than half of the nitrite lost during the first days of storage was recovered as protein-bound nitrite. This bound nitrite increased to a maximum and then gradually declined with kinetics similar to those for the depletion of free nitrite. Addition of Fe²⁺ at 0.1 or 1 mmol/kg resulted in more protein-bound nitrite, whereas EDTA showed the opposite effect. This effect was more pronounced at pH 6.2 than at pH 5.65, and it was completely lost at pH 5.1 (179). In 1974 Ando (4) reported that Fe²⁺ significantly decomposed nitrite in the absence of ascorbate, whereas Mg²⁺, Ca²⁺ and Zn²⁺ enhanced nitrite decomposition to some extent only in the presence of ascorbate. The effects of Fe²⁺ and Fe³⁺ in the presence of ascorbate were considerable. Also glutamate, succinate, nicotinic

<table>
<thead>
<tr>
<th>Product</th>
<th>Added nitrite (mg/g)</th>
<th>Final cooking temperature (°C)</th>
<th>Storage temperature (°C)</th>
<th>Days after cooking</th>
<th>Replications</th>
<th>Additive</th>
<th>Nitrite depleted (% of added)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned. comminuted pork</td>
<td>50</td>
<td>68.5</td>
<td>—</td>
<td>0</td>
<td>A,a</td>
<td>—</td>
<td>30</td>
<td>(268)</td>
</tr>
<tr>
<td>Pasteurized pork curd</td>
<td>175</td>
<td>70.0</td>
<td>—</td>
<td>0</td>
<td>A,a</td>
<td>—</td>
<td>29</td>
<td>(212)</td>
</tr>
<tr>
<td>Canned. comminuted pork</td>
<td>50</td>
<td>68.5</td>
<td>—</td>
<td>0</td>
<td>A</td>
<td>0%hemoglobin</td>
<td>40</td>
<td>(271)</td>
</tr>
<tr>
<td>Canned. comminuted pork</td>
<td>150</td>
<td>68.5</td>
<td>7.0</td>
<td>0</td>
<td>A</td>
<td>1%hemoglobin</td>
<td>88</td>
<td>(40)</td>
</tr>
<tr>
<td>Wieners</td>
<td>150</td>
<td>71.0</td>
<td>27.0</td>
<td>0</td>
<td>B</td>
<td>b</td>
<td>—</td>
<td>(123)</td>
</tr>
</tbody>
</table>

1Selected and calculated from the references listed.
2Laboratory.
3Meat lot.
that ascorbate and endogeneous meat reductants to say that a large proportion of the nitrite week of storage when around 40% of the initial nitrite was converted to nitrate. In another study with the same product (bacon), nitrate was detected after processing and during storage at 7 and 27 C even though it was not in the product formulation (41). The quantity of nitrate found was related to the ingoing nitrite concentrations (41). In a study (87) on the fate of nitrite in a model system composed of nitrite, myoglobin and ascorbate, most of the nitrite nitrogen was recovered as nitrate. The remaining was found as nitrosomyoglobin or gases. These findings were confirmed by Lee et al. (152) in model and cured meat systems. Lee et al. (152) suggested that ascorbate and endogeneous meat reductants reduced metmyoglobin to myoglobin which was then oxidized simultaneously with nitrite to form nitrate.

The fate of nitrite in meat during curing was reviewed by Cassens et al. (33). It was stated that it is unacceptable to say that a large proportion of the nitrite “disappears.” About 10-20% of the ingoing nitrite combines with porphyrin-containing compounds such as myoglobin. Other compounds shown to be formed are nitrate, gaseous products (N₂, NO, N₂O), nitrosothiols (protein-bound nitrite), and in the pH range of 2-3, S-nitrosocysteine can be formed through nitrite reaction with cysteine.

Generally the fate and rate of nitrite depletion differs from one system to another since different variables and several depletion pathways can be involved.

Nitrates toxicity-Nitrosamines

Concern over the nitrate and nitrite intake and its effect on human health goes back to the beginning of the century. In 1907 Richardson (197) asserted that most of the nitrate ingested was from vegetables. Kerr et al. (143) in their studies on the use of nitrite in meat curing gave special consideration to public health aspects. Nitrite is considered toxic at high concentrations and it is also implicated in carcinogenic nitrosamine formation. A recent report implicated nitrite itself to be a carcinogen (279). Used under the existing regulations, nitrite is not considered as a health hazard. Some rare toxic episodes have occurred, mostly due to accidental overuse. The lethal nitrite dose is 300 mg/kg of body weight (252). Nitrite can react as vasodilator and hypotensive agent (219); it can reduce the storage of vitamin A in the liver and it can also disturb thyroid function (71). DiFate (56) found nitrite to be mutagenic. It is well known that nitrite can oxidize hemoglobin to methemoglobin, thereby lowering the ability of the bloodstream to carry oxygen. This disturbance is called methemoglobinemia; it can be fatal and most often occurs in infants. Two methemoglobinemia outbreaks involving 19 cases occurred in Los Angeles (36). They were traced to nitrite which had been repackaged and accidentally sold as monosodium glutamate (MSG).

