

Effect of Sodium Chloride Levels in Frankfurters on the Growth of *Clostridium sporogenes* and *Staphylococcus aureus*

R. C. WHITING, R. C. BENEDICT, C. A. KUNSCH, and J. H. WOYCHIK

ABSTRACT

Frankfurters were made with 100%, 80%, and 60% of the normal 2.5% added NaCl, plus 150 ppm NaNO₂ and 430 ppm ascorbate. They were either untreated or inoculated with *Staphylococcus aureus* or *Clostridium sporogenes* and incubated at a moderate abuse temperature of 11°C for up to 11 days. A slightly more rapid growth of the natural flora was noted in the frankfurters containing 60% of the normal salt compared to those containing higher levels of salt. No outgrowth of clostridial spores occurred in any inoculated sample. Staphylococcal growth was slow and inconsistent, with no effect related to salt levels. Incubating frankfurters at higher temperatures showed that abuse temperature had a greater effect on total aerobic and staphylococcal growth than did the salt levels tested.

INTRODUCTION

REDUCTION of the sodium content in the American diet has been recommended as an important step in reducing the incidence of hypertension and the subsequent occurrences of cardiovascular disease, stroke, renal failure, and decreased life span in the 20% of the population susceptible to this condition (Abernethy, 1979; Kolari, 1980; Pearson and Wolzak, 1982). Processed meat products contribute over one-quarter of the estimated total dietary sodium chloride intake of 10-12g per day (IFT, 1980; Marsden, 1980). An average frankfurter, for example, contains about 0.6g sodium chloride (USDA, 1980).

In addition to the contribution of salt to the flavor of meat products, it plays an essential role in solubilizing proteins for good emulsification, binding and gel formation, and in contributing to the suppression of microbial growth (Ingram and Kitchel, 1967; Marsden, 1980). Several authors have reported on flavor and textural changes resulting from the reduction of NaCl or its substitution by KCl, phosphates, or other salts (Seman et al., 1980; Terrell and Brown, 1981; Whiting and Jenkins, 1981; Hand et al., 1982).

The microbiological preservation and safety of most meat products is due to the unique combination of salt, nitrite level, heat processing, pH, vacuum packaging, and refrigeration. Few studies have reported on changes in microbiological growth in semi-preserved meat products with reduced salt levels (Riemann et al., 1972; Smith et al., 1983). The objective of this study was to determine the effect of reduced NaCl levels in frankfurters on microbiological growth, especially *Clostridium sporogenes*, a nontoxigenic anaerobic sporeformer similar to the proteolytic strains of *Clostridium botulinum*, and *Staphylococcus aureus*.

MATERIALS & METHODS

Meat

Beef chuck and pork backfat were obtained from local abattoirs, and stored at 1°C until used. The beef was trimmed of excess fat

Authors Whiting, Benedict, Kunsch, and Woychik are with the USDA-ARS, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118.

before grinding through 1/2 and 3/8 in. plates. The pork fat was ground through 1/2 in. plates.

Frankfurter manufacture

The basic frankfurter formulation was 4.00 kg lean beef, 1.35 kg pork fat, 1.55 kg ice, 105g sugar, 80g spice (Baltimore Spice Co.), 0.85g NaNO₂ (150 ppm), and 2.81g sodium ascorbate (430 ppm). The standard salt addition was 132g (2.5% of the meat block). The emulsion was chopped in a Schnellkutter to 15.5°C, stuffed into #25 cellulose casings, and tied into 4 in. links. The frankfurters were smoked by an atomized liquid smoke, cooked in a humidity controlled smokehouse to an internal temperature of 71°C, and cooled with a water spray. In one experiment, some frankfurters were smoked with natural wood smoke.

Casings were removed and three frankfurters (approximately 100g) were placed in vacuum pouches. Some were sealed with a cotton plug for aerobic storage, others were vacuum sealed (Smith Equipment Co., model GK 120/T) at 0.97 bar vacuum. Packages were stored at 11°C to simulate mild abuse. This temperature is sufficiently high for proteolytic clostridial spore outgrowth and vegetative cell growth, although the expected growth would be slow (Riemann et al., 1972; Holley, 1981; Sperber, 1982). In some experiments, 5°C and 16°C storage temperatures were used; additional packages were held at 5°C for 13 days before being given an extreme abuse of 20°C or 30°C.

