Role of Curing Agents in the Preservation of Shelf-stable Canned Meat Products

CHARLES L. DUNCAN AND E. M. FOSTER

Department of Bacteriology and Food Research Institute, University of Wisconsin, Madison, Wisconsin 53706

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Experiments were conducted to gain a better understanding of the mechanism by which sodium chloride, sodium nitrate, and sodium nitrite supplement the action of heat in preserving canned cured meat products. Heated spores of putrefactive anaerobe 3679h were less tolerant of all three curing agents in the outgrowth medium than were unheated spores. When the curing agents were added to the heating menstruum, but not to the outgrowth medium, sodium chloride and sodium nitrate tended to protect the spores against heat injury, but sodium nitrite did not. When the spores were both heated and cultured in the presence of the curing agents: (i) nitrate and salt increased the apparent heat resistance at low concentrations (0.5 to 1%) but decreased it at concentrations of 2 to 4%; (ii) nitrite was markedly inhibitory, especially at pH 6.0. At the normal pH of canned luncheon meats (approximately 6.0), nitrite appears to be the chief preservative agent against spoilage by putrefactive anaerobes.

Table-ready, shelf-stable canned luncheon meats represent an important group of food products. They contain the usual curing ingredients (sodium chloride, sodium nitrate, and sodium nitrite) and they are heat-processed after canning. However, the heat treatment is far below that required to effect sterilization, usually amounting to about F 0 = 0.3 [equivalent to 0.3 min at 250 F (121 C)]. Nevertheless, these products can be stored for months at room temperature without spoiling. Several billion pounds of luncheon meats have been consumed in the United States without a single known—or even suspected—case of food poisoning.

Numerous workers have commented on the remarkable stability of these products (9, 11). This stability is achieved even though the curing ingredients by themselves are not sufficient to prevent spoilage of the meat, and the relatively mild heat treatment used is not nearly sufficient to destroy all the bacterial spores (5, 6, 12).

A heat treatment which does not necessarily result in complete inactivation of spores may damage them enough to prevent growth and multiplication under the storage or test conditions that prevail after heating (2, 3, 7). This is generally thought to be the case in the preservative system of cured meats.

Recently, Roberts and Ingram (10) reported that spores given increasing heat treatments became progressively more sensitive to the effect of curing agents in the outgrowth medium. The effect of nitrate was roughly equal to that of salt. Nitrite concentrations of the order of 50 ppm had a decisively inhibitory effect on the outgrowth of heated spores.

Surprisingly little is known as to whether the primary effect of the curing salts is exhibited during the actual heat treatment, or later during outgrowth of the bacterial spores. This study was undertaken in an attempt to gain a better understanding of the mechanism by which the curing ingredients supplement the action of heat in effecting preservation.

MATERIALS AND METHODS

Preparation of spore suspensions. Putrefactive anaerobe 3679 strain h (PA 3679h), obtained from Hilmer A. Frank, was used in all experiments. This organism was described by Campbell and Frank (1).

The activated culture technique of Uehara, Fujioka, and Frank (13) was employed to prepare the inoculum for sporulation in the following medium: Trypticase, 6%; glucose, 0.1%; sodium thioglycolate, 0.1%; pH 7.0. Cultures were incubated for 1 week at 37 C and for an additional week at 20 C; then the spores were harvested by centrifugation and washed five times at 4C with cold sterile deionized water. They were cleaned with Carbowax 4000 (8) and washed 10 more
When Pyrex tubes (16 X 150 mm) containing 12 ml of Difco Liver Veal Agar (LVA). The tubes were capped with about 3 ml of 2% agar containing 0.2% sodium thiglycylate. When used, sodium chloride and sodium nitrate were added to the medium before sterilization. The amounts indicated in the figures are additional to the respective 0.5 and 0.2% present in the formulated LVA. Sodium nitrite was added to the tubes of melted LVA from a filter-sterilized 10% stock solution just before inoculation. The pH of the LVA was adjusted to 6.0 or 7.0 with 3 N NaOH or 3 N HCl before sterilization. Colonies were counted after 5 days at 30°C.