On February 5, 1972 the FDA-USDA announced that nitrosopyrrolidine, a nitrosamine, was formed in retail-purchased and fried bacon. The levels recorded in the fried product ranged from 30 to 106 parts per billion (ppb), while the raw bacon was found free of nitrosopyrrolidine (114). Over 65 different nitrosamines have been found to be carcinogenic (158). Nitrosamines can be produced by a combination reaction of nitrite or nitrous acid and secondary or tertiary amines (225). Since cured meats contain both nitrite and amines, there is a potential for nitrosamine formation under the appropriate reaction conditions. Excellent reviews on the subject of nitrosamines are given by Sebranek and Cassens (225), and Crosby and Sawyer (49).

In recent years the concern over the nitrosamine question in cured meat products has greatly increased, and nitrite usage in meat curing has come under attack. Of all the cured meat products, fried bacon is the one product in which nitrosamines (mostly nitrosopyrrolidine) have been mostly found (232). Bacon is high in the amino acids proline and hydroxyproline, and other nitrosopyrrolidine precursors. The most likely pathway for nitrosopyrrolidine formation is that proline is first nitrosated and then decarboxylated (77). An excellent review on the subject is given by Gray (95). Nitrosopyrrolidine concentrations in bacon as high as 108 ppb have been reported (75), but the levels usually found fall in the range of 10 to 20 ppb (148). Nitrosopyrrolidin-4 levels up to 139 ppb were confirmed in fried bacon by Havery et al. (111). It was also isolated from raw bacon (95,148). Wasserman et al. (292) reported finding nitrosopyrrolidine and nitrosodimethylamine, another nitrosamine, in 25 home-cooked bacon samples. Ten of the 25 samples tested contained nitrosamine levels above 10 and up to 39 ppb. Besides fried bacon, nitrosopyrrolidine was also found in some samples of severely fried (170 C, 12 min) country-style ham (100).

In other cured meat products nitrosamines have not been found or are only sporadically encountered. Fazio et al. (75) and Fiddler et al. (77) found nitrosopyrrolidine present in bacon but not in other cured meat products. Wiener samples tested for 14 nitrosamines at the 10 ppb sensitivity level were found negative (123). Canned comminuted pork (40), fermented sausage (147) and thuringer sausage (52) were also found nitrosamine-free. Wasserman et al. (289) reported occasional samples of commercial frankfurters at the retail level to show dimethylnitrosamine formation. Under controlled laboratory and normal heating conditions no nitrosamines were formed in frankfurters formulated with nitrite levels up to 750 µg/g. A nitrite concentration of 1500 µg/g was necessary for dimethylnitrosamine formation at levels of
10-11 ppb. A doubling of the smoking and heating process was necessary for the dimethylnitrosamine levels to reach 22 ppb (80). The researchers (80) concluded that frankfurters conforming to the United States Federal Regulations contained insufficient nitrite for nitrosamine formation.

Cured meat products can be divided into two groups on the basis of nitrosamine occurrence (96, 220). One group includes bacon, where nitrosamines have mostly been found, and the other group includes ham, wiener, bologna and similar products which are less involved with nitrosamines. In bacon the nitrosopyrrolidine concentration has been found to be higher in the fat cookout than in the cooked edible portion (75, 77, 114, 183, 230). Removal of the fat before frying inhibited or at least diminished nitrosamine formation in the lean portion of bacon (77). The extent of nitrosamine formation in bacon is affected by ingoing nitrite concentration (232), cooking conditions (183) and post-processing age of bacon (111). Under certain conditions, several substances (ascorbate, ascorbyl palmitate, cysteine, glutathione, hydroquinone, alpha-tocopherol and tertiary butylhydroquinone) have been shown to decrease nitrosamine formation in cured meat products (3, 78, 79, 97, 99, 114, 167, 231). Addition of nitrite to spice premixes resulted in formation of nitrosopyrrolidine from pepper, and nitrosopyrrolidine from paprika (229). Recently nitrite incorporation into spice premixes was disallowed in the U.S., but the reaction is rapid at pH values close to those of the human stomach. Some important considerations when dealing with toxic compounds such as nitrosamines are the significance of a 10-ppb level, the no-effect level, and possible advances in techniques to detect ppt (trillion) levels.