Chemical analyses

The protein, fat, and water content of the frankfurters were determined by Kjeldahl, Soxhlet, and oven drying procedures, respectively (AOAC, 1975). The sodium content was obtained by dry ashing at 525°C, dissolving in nitric acid, and measuring by atomic absorption spectroscopy (AOAC, 1975).

Microbiological methods

Aerobic plate counts were made by homogenizing the entire contents of a pouch with an equal weight of 0.1% peptone water in a Stomacher 400 for 2 min. Dilutions were made with peptone water and a 0.1 ml aliquot was spread on APT agar (Difco) and incubated at 20°C. The minimum detectable counts were 10²/frankfurters.

The *Staphylococcus aureus* 196E stock culture was grown up in 250 ml BHI broth (Difco) at 37°C for 48 hr on a shaker. One or 10 ml aliquots were placed into vials, frozen in dry-ice acetone, and stored at -13°C. Thawed samples plated on TSA agar (Difco) containing 7.0% additional salt (TSAS agar) had a viable count of 7 × 10⁷ colonies/ml. The added salt inhibits most bacterial species and this strain of *S. aureus* grows as golden-colored colonies.

To inoculate the frankfurters, the *S. aureus* stock culture was thawed and diluted to 7 × 10⁶ colonies/ml. An aliquot of 0.14 ml was pipetted onto the frankfurters in the vacuum pouches to give an inoculum of 1 × 10⁴ organisms/g frankfurter before the bags were sealed.

After a predetermined storage time, the pouch was weighed, and its entire contents transferred to a Stomacher bag. The pouch was rinsed with peptone water which was then added to the Stomacher bag. The *S. aureus* were enumerated as golden colonies on TSAS agar after incubation at 37°C for 48 hr.

The *C. sporogenes* spore suspension was prepared in Beef Heart Infusion broth according to Santo-Goldoni et al. (1980), and contained 7 × 10⁸ spores/ml. The spores were inoculated into the raw frankfurter emulsion at 1.0 ml/kg emulsion (7 × 10⁵ spores/g frankfurter), and kneaded into the raw emulsion before stuffing and cooking. After the desired storage, the pouch contents were homogenized in the Stomacher and diluted with peptone water. Aliquots were pour plated on Botulinum Assay Medium (BAM) (Huhtanen,

1975) and incubated at 30°C for 2 days in an anaerobic chamber flushed with a N₂-H₂-CO₂ gas mixture. This medium permits growth of anaerobes and facultative anaerobes, and therefore enumeration of *C. sporogenes* was determined by the difference between inoculated and uninoculated frankfurters.

RESULTS & DISCUSSION

THE PROXIMATE ANALYSES of the frankfurters averaged 13.4% protein, 21.9% fat, and 59.1% water. These values fall between the composition of red meat frankfurters and poultry frankfurters (USDA, 1980). Our sodium analyses averaged 9.4 mg Na/g for the 2.5% NaCl and 5.3 mg Na/g for the 1.5% NaCl frankfurters, contrasting with reported U.S. averages of 10.2 mg Na/g for all beef, 11.2 mg/g for beef and pork, and 14.0 mg/g for poultry frankfurters (USDA, 1980). European sodium levels tend to be lower (Rust and Olson, 1982). Our higher salt frankfurters, therefore, contained a slightly below average level of sodium, probably because of use of a low salt spice mixture in the formulation. Use of only 1.5% salt without other additives resulted in a soft and mushy frankfurter. Many batches had water inside the casing after cooking and a greasy feel upon peeling. Fat caps, however, were not observed.

The percent brine can be estimated from the total sodium content and percent water in the frankfurters. For the 2.5% and 1.5% salt additions, the brine percentages were 3.8% and 2.1%, respectively. Terrell and Brown (1981) described frankfurters with medium brine contents as having 3.5–4.5% brine.

Total aerobic plate counts

The mean growth of aerobes at 11°C in uninoculated frankfurters is shown in Fig. 1. The frankfurters usually had no colonies on the 10⁻² dilution plates after 1 day of storage. This apparent pasteurization by the cooking process was also noted by Palumbo et al. (1974). Vacuum packaging slowed bacterial growth; the aerobically packaged frankfurters reached 10⁶ organisms in 5–6 days while the vacuum-packaged frankfurters required 8–9 days.

Salt level had only a minor influence on aerobic growth; the two curves differed by less than one log cycle. Although the averaged runs show greater growth with 1.5% salt, this occurred in just three of the five runs. Terrell and Brown (1981) found that only a high brine content above 4.5% reduced the growth of aerobic bacteria in vacuum-packaged frankfurters.