Preparation of survivor curves. The basal heating menstruum was 0.067 m phosphate buffer (pH 6.0 or 7.0) prepared with Na₂HPO₄ and KH₂PO₄. When sodium chloride or sodium nitrate was added, the final pH was adjusted with 3 N NaOH or 3 N HCl before sterilization. Filter-sterilized sodium nitrite was added to the heating menstrum from a 10% stock solution.

Spores were heated at 98°C in a 200-ml three-neck round bottom flask, which was submerged in a bath of high-temperature propylene glycol. The apparatus was similar to the one described by Daoust, Read, and Litsky (4). The flask was fitted with a thermometer and a motor-driven agitator. In preparation for an experiment, 198 ml of the appropriate sterile menstruum was added to the flask and equilibrated at 98°C. Then 2 ml of suspension containing approximately 3.6 X 10⁶ spores was added. Thus, the spores reached the desired temperature almost instantaneously.

Samples were removed at intervals, cooled immediately, and diluted as necessary for preparation of quadruplicate counts in LVA tubes with or without the curing salts. Heating was continued long enough to reduce the viable spore count by at least 99.99%.

A survivor curve was prepared for each experimental variable by plotting per cent survivors against time on semilog paper. The initial value (100% survival) was the count of unheated spores in the particular outgrowth medium being tested (LVA with or without curing salts).

Experimental design. Experiments were planned to allow the separation of effects during heating from those occurring after heating (i.e., effect on outgrowth). All trials were run both at neutrality and at pH 6.0, a value near that of canned luncheon meat. Three types of experiments were run:

(i) Spores were heated in phosphate buffer, then cultured in LVA containing various concentrations of the curing salts. This approach should reveal the effect of the salts on outgrowth of spores after heat injury.

(ii) Spores were heated in phosphate buffer containing various concentrations of the curing salts. Then the spores were removed by centrifugation, washed three times in cold deionized water to remove the salts, and cultured in plain LVA. This treatment should reveal the additive effects of the chemicals during heating independent of their effect in the outgrowth medium.

(iii) Spores were heated in phosphate buffer containing various concentrations of the curing salts, then cultured in LVA containing the corresponding chemicals. This treatment should reveal the combined effects of heat and chemicals and most nearly approaches the conditions that exist in practice.

Control survivor curves were prepared for each set of test conditions by omitting the curing salts.

Expression of results. The foregoing plan produced 120 survivor curves. For convenience in comparing the relative resistance of the spores under different conditions of heating, the D value of each survivor curve was plotted against concentration of the curing salt. (D value is the time in minutes required to kill 90% of the spores at a specified temperature.) Thus, a D value higher than the control (survivor curve without the curing salt) shows increased resistance (i.e., protection) of the spores, whereas a D value lower than the control indicates decreased resistance or sensitization to the curing agents.

Differences in D values of control survivor curves between experiments reflect different batches of spores produced at various times.

RESULTS

Effect of curing agents on outgrowth of heat-injured spores. That heating injures spores in a manner that reduces their ability to grow in the
(ii) sodium chloride tended to protect the spores against heat injury. When the spores were both heated and cultured in the presence of NaCl, the protective effect predominated at the lower concentrations and the sensitizing effect predominated at the higher concentrations. The protective effect was more pronounced at pH 6.0 than at pH 7.0.

Fig. 2. Effect of prior heating on outgrowth of PA 3679h spores in LVA containing 0 to 2% NaNO₃ at pH 6.0 and 7.0. Experimental conditions and symbols as in Fig. 1.

Fig. 3. Effect of prior heating on outgrowth of PA 3679h spores in LVA containing 0 to 0.06% NaNO₂ at pH 6.0 and 7.0. Experimental conditions and symbols as in Fig. 1.