### TABLE 4. Nitrosopyrrolidine (ppb) in bacon.

<table>
<thead>
<tr>
<th>Year</th>
<th>Average</th>
<th>Range</th>
<th>No. of samples</th>
<th>No. determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1973</td>
<td>31.8</td>
<td>10-67</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>1974</td>
<td>21.3</td>
<td>7-47</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>1976</td>
<td>4.3</td>
<td>0-9</td>
<td>16</td>
<td>42</td>
</tr>
</tbody>
</table>

1 Calculated from data submitted to USDA (2).  
2 Multiple determinations from different laboratories.

### TABLE 5. Residual nitrate and nitrite in frankfurters and bacon.

<table>
<thead>
<tr>
<th>Year</th>
<th>Frankfurters</th>
<th>Bacon</th>
<th>Nitrate (µg/g)</th>
<th>Frankfurters</th>
<th>Bacon</th>
<th>Nitrite (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Range</td>
<td>Average</td>
<td>Range</td>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>1936</td>
<td>710</td>
<td>570-880</td>
<td>2650</td>
<td>1550-3450</td>
<td>84</td>
<td>55-146</td>
</tr>
<tr>
<td>1937</td>
<td>714</td>
<td>340-1117</td>
<td>1200</td>
<td>300-1900</td>
<td>75</td>
<td>25-135</td>
</tr>
<tr>
<td>1970</td>
<td>38</td>
<td>15-80</td>
<td>30</td>
<td>10-55</td>
<td>38</td>
<td>15-80</td>
</tr>
<tr>
<td>1972</td>
<td>4.2</td>
<td>1-11</td>
<td>5.5</td>
<td>1-12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Calculated from data given (3, 22).

Only 25-33% of the human nitrite intake comes from cured meats, while the rest comes from the saliva where it is formed from nitrate by the microflora of the mouth (253). High in nitrate (220, 295). Another estimation (295) has indicated that only 10% of the ingested nitrate and 21% of the nitrite comes from cured meats, whereas vegetables contribute 86% of the nitrate and saliva 77% of the nitrite. A recent report (253) indicated that only 2% of the nitrite humans are exposed to comes from cured meats, another contributor being in vivo nitrite formation by microorganisms from degraded proteins. Tannenbaum et al. (255) found that under certain conditions salivary nitrite may reach concentrations of hundreds of µg/g. The importance of salivary nitrite to the in situ formation of nitrosamines has been discussed by Tannenbaum et al. (254). The kinetics of the reaction in model systems were studied by Mirvish (166) who showed that secondary amines and nitrite react only slowly at ambient temperatures and neutral pH values, but the reaction is rapid at pH values close to those of the human stomach. Some important considerations when dealing with toxic compounds such as nitrosamines are the significance of a 10-ppb level, the no-effect level, and possible advances in techniques to detect ppt (trillion) levels.

**SORBATE**

**History, chemistry, safety**

Sorbic acid was first discovered in 1859 in London by a German chemist named A. W. Hofmann. It was formed by the reaction of rowan berry oil with strong alkali. It was first discovered in Germany in 1939 and in the U.S. in 1940 by E. Müller and C. M. Gooding, respectively. Industrial production of the compound started in the 1950's, and its use as a food preservative increased gradually after being permitted in most countries (cited from 156).

Sorbic acid is the trans-trans form of hexa-2,4-dienoic acid and has the structure CH₂CH = CH CH = CH COOH. It is a di-unsaturated, aliphatic, straight chain compound was clarified in the period 1870-1890 and it was first synthesized by O. Doebner in 1900. The antimicrobial properties of sorbic acid were first discovered in Germany in 1939 and in the U.S. in 1940 by E. Müller and C. M. Gooding, respectively. Industrial production of the compound started in the 1950's, and its use as a food preservative increased gradually after being permitted in most countries (cited from 156). Sorbic acid is the trans-trans form of hexa-2,4-dienoic acid and has the structure CH₂CH = CH CH = CH COOH. It is a di-unsaturated, aliphatic, straight chain compound was clarified in the period 1870-1890 and it was first synthesized by O. Doebner in 1900. The antimicrobial properties of sorbic acid were first discovered in Germany in 1939 and in the U.S. in 1940 by E. Müller and C. M. Gooding, respectively. Industrial production of the compound started in the 1950's, and its use as a food preservative increased gradually after being permitted in most countries (cited from 156).
monocarboxylic acid (7,156).

The solubility of sorbic acid in water at room temperature is relatively low (0.16%). It dissolves more easily in hot water or ethyl alcohol, which is an excellent solvent for the compound. Of the acids found in foods, acetic is by far the best solvent for sorbic acid. The pH of a sorbic acid-distilled water solution is 3.1, and in buffered solutions with pH above 3.1, more sorbic acid can be dissolved due to its partial conversion to its highly soluble salts. Sorbic acid is about three times more soluble in edible oils and fats than in water, and salt noticeably lowers its solubility. The major advantage of the alkali salts of sorbic acid is their good water solubility. Potassium sorbate, the salt most widely used in food applications, can be made into solutions of more than 50% in cold water without difficulty (91,156).

Sorbic acid is utilized in the body in a way similar to other fatty acids. Only the first step of β-oxidation is omitted, α, β-dehydrogenation, since sorbic acid already has an α, β double bond. The half-life of sorbic acid in the body is 40-110 min, depending on the dosage. No sorbic acid residues were found in the muscular tissues of domestic animals that were given feed containing sorbic acid. Under normal conditions of alimentation, it is completely oxidized to CO₂ and H₂O, yielding its potential energy as calories (53,54,156).