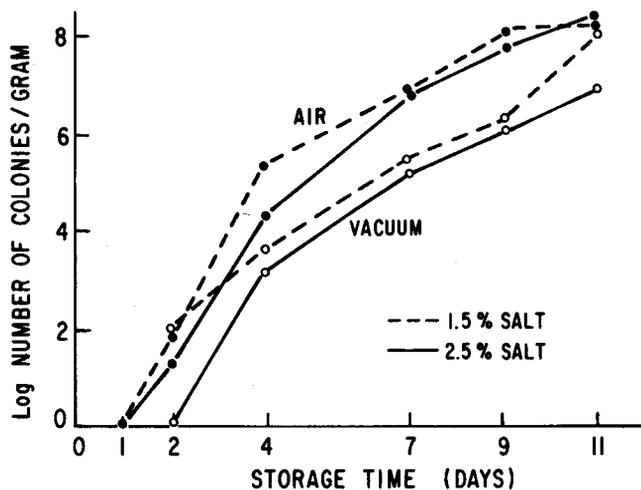


Fig. 1—Aerobic bacterial growth in uninoculated frankfurters stored at 11°C. Values are averages of five runs.

Clostridium sporogenes

Growth of anaerobes and facultative anaerobes in aerobic and vacuum packages is shown in Fig. 2a and 2b, respectively. These frankfurters were not inoculated; therefore, these counts indicate that small numbers of spores or anaerobes survived processing or came from post-processing contamination. Vacuum packaging had little effect on these organisms. The salt level had a small but consistent effect; counts were greatest for frankfurters with 1.5% salt.

The viability of the spores during storage was shown by BAM counts from frankfurters inoculated with 7 x 10⁵ spores/g (Fig. 3a, b). Counts of approximately 10⁵ microorganisms after 1 day of storage indicated survival of the spores. However, no growth was evident in either aerobic or vacuum packages. The increase observed after 9 days storage resulted from growth of uninoculated bacteria exceeding 10⁵ colonies/g (Fig. 2b).

There was no vegetative growth from spores at any salt level. Salt by itself at neutral pH must exceed 10% brine concentration, with a water activity below 0.93, to prevent growth of proteolytic clostridia (Sperber, 1982). However, the synergistic combination of salt, pH, and nitrite has been recognized as an important factor in the safety of meat products (Roberts et al., 1981). The frankfurters in this study had initial levels of 150 ppm sodium nitrite and 430 ppm ascorbate. These levels were apparently sufficient to prevent outgrowth at 11°C even with the large inoculation.

Several workers have studied the salt and nitrite levels in model systems and meat products. Nordin et al. (1975) found little growth of clostridia with 100–200 ppm nitrite, but outgrowth did occur with lower levels of nitrite. Decreasing the salt from 2.5% to 1.5% permitted more outgrowth. Hauschild (1982) reported that when the brine was lower than 4% salt, more than 100 ppm nitrite was required to inhibit outgrowth. With concentrations above 4.5% salt, considerable protection against *C. botulinum* existed without any nitrite.

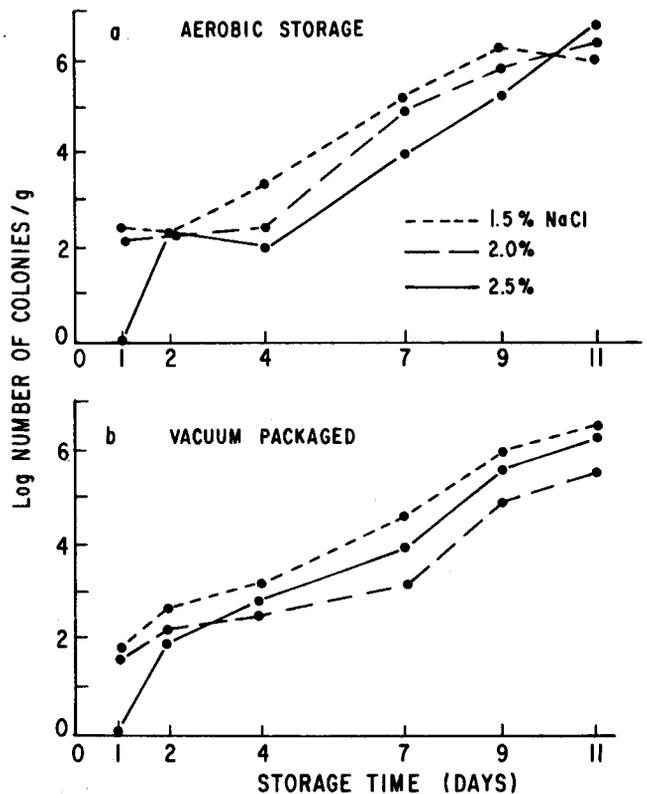


Fig. 2—Growth of anaerobic bacteria in uninoculated frankfurters stored at 11°C. Values are averages of two runs.