Fig. 4. Effect of prior heating on outgrowth of PA 3679h spores in LVA containing mixed curing agents (0.1% NaNO₃, 0.03% NaNO₂, and 0 to 4% NaCl) at pH 6.0 and 7.0. Experimental conditions and symbols as in Fig. 1.

Fig. 5. Effect of NaCl on D values of PA 3679h spore survivor curves at 98°C. Symbols: O, spores heated in 0.067 M phosphate buffer, pH 6.0 or 7.0, containing 0 to 4% NaCl, then washed and cultured in LVA at the corresponding pH without NaCl; ●, spores heated in 0.067 M phosphate buffer, pH 6.0 or 7.0, then cultured in LVA at the corresponding pH with 0 to 4% NaCl; ▲, spores heated in 0.067 M phosphate buffer, pH 6.0 or 7.0, containing 0 to 4% NaCl, then cultured in LVA at the corresponding pH and NaCl concentration.

Effect of NaCl. Two effects were observed (Fig. 5): (i) heat increased the sensitivity of spores to sodium chloride in the outgrowth medium, and presence of curing salts is shown in Fig. 1-4. In every instance, spores that survived 98°C for 10 min were less tolerant of the curing salts than were unheated spores. The effect was more marked at pH 6.0 than at pH 7.0. Nitrite was especially inhibitory at the lower pH value. In fact, it completely inhibited outgrowth of both heated and unheated spores at the lowest concentration tested (Fig. 3). The combined curing ingredients (Fig. 4) had essentially the same effect as nitrite alone.

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In canned luncheon meats, the effective salt concentration (per cent salt in the aqueous phase) usually is between 4 and 6%; hence, NaCl should play a significant role in preservation.

Effect of NaNO₃. Nitrate had essentially the same effect as NaCl (Fig. 6). However, at the concentrations normally used in canned luncheon meats (up to 0.1% based on the weight of the product), it is doubtful that this agent would have a significant preservative effect.

Effect of NaNO₂. In contrast to NaCl and NaNO₃, nitrite had no detectable protective effect during heating of the spores (Fig. 7). Heating in the presence of as little as 0.01% NaNO₂ reduced the ability of the spores to grow in its absence. Even more marked was the inhibitory effect of nitrite in the outgrowth medium, particularly at pH 6.0.

**Effect of mixed curing salts.** The interaction of the curing salts with their combined protective and sensitizing effects is shown in Fig. 8. At pH 7.0, the protective effect of NaCl and NaNO₃ predominated when the spores were heated with the curing salts and cultured in their absence. However, the spores showed the expected sensitivity when heated in buffer and then cultured in the presence of the curing salts. The inhibitory effect of nitrite overcame the protection afforded by NaCl and NaNO₃ when the spores were both heated and cultured with the mixed curing salts.

The importance of nitrite in preservation of cured meats is clearly shown in the results for pH 6.0. It had little effect during heating but was decisively inhibitory when present in the outgrowth medium.

**DISCUSSION**

This work was done prior to publication of and without knowledge of similar experiments being performed in England (10). The results show good agreement. As reported by Roberts and Ingram (10), heat in some way injures or sensitizes bacterial spores, making them less tolerant of meat-curing salts in the outgrowth medium. The result-
ing inhibitory effect of the curing agents is considerably greater at pH 6.0, which is near the usual pH of canned cured meat products, than at pH 7.0. No doubt these circumstances contribute to the remarkable stability of canned cured luncheon meats.

The experimental plan of the study reported here allowed the separation of effects during heating from those during outgrowth. At relatively low concentrations, sodium chloride and sodium nitrate actually tended to protect the spores against heat injury, as was shown by Viljoen with sodium chloride over 50 years ago (14). Nitrite, on the other hand, enhanced heat injury.

Examination of the combined effects of heat and the mixed curing salts (Fig. 8) clearly shows the significance of nitrite and pH 6.0 in preserving canned cured meat products. Sodium chloride is contributory (Fig. 5), but sodium nitrate probably has no significant effect at the concentrations normally used in practice (Fig. 6).

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LITERATURE CITED