Deuel et al. (53) reported that sorbic acid was harmless to rats and dogs when incorporated in their diets to the extent of 5%. Its toxicity was lower than that of sodium benzoate which must be detoxified in the liver (53,109). The acute toxicity dose of sorbic acid (LD₅₀) is about 10 g/kg of body weight. The safety of sorbic acid use can be understood by comparing the above value with the corresponding LD₅₀ of cooking salt, which is about 5 g/kg of body weight, and taking into consideration the respective quantities of salt and sorbic acid added to foods (156). Sorbic acid and its potassium salt have been cleared for use and listed as products “generally recognized as safe” (GRAS) by the FDA. On March 10, 1978 the FDA published a proposal to reaffirm the GRAS status of sorbic acid and potassium sorbate (276). The Select Committee concluded that these two substances demonstrated very low acute or chronic toxicity for experimental animals; and they were metabolized in the animal by the normal fatty acid pathway (276). The World Health Organization has confirmed the harmlessness of sorbic acid by stipulating for it the highest acceptable daily intake (25 mg/kg of body weight) among food preservatives (156).

Preservative action

Sorbic acid and its salts inhibit growth of fungi, yeasts and many bacteria, even though their action against bacteria is not as comprehensive as that against fungi and yeasts. The antimicrobial activity of sorbates depends on the pH of the substrate. At low pH values the amount of free undissociated acid, the effective form, increases (7,9,156,196). The inhibitory effect of sorbic acid against catalase-positive cultures was influenced by pH and it was found most effective in the pH range 5.0 to 5.5 (70). Gooding et al. (91) reported that the activity of sorbic acid in protecting foods against mold and yeast spoilage was higher in an acid environment. Raevuori (196) stated that the free acid enters the bacterial cell and inhibits several enzyme systems. In practice, effects against microorganisms can be obtained if the pH of the substrate is lower than 6.5. At that pH, the potassium salt is also hydrolyzed to free acid. In weakly acid environments, sorbic acid contains a considerable proportion of the undissociated form and thus sorbic acid is a better preservative at these hydrogen-ion concentrations compared to other preservative acids (156). The upper pH limit for sorbate efficacy has been reported to be 6.5; for propionates, 5.5; and for benzoates, 4.5. Parabens (esters of para-hydroxybenzoic acid) are generally used for foods with a pH of 7.0 or higher, but their astrangent medicinal taste may be objectionable. The increased upper pH limit of sorbate efficacy (6.5), compared to other preservatives, extends its use in a wider variety of mildly acid foods. Sorbate can also frequently be used in more acidic foods to avoid the off-taste from benzoate, and/or in combination with benzoate to cover a broader range of microorganisms. The optimum effectiveness of sorbate, which is greater than that of benzoate and propionate is at pH values below 6.0. It also has some effectiveness at pH around 6.5, but at pH 7.0 and above it is ineffective. Sorbate is more effective than benzoate and propionate even at lower pH values (2.5-4.0) where they show their greatest effectiveness (7).

Luck (156) stated that “if the literature and practice of various countries are studied it will be found that sorbic acid has been tested for nearly all groups of food and also that it is used for all of these groups separately or collectively somewhere in the world.” Sorbate has been used to preserve foodstuffs such as edible fat emulsions, cheese, meat and fish, fermented vegetables, pickled vegetables, tomato products, dried fruit, fruit juices and fruit syrups, drinks, fruit preserves, bakery goods, sugar and confectionery. Also it has found application on packaging material as a fungistatic agent, in pharmaceuticals and cosmetic products and in animal feeds (156). The inhibitory properties of sorbic acid were first noted by Gooding (90). A concentration of 0.1-0.2% sorbic acid was used by Tanaka et al. (250) to preserve sausages. Boyd and Tarr (27) found better keeping quality in smoked fish with sorbic acid addition, and Amano et al. (2) found a tylosin-sorbic acid combination (40 μg/g-0.1%, respectively) most effective in preserving fish sausage at elevated temperatures (30 and 37°C). Flaked ice containing glycol diformate and sorbic acid was found somewhat effective in preserving poultry (141). The shelf-life of eviscerated, cut-up “dry-pack” poultry was extended by the synergistic effect of a two-step process in work reported by Perry et al. (186). The process consisted of acidic hydration and sorbic acid application. Control samples spoiled after 5 days at
reduction of storage, whereas treated samples did not spoil until about 18 days. It was also noted that putrid odor development was suppressed markedly even during the latter days of storage and despite the eventual increase in microbial growth. The efficacy of potassium sorbate dip in extending shelf-life of broiler-parts and salmonellae growth was examined by Robach and Ivey (203). The total number of viable bacteria was significantly reduced with dipping in potassium sorbate solution. A 10% dip significantly reduced the total plate count compared to control parts after 5 days at 22 C. The 10% dip also resulted in salmonellae counts significantly lower than the untreated parts after 7 days at 10 C. A 5% or greater dip reduced the growth rate of salmonellae at 10 and 22 C. A 2.5% sorbate dip gave 0.05% sorbic acid concentration on poultry parts, while a 5.0 and a 10.0% dip gave a 0.13 and 0.31% sorbic acid concentration, respectively.