- Holly (1981) summarized the generally safe conditions as 75-150 ppm nitrite, 1.5-2.0% NaCl, and storage below 10°C. However, with temperature abuse, clostridial growth can occur even with nitrite levels above 300 ppm. Hauschild (1982) and Hauschild et al. (1982) pointed out that meat products, including liver sausage and turkey rolls, are presently marketed containing marginal salt and nitrite levels. These products rely primarily on low spore counts and refrigeration for their safety.

Staphylococcus aureus

Fig. 4a and 4b show the growth in frankfurters inoculated with 9×10^3 colonies *S. aureus*/g and stored aerobically and vacuum packaged, respectively. Uninoculated frankfurters were also plated on TSAS agar but no staphylococci were present in any of the samples, agreeing with the results of Bayne and Michener (1975). Frankfurters inoculated with 9×10^3 colonies/g had a level of 2×10^2 colonies/g on the following day, indicating a slight loss from adaptation to the new medium.

Under aerobic storage (Fig. 4a), the samples inoculated with staphylococci initially showed much higher counts than uninoculated samples (Fig. 1), but by day 7 the staphylococcal counts were one log cycle less than the total aerobic counts in the inoculated frankfurters. Growth under vacuum packaging (Fig. 4b) was slower than when aerobically packaged and the staphylococci appeared to compete more effectively with the total aerobic flora. The salt levels did not affect staphylococcal growth. Toxin production was not measured in this study but it is known to be greater under aerobic conditions (Riemann et al., 1972; Smith et al., 1983).

S. aureus is halo-tolerant but prefers lower salt concentrations for optimum growth (Minor and Marth, 1976;

Smith et al., 1983). *S. aureus* growth and toxin production can be inhibited by other bacteria. Possibly the salt reduction allowed other bacteria to compete more effectively with the staphylococci (Troller and Frazier, 1963; Koenig and Marth, 1982; Smith et al., 1983). The effectiveness of a particular salt concentration in preventing growth and toxin production is partially dependent on the size of the staphylococcal inoculation (Riemann et al., 1972). Despite the large inocula used in these experiments, no influence of salt concentrations was observed.

Most workers consider nitrite to have little effect on *S. aureus* in meat products (Lechowich et al., 1956; Minor and Marth, 1976; Holley, 1981; Smith et al., 1983) including frankfurters (Bayne and Michener, 1975).

The possibility of *S. aureus* inhibition by the liquid smoke applied to the frankfurters' surface was examined. Natural wood smoke and liquid smoke were shown to have antimicrobial activities toward many bacteria including staphylococci (Erdman et al., 1954; Riemann et al., 1972; Minor and Marth, 1976). Fig. 5a and 5b show the aerobic and staphylococcal growth on frankfurters made with 1.5% and 2.5% salt and given liquid smoke, natural wood smoke, or no smoke treatments. All were inoculated with *S. aureus*, vacuum packaged, and stored at 11°C.

No differences in overall total aerobic or staphylococcal growth were observed between the 1.5% and 2.5% salt frankfurters. The total aerobic plate counts tended to be slightly higher in the unsmoked frankfurters, but by less than one log cycle. The initial decreases in *S. aureus* counts

