Journal of Industrial Microbiology, 12 (1993) 162–167

Published by The Macmillan Press Ltd

Development and use of probability models: the industry perspective

Melanie R. Maas

Oscar Mayer Foods Corporation, Research and Development, PO Box 7188, Madison, Wisconsin 53707, USA

(Received 23 September 1992)

Key words: Clostridium botulinum; Sodium lactate; Predictive modeling

SUMMARY

In the processed meat industry, food safety and microbiological shelf life issues lend themselves to the use of probability modeling. Our research concentrated on predicting the effectiveness of sodium lactate as an antibotulinal agent in vacuum packaged, uncured and cured turkey breast model systems. In uncured turkey breast containing 1.4% NaCl, 0.3% Na phosphate, and 0–3% Na lactate, the antibotulinal effect of sodium lactate can be predicted using the following model: Days to toxicity = 3.13 + 0.39(Na lactate)^2. Using cured turkey breast with 0.3% Na phosphate, 0.2% sucrose, 0–3% Na lactate, the time to toxicity can be predicted from the following model: Days to toxicity = 1.69 + 4.88(NaCl) − 11.16(Na lactate) + 7.23(Na lactate)^2. Probability models have also been developed to predict the refrigerated shelf life of specific processed meat products. The usefulness of the predictive modeling for food safety and quality in the food industry will also be discussed.

INTRODUCTION

Producers of processed meat and poultry products are interested in the use of predictive models in the area of food safety and refrigerated shelf life. Regarding food safety, our research has concentrated on predicting the efficacy of sodium lactate as an antibotulinal agent in both uncured and cured, low salt turkey breast model systems. Also, our research has been directed toward developing predictive refrigerated shelf life models using naturally contaminated products.

Vacuum packaged, cook-in-bag turkey products are by definition processed and marketed in the same package. The heat treatment destroys all vegetative cells leaving only spores in the finished product. The uncured products contain sufficient nutrients, a low level of salt, no sodium nitrite, a pH near neutrality and an oxygen-free environment, all of which permit the growth of and toxin production