Gooding et al. (91) indicated salt and sugar to have a marked synergistic effect on sorbic acid fungistasis. In high sugar systems the increased sorbic acid effect occurred even at pH values above 6.5. The same authors (91) reported sorbic acid to be about four times as effective as propionates in protecting cheese, bread and cake products. Smith and Rollin (238) found sorbic acid superior to sodium benzoate as a fungistatic agent in protection of yeast dough. Emard and Vaughn (70) found that besides salmonellae, some Streptococcus faecalis strains were also inhibited by 0.12% sorbic acid, while S. aureus was inhibited by 0.07% sorbic acid in glucose yeast extract media and 0.12% in liver infusion media. Sorbic acid was also reported being effective in suppressing the growth of aerobic sporeforming bacilli (70, 284).

Sorbic acid has been recommended as a selective agent in culture media for isolation of catalase-negative lactobacilli and clostrdia (70, 279, 280, 284). It was reported by Vaughn and Emard (284) and Emard and Vaughn (70) that sorbic acid-containing media could be used for selective enrichment and isolation of the catalase-negative lactic acid-producing bacteria. The authors suggested that sorbic acid media might be useful for enrichment and isolation of the catalase-negative clostridia. A concentration of 0.1% sorbic acid in liver infusion agar exerted a marked inhibitory effect on most catalase-positive cultures tested (70). The authors also reported some indication that the pH of the medium might affect the inhibitory activity of sorbic acid. No data showing possible usefulness of sorbic acid as a selective agent for clostridia were presented and the pH effect was not tested for clostridia. It was concluded (70) that the effectiveness of sorbic acid was dependent upon the concentration used, the basal medium, and the pH of the medium. A total of 47 cultures representing 20 species and types of Clostridium, including vegetative cells and spore suspensions, were tested for their resistance to sorbic acid in beef liver infusion medium by York and Vaughn (279). Vegetative cells and spores of C. botulinum types C, D and E were inhibited by 0.5% sorbic acid, while 3.0% sorbic acid did not inhibit C. parabotulinum types A and B. Nothing was mentioned by these workers (279) about the solubility of sorbic acid in the medium; the pH of the medium was 6.7-6.8; and considerable variation in resistance to sorbic acid was observed among the Clostridium species tested. In another study, Hansen and Appleman (109) tried to determine if sorbic acid was a growth stimulant for clostridia. It was reported that 0.12% sorbic, propionic, and caproic acids were neither inhibitory nor stimulatory to C. sporogenes and C. botulinum types A and B. Continuing their efforts, York and Vaughn (280) investigated the sorbic acid resistance of C. parabotulinum types A and B, and C. botulinum types C, D and E.
They concluded that sorbic acid was not a particularly effective inhibitor of food-poisoning bacteria in a suitable medium. These investigators (279,280) failed to report whether or not the growth of clostridia in the presence of sorbic acid was better or poorer compared to the growth in sorbic acid-free media.

Besides sorbic acid solubility, the pH of the medium is another factor that might have affected the conclusions reported from work related to sorbic acid effectiveness against clostridia. York and Vaughn (279) found that the ability of different clostridial spore cultures to germinate in 7 days was markedly decreased as the pH of the medium was reduced from 5.8 to 5.0. The authors stated that “the pH range must be increased to at least 6.0 or above if the sorbic acid medium is to be used for enrichment and isolation of the more fastidious species of Clostridium.” However, in the higher pH range, 0.12% sorbic acid in liver infusion medium was not as effective in preventing growth of the catalase-positive microorganisms as in the recommended range of pH 5.0 to 5.5 (279).

The same authors, York and Vaughn (280), reported that sorbic acid was more efficient at acid conditions (pH below 4.5), especially when the maximum concentration used was only 0.1% for C. parabotulinum types A and B and 1.0% for C. botulinum types C, D and E. In liver infusion, at pH 6.7-6.8, a sorbic acid concentration of 2.5% was necessary for inhibition to start, while 4.0% inhibited all strains tested. Generally, the studies recommending sorbic acid as a selective agent for the isolation of clostridia were made at high pH values, using sorbic acid to more strongly inhibit competing microorganisms and to eventually allow growth of clostridia.

York and Vaughn (279) reported that sorbic acid was used as a carbon source by some of the species tested, and the same authors (280) found that sorbic acid concentrations of 0.12 to 1.0% in the medium were used by all C. parabotulinum cultures examined. Melnick et al. (161) reported that a,β-unsaturated fatty acids such as sorbic acid were normal transitory metabolites in the oxidation of saturated fatty acids by molds, and that molds could also metabolize the compounds even during the period of growth inhibition. They suggested that since molds could metabolize sorbic acid, a “high” initial concentration was capable of inhibiting the dehydrogenase enzyme system in molds. They concluded that inhibition of that important enzyme system (dehydrogenase) was responsible for sorbic acid exhibiting fungistatic and under certain conditions even fungicidal activity.