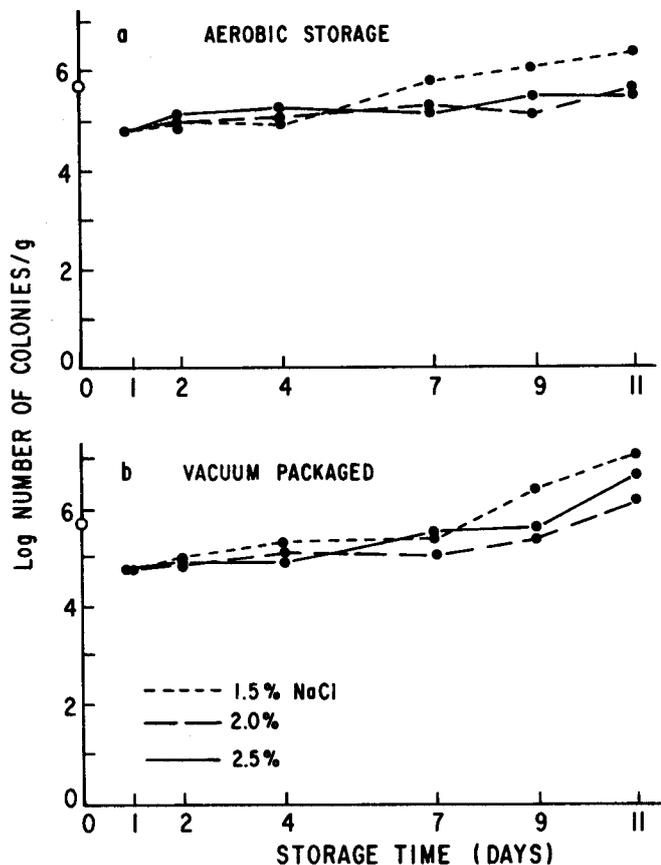


Fig. 3—Growth of *Clostridium sporogenes* in frankfurters stored at 11°C. Values are averages of two trials. The open circles on the y axis indicate the level of the *C. sporogenes* inoculum (7×10^5 spores/g).

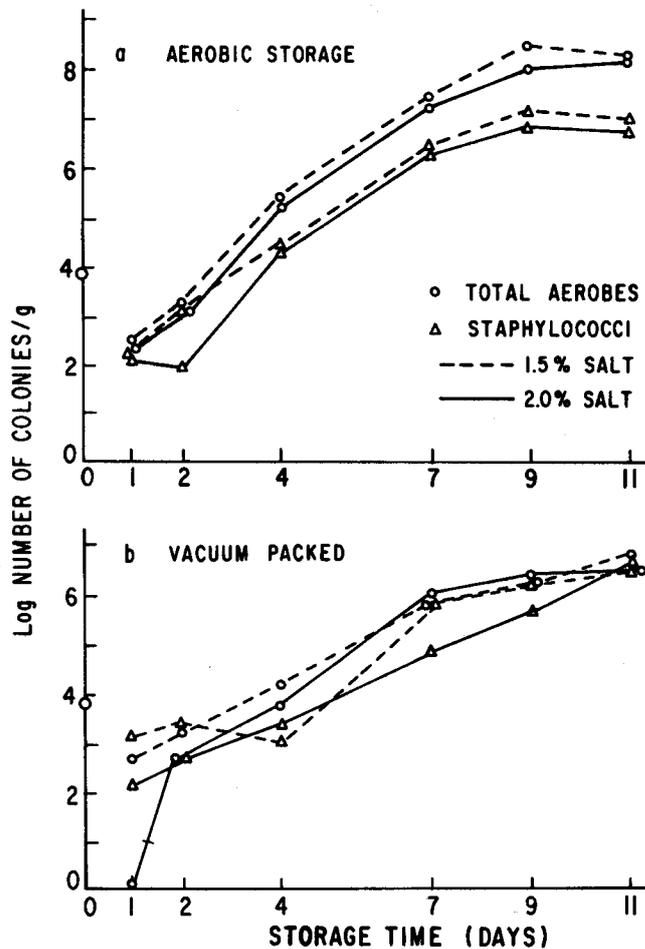


Fig. 4—Growth of aerobes and *Staphylococcus aureus* in inoculated frankfurters that were aerobically or vacuum packaged and stored at 11°C. Values are averages of two trials. The open circles on the y axis indicate the level of *S. aureus* inoculums.

MICROBIAL GROWTH WITH REDUCED SALT . . .

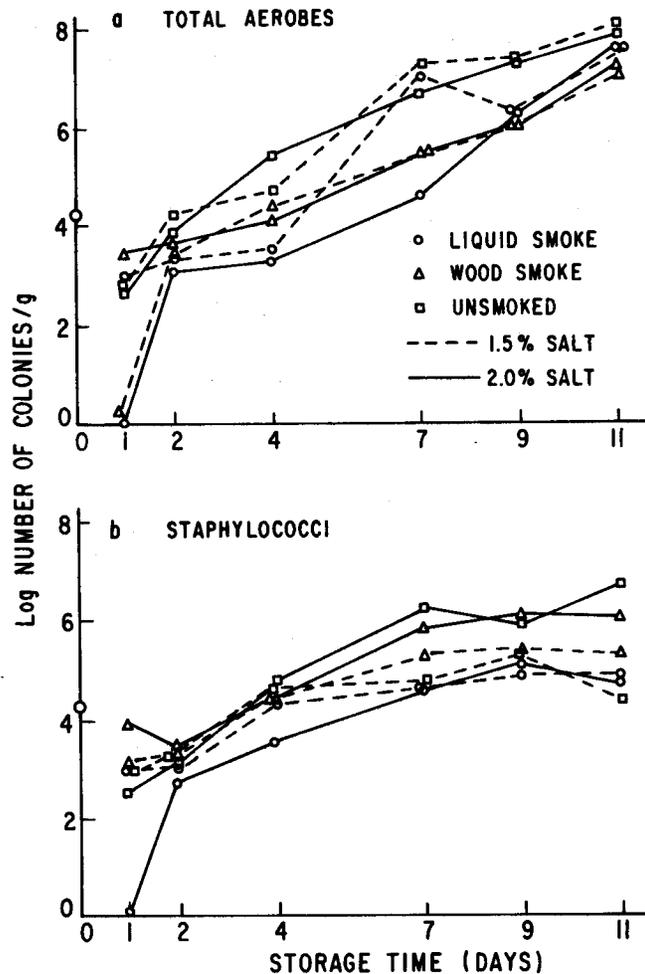


Fig. 5—Growth of aerobic bacteria and *Staphylococcus aureus* on frankfurters that were unsmoked, liquid-smoked, or wood-smoked. All frankfurters were inoculated with *S. aureus*, vacuum packaged, and stored at 11°C. The open circles on the y axis indicate the level of the *S. aureus* inoculum.

were again observed, in some treatments counts were below detectable levels (10^2 /g), but the staphylococci were not affected by the smoke treatment. If the surface pH were decreased by smoking, the expected staphylococcal growth would have been reduced (Riemann et al., 1972). However, the pH measured with a surface electrode averaged 5.90, 5.80, and 5.85 for the unsmoked, wood-smoked, and liquid-smoked frankfurters, respectively.

Temperature

Because temperature greatly affects growth, frankfurters made with 1.5% or 2.5% salt were inoculated with *S. aureus* or *C. sporogenes*, vacuum packaged, and stored at 5°C, 11°C, or 16°C. Additional packages were stored at 5°C for 13 days and then abused by storage at 20°C or 30°C.

The total aerobic counts of the uninoculated frankfurters showed the effect of temperature (Fig. 6). At 16°C growth reached 10^6 colonies/g in 6 days, while at 11°C this required 10–12 days. Frankfurters stored at 5°C showed little growth, even at 13 days. To simulate a probable pattern of abuse, samples were stored at 5°C for 13 days and then subjected to 20°C or 30°C for 1 or 2 days. When these samples were raised to 20°C and 30°C growth was very rapid, reaching 10^6 colonies/g after just 1 day. As before, the differences between the salt concentrations were not consistent or large.

Frankfurters inoculated with 10^5 spores/g of *C. sporogenes* showed no growth at any temperature (Fig. 7). The

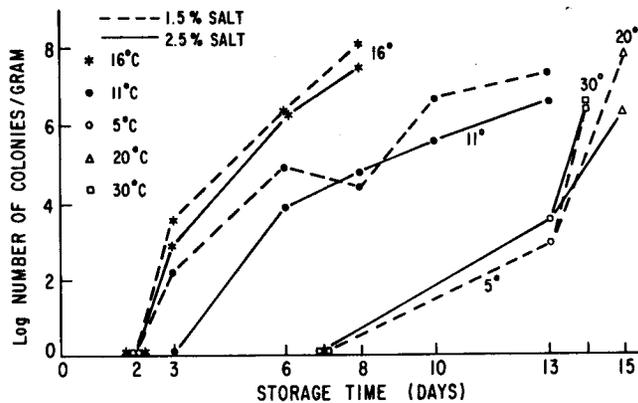


Fig. 6—Total aerobic plate count in uninoculated, vacuum packaged frankfurters stored at various temperatures.