Since sorbic acid is a fatty acid, a brief review of the effects of other fatty acids on bacteria, and especially clostridia, seems necessary. Humfeld, (122) in 1947, summarizing the pertinent literature, indicated that unsaturated C18 fatty acids, and particularly oleic, were known to inhibit a great variety of bacteria. Inhibition of germination of C. botulinum spores by as little as 0.1-1 μg of oleic, linoleic and linolenic acids/ml was reported by Foster and Wynn (83). Foster and Wynn (82) also found that 10 μg of oleic acid/ml completely inhibited C. botulinum spore germination. Some variation in susceptibility among strains was reported, but all were affected, and linoleic and linolenic acids gave results comparable to oleic. Vegetative cells of C. botulinum were not inhibited by the acids, and starch neutralized their inhibitory effect. An oleic acid concentration of 100 μg/ml prevented germination over a 4.5-month period, and it was not sporidal in distilled water but was in brain-heart medium. Spores of C. perfringens and PA 3679 were less inhibited; spores of Clostridium histolyticum and Clostridium chauvei were only slightly affected; and spores of aerobic species were unaffected (82). In 1952, Roth and Halvorson, (218) demonstrated that oxidative rancidity in lard and corn oil was a definite inhibitory factor for PA 3679. C. botulinum and Clostridium pasteurianum spore germination. The effect of methyl esters of oleic, linoleic and linolenic acids on spores of PA 3679 was also studied by the same scientists (218). Fresh unoxidized methyl esters at 0.01% concentration were not inhibitory, whereas the oxidized forms showed significant inhibition at the same concentration. Methyl-linoleate and methyl-linolenate caused 95% inhibition, and methyl-oleate 50% inhibition. All three acids were inhibitory in the rancid state and inhibition increased with unsaturation. A spore inhibition of the same magnitude as that of the fatty acids was observed with benozyl peroxide. Addition of 0.01% catalase was able to partially overcome the inhibition of spore germination, indicating that the fatty acid action was actually due to peroxide. Sorbic acid however, is resistant to autoxidation (160). The relationship of the above to the antimicrobial activity of sorbic acid is not known. However, sorbic acid is also an unsaturated fatty acid (two double bonds) as is oleic, linoleic and linolenic, differing in that sorbic has only 6 carbons while the others have 18.

The antimicrobial action of sorbate against six Bacillus species was examined by Gould (93). At sodium sorbate concentrations of 0.015 to 0.05% and pH 6.0, germination occurred and spore walls were ruptured or were lysed and vegetative cells emerged and elongated but failed to multiply. A depressed rate of germination was observed at sodium sorbate concentrations above 0.04% and pH 6.0. The inhibitory effect of sorbate was five times larger at pH 6.0 than at pH 7.0. D-α-hydroxyisocaproic, L-α-hydroxyisocaproic and DL-α-hydroxyisocaproic acids (caproic acid is the saturated form of sorbic acid) in combination with L-alanine were shown to cause 74.87% inhibition of germination of C. botulinum type A and E spores (4).

The exact effects of sorbic acid on the growth of bacteria, and especially clostridia, and the mechanism of such effects, especially in food systems, are not fully understood. The findings reviewed here can give some indications or directions in the search for the answers. Sorbate in meat products

The selectivity of bacterial inhibition by sorbic acid
and the early reports implicating it in enhancing, or at least not restricting, growth of clostridia might have been the reasons that the compound was neither tested nor used as an antimicrobial agent in meat products until recently. The only approved application of sorbic acid in meat products is that of dipping the dry sausage casings in a 2.5% potassium sorbate solution to inhibit mold growth on the surface of the product during the drying period.

Tompkin et al. (267) examined the possibility of increasing the public health hazard by using potassium sorbate to retard mold spoilage in cooked pork sausage. If sorbate enhanced clostridial growth at elevated temperatures, the product could become toxic. To the contrary, the data demonstrated that the addition of potassium sorbate reduced the public health hazard. Skinless, uncured sausage links with or without 0.1% (wt/wt) potassium sorbate were inoculated with salmonellae, *S. aureus, C. perfringens* and *C. botulinum* and stored at 27 °C. The normal spoilage flora was delayed one day by sorbate, and salmonellae growth was markedly retarded. Growth of *S. aureus* was delayed 1 day with sorbate, after which growth occurred to the same extent as in product without sorbate. *C. perfringens* declined to undetectable levels within the first day in products with or without sorbate. The most important and probably unexpected finding was that sorbate retarded growth and toxin production by *C. botulinum*. Toxin was detected after 4 days in product without sorbate but not until after 10 days in product with sorbate.

One of the recommendations of the Expert Panel on Nitriles, Nitrates and Nitrosamines was that alternate preservatives with the potential to replace nitrite in cured meats should be evaluated. As a result of the general antimicrobial properties attributed to sorbate, the recent findings of Tompkin et al. (267) and the Expert Panel recommendation, research on the efficacy of sorbate to control botulinal toxicity in cured meat products has been initiated during the last 3 years.

Based on results of such research, on May 15, 1978 the USDA in accepting an April 27, 1978 petition, proposed that in the future, bacon may be produced with 40 µg nitrite/g and 0.26% potassium sorbate. The proposal will become effective in 1 year, unless data are presented to show that its adoption will result in bacon with inadequate botulinal protection or that the bacon will contain confirmable levels of nitrosamines after frying (277). A summary of the research that led to the above proposal follows.

Ivey et al. (131), in an effort to reduce the initial nitrite levels in the curing of bacon and still assure botulinal safety, tested low nitrite (0 and 40 µg/g) and potassium sorbate (0.13 and 0.26%) concentrations in product inoculated with 1100 spores per gram and incubated at 27 °C for 110 days. Commercial bacon and bacon samples formulated with 80 and 120 µg of nitrite/g were included in the study. The results indicated that potassium sorbate significantly reduced the number of toxic-swollen packages and lengthened the time before toxicity was observed. The presence or absence of 40 µg of nitrite/g had no significant effect on the sorbate inhibition. Nitrosopyrrolidine occurrence was reduced with decreased nitrite levels, and microbial growth on uninoculated samples was retarded by sorbate.

Tanaka et al. (251) tested nitrite, sorbate, sodium acid pyrophosphate (SAPP), pH and salt in a pork-macerate system. Sorbate did not show significant botulinal inhibition at pH 6.3, but it showed a rather strong effect at pH 5.5. At pH 6.0 some sorbate inhibition could be seen. Addition of low nitrite levels (25 or 50 µg/g) in the presence of sorbate and/or SAPP greatly increased the inhibitory effect. Sorbate and SAPP combined were much more inhibitory than sorbate or SAPP alone.

AF-2(2-furyl)-3-(5-nitro-2-furyl)acrylamide was used as a fish sausage preservative in Japan until it was prohibited in July, 1974. Since the above compound was replaced by sorbic acid, Wada et al. (287) examined the preservative effects of sorbic acid for fish sausage in connection with pH and storage temperature. The results indicated that addition of 0.1 or 0.2% sorbic acid prolonged the shelf-life of fish sausage by two or three times, respectively, at 30 °C and pH 5.9. The authors concluded that a 0.2% sorbic acid level could preserve fish sausage for at least 2 months at about 10 °C, if the pH was adjusted to the range of sorbic acid effectiveness.

As stated before and reported by Tanaka et al. (251), the inhibitory effect of sorbate is greater at lower pH values and inhibition diminishes at pH values above 6.0-6.5. Addition of sorbate and especially sorbic acid to foods causes a pH decrease in the product. Tompkin et al. (267) reported that uncured sausage links with 0.1% potassium sorbate had a pH of 6.4 while samples without sorbate had a pH of 7.1. Raevuori (196), working with the rice filling of Karelian pastry, presented data showing that addition of 0.4% potassium sorbate to the samples did not give a different pH than the control, either before or after baking, and after 6 days at 23 °C. In contrast, addition of 0.3% sorbic acid decreased the pH by one unit and it did not change during baking and 6 days at 23 °C. A question to be answered is whether the antimicrobial activity of sorbic acid is not only pH dependent, but that part of the effect is due to a restrictive pH for bacterial growth. Tompkin et al. (267) stated that the pH value of 6.4 in their sausage links with potassium sorbate was high enough to exclude the possibility that pH was a factor in inhibition of botulinal growth and toxin production. Therefore, the effectiveness recorded was attributed solely to sorbate.

The effectiveness of sorbic acid and low nitrite concentrations to inhibit botulinal growth in a canned comminuted pork product was studied by Ivey and Robach (130). Sorbic acid at the 0.2% level delayed growth and toxin production, while 0.1% sorbic acid was ineffective. Low nitrite concentrations (50 µg/g) and 0.1 or 0.2% sorbic acid greatly retarded growth and toxicity. Inclusion of either SAPP or sodium hexametaphosphate had a synergistic effect with sorbic acid. It was
stated that 0.2% sorbic acid and either phosphate with or without 50 μg of nitrite/g might be a potential alternative preservative to high nitrite levels in canned comminuted pork products.

The antibotulinal activity of sorbic acid has also been tested in a comminuted chicken product. Robach et al. (204) found that addition of 0.1 and 0.2% sorbic acid extended the time for swelling. Combinations of 20 μg of nitrite/g and 0.1 or 0.2% sorbic acid delayed botulinal growth even further. In another bacon study, Pierson (189) confirmed the effectiveness of potassium sorbate in delaying botulinal toxicity. A concentration of 0.13% potassium sorbate (equivalent to 0.10% sorbic acid) showed a minor effect, while 0.13% potassium sorbate in combination with 40 μg of nitrite/g was very effective. An even larger effect was found with 0.26% potassium sorbate (0.20% sorbic acid) and 40 μg of nitrite/g. In all cases the effectiveness increased in samples inoculated with sand-dried spores, compared to samples inoculated with aqueous spore suspensions.

Work in our laboratory (239,240,241,242) has indicated that sorbic acid (0.2%) inhibited botulinal spore germination (loss of heat resistance) in mechanically-deboned chicken meat, beef, and pork frankfurter emulsions abused at 27 C. Nitrite (20-156 μg/g), as indicated earlier in the text, did not affect spore germination, but at increased concentrations (156 μg/g) delayed toxin production. The above sorbic acid inhibitory effect on botulinal spore germination, outgrowth, and toxin production was pH-dependent and it started appearing at pH values below 6.0. When nitrite (40, 80, 156 μg/g) was added to the formulation, the sorbic acid effectiveness was greatly increased in magnitude and it started at pH values (6.20) higher than those for sorbic acid alone (< 6.0). Also, when sorbic acid (0.2%) was included in the formulation, nitrite depletion from the product was slower during temperature (27 C) abuse. This is important, since our studies indicated that even when sorbic acid (0.2%) was added in combination with nitrite (40-156 μg/g), botulinal toxicity developed when the residual nitrite in the product, and under our conditions, decreased to 5-15 μg/g. The above results, besides demonstrating the effectiveness of sorbic acid (0.2%-nitrite (40-156 μg/g) combinations to extend the botulinal safety of emulsified cured meat products, can also facilitate the search for nitrite and/or sorbic acid mechanisms for such action.

An aspect of concern with incorporation of sorbate in food products is that of a potential undesirable flavor.Luck (156) reported that its flavor is neutral. In the study of Raevuori (196) organoleptic analysis of the sorbic acid-protected Karelian pastry was performed. A sorbic acid concentration of 0.08% in the rice filling could not be distinguished from the control, whereas a 0.15% concentration was easily detectable and judged as an off-flavor. It was recommended that a sorbic acid concentration of 0.1% could be introduced without altering the organoleptic quality of the product. In their bacon study, Ivey et al. (131) using experienced judges to evaluate flavor, found that 0.26% potassium sorbate decreased product preference only slightly. Perry et al. (186) reported that sorbic acid did not impart off-flavors to poultry when used at recommended levels. To assure flavor acceptability of sorbic acid-treated products, more work is necessary with different products to establish no-effect levels and to possibly mask potential undesirable flavor.

Sorbate-nitrite combinations have been shown to greatly extend the botulinal safety of cured meat products, even more than nitrite or sorbate alone. One explanation of the effectiveness of such combinations, would be that nitrite and sorbate react and form a more potent inhibitor(s). Kada (139) reported that a mixture of nitrite and sorbic acid in an aqueous medium became positive upon heating in a sensitivity test named "rec-assay" (140) of Bacillus subtilis. According to the author, this indicated production of DNA-damaging compounds. Hayatsu et al. (112) and Namiki and Kada (173) further examined the possible reaction of nitrite and sorbic acid. Namiki and Kada (173) isolated and identified ethylnitrolic acid as a reaction product between a sodium nitrite solution and a sorbic acid partial suspension in distilled water in a 90 C water bath for 1 h. The pH of the mixture, from 4.3 at the beginning reached the value of 6.0 in the final state. It should be stressed that the reaction took place under special in vitro experimental conditions and in acid solution, as the authors stated (173). Testing ethylnitrolic acid against Escherichia coli, it was effective at 25-50 μg/ml, compared to 2000-4000 and 1506-3000 μg/ml for sorbic acid and nitrite, respectively. An extensive effort was undertaken by DiFate (56) to form ethylnitrolic acid by different methods and to test its mutagenicity. The results demonstrated that ethylnitrolic acid was not mutagenic, whereas nitrite was. DiFate (56) attributed the mutagenicity reported by Kada (139) and Namiki and Kada (173) to possibly free nitrite contamination in the ethylnitrolic acid solution. Mirna and Coretti (165) reported that the reaction of nitrite with carbonyl compounds containing an activated methylene group (like some ketones of fat degradation products) might lead to formation of nitrolic acids. Ethylnitrolic acid was tested against Micrococcus sp., Enterobacter liquefaciens, Escherichia coli and Staphylococcus aureus and inhibition was observed even at concentrations of 10 μg/g. Propylnitrolic acid inhibited the same microorganisms at about 100 μg/g (164). The relevance of the above to real cured meat products is unknown and debatable. However, the potential of such inhibitors being formed in cured meats by nitrite and sorbic acid exists and as the microbiological session of the Second International Symposium on Nitrite in Meat Products concluded and recommended, "attention should be directed especially to nitrolic acids" as possible botulinal inhibitors of the potent Perigo-type (264).

Sorbic acid can be considered as a unique food...
additive since it is a metabolizable fatty acid and its use would represent one food protecting another. Usage of nitrite-sorbate mixtures to protect cured meats against botulinal toxicity is an attractive alternative due to the following factors (5): (a) with lower nitrite levels (e.g. 40 μg/g) the nitrosamine formation potential would be minimized, (b) Clostridium botulinum would be inhibited at least as well or even better compared to present formulations, (c) the low nitrite level used would give the characteristic cured meat color and flavor, (d) the shelf-life of the products would increase, (e) sorbate would not cause health problems, as being a metabolizable GRAS substance and (f) the current processing procedures would not have to be changed.

REFERENCES
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