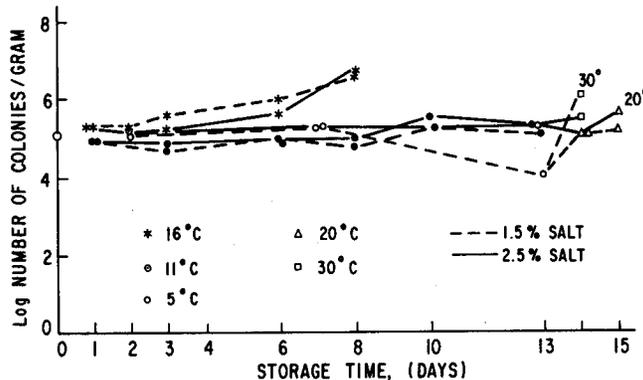


Fig. 7—Growth of anaerobic bacteria in vacuum packaged frankfurters inoculated with *Clostridium sporogenes* and stored at various temperatures. The open circle on the y axis indicates the level of *C. sporogenes* inoculation.

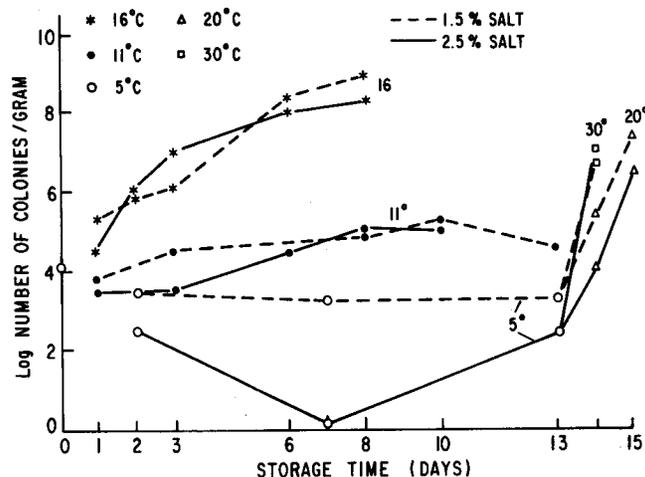


Fig. 8—*Staphylococcus aureus* growth on vacuum packaged frankfurters inoculated with *S. aureus* and stored at various temperatures. The open circles on the y axis indicate the level of *S. aureus* inoculation.

increase observed after 8 days at 16°C was from uninoculated anaerobes (data not shown), as seen previously on Fig. 3. Even the 20°C and 30°C abuse storage showed no effects. The apparent increase with 1.5% salt probably resulted from an abnormally low 13-day value. No effect from the salt concentration was observed. Should nitrite levels also be reduced in meat products, however, the

lowering of the salt may become a significant factor in permitting clostridial growth (Hauschild, 1982).

Staphylococcal growth was markedly affected by temperature (Fig. 8), but again no differences were apparent due to the salt content of the frankfurters. The decline in *S. aureus* in the 2.5% salt frankfurters at 5°C may be an actual decrease or may just reflect the inaccuracy of counting low numbers of bacteria. The staphylococci were present and capable of extremely rapid growth when the temperature was raised to 20°C or 30°C after 13 days at 5°C.

In summary, reduction of the salt content in frankfurters from 9.4 mg sodium/g to 5.3 mg/g did not affect growth of aerobic bacteria and *S. aureus* to any great extent. The temperature of storage or abuse had a much greater effect than did the salt content on the growth of these organisms. The 150 ppm nitrite and 430 ppm ascorbate used in this study effectively prevented outgrowth of *C. sporogenes* at the reduced salt levels tested, even with the large inoculation and elevated abuse temperatures.

REFERENCES

- Abernethy, J.D. 1979. Sodium and potassium in high blood pressure. *Food Technol.* 33: 57.
- AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.
- Bayne, H.G. and Michener, H.D. 1975. Growth of *Staphylococcus* and *Salmonella* on frankfurters with and without sodium nitrite. *Appl. Microbiol.* 23: 844.
- Erdman, A.M., Watts, B.M., and Elias, L.C. 1954. Smoke flavor and ascorbic acid as preservatives for fatty fish. *Food Technol.* 8: 320.
- Hand, L.W., Terrell, R.N., and Smith, G.C. 1982. Effects of chloride salts on physical, chemical, and sensory properties of frankfurters. *J. Food Sci.* 47: 1800.
- Hauschild, A.H.W. 1982. Assessment of botulism hazards from cured meat products. *Food Technol.* 36: 95.
- Hauschild, A.H.W., Hilsheimer, R., Jarvis, G., and Raymond, D.P. 1982. Contribution of nitrite to the control of *Clostridium botulinum* in liver sausage. *J. Food Prot.* 45: 500.
- Holley, R.A. 1981. Review of the potential hazard from botulism in cured meats. *Can. Inst. Food Sci. Technol. J.* 14: 183.
- Huhtanen, C.N. 1975. Some observations on a Perigo-type inhibition of *Clostridium botulinum* in a simplified medium. *J. Milk Food Technol.* 38: 762.
- IFT. 1980. Dietary Salt, A Scientific Status Summary. Institute of Food Technologists' Expert Panel of Food Safety and Nutrition and the Committee on Public Information. *Food Technol.* 34: 85.
- Ingram, M. and Kitchell, A.G. 1967. Salt as a preservative for foods. *J. Fd. Technol.* 2: 1.
- Koenig, S. and Marth, E.H. 1982. Behavior of *Staphylococcus aureus* in cheddar cheese made with sodium chloride or a mixture of sodium chloride and potassium chloride. *J. Food Prot.* 45: 996.
- Kolari, O.E. 1980. Salt dietary concerns. *Proc. Meat Ind. Res. Conf. Am. Meat Inst. Found.*, p. 89, Arlington, VA.
- Lechowich, R.V., Evans, J.B., and Niven, C.F., Jr. 1956. Effect of curing ingredients and procedures on the survival and growth of staphylococci in and on cured meats. *Appl. Microbiol.* 4: 360.
- Marsden, J.L. 1980. The importance of sodium in processed meats. *Proc. Meat Ind. Res. Conf. Am. Meat Inst. Found.*, p. 77, Arlington, VA.
- Minor, T.E. and Marth, E.H. 1976. "Staphylococci and Their Significance in Foods." Elsevier Scientific Pub. Co., New York.
- Nordin, H.R., Burke, T., Webb, G., Rubin, L.J., and van Binnendyk, D. 1975. Effect of pH, salt and nitrite in heat processed meat on destruction and outgrowth of P.A. 3679. *Can. Inst. Food Sci. Technol. J.* 8: 58.
- Palumbo, S.A., Huhtanen, C.N., and Smith, J.L. 1974. Microbiology of the frankfurter process: *Salmonella* and natural aerobic flora. *Appl. Microbiol.* 27: 724.
- Pearson, A.M. and Wolzak, A.M. 1982. Salt — Its use in animal products — A human health dilemma. *J. Anim. Sci.* 54: 1263.
- Riemann, H., Lee, W.H., and Genigeorgis, C. 1972. Control of *Clostridium botulinum* and *Staphylococcus aureus* in semi-preserved meat products. *J. Milk Food Technol.* 35: 514.
- Roberts, T.A., Gibson, A.M., and Robinson, A. 1981. Prediction of toxin production by *Clostridium botulinum* in pasteurized pork slurry. *J. Fd. Technol.* 16: 337.
- Rust, R.E. and Olson, D.G. 1982. Salt reduction in processed meats. *Meat Processing* 21 (June).
- Santo-Goldoni, J., Kojima, S., Leonard, S., and Heil, J.R. 1980. Growing spores of P.A. 3679 in formulations of beef heart infusion broth. *J. Food Sci.* 45: 467.
- Seman, D.L., Olson, D.G., and Mandigo, R.W. 1980. Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. *J. Food Sci.* 45: 1116.
- Smith, J.L., Buchanan, R.L., and Palumbo, S.A. 1983. Effect of food environment on staphylococcal enterotoxin synthesis: A review. *J. Food Prot.* 46: 545.
- Sperber, W.H. 1982. Requirements of *Clostridium botulinum* for growth and toxin production. *Food Technol.* 36: 89.
- Terrell, R.N. and Brown, J.A. 1981. Salt, water, and oilseed proteins affect brine content of sausages. *J. Food Prot.* 44: 43.
- Troller, J.A. and Frazier, W.C. 1963. Repression of *Staphylococcus aureus* by food bacteria. 1. Effect of environmental factors on inhibition. *Appl. Microbiol.* 11: 11.
- USDA. 1980. *Composition of Foods, Agriculture Handbook No. 8-7*. U.S. Government Printing Office, Washington, DC.
- Whiting, R.C. and Jenkins, R.K. 1981. Partial substitution of sodium chloride by potassium chloride in frankfurter formulations. *J. Food Quality* 4: 259.

Ms received 7/29/83; revised 9/26/83; accepted 10/20/83.

Reference to a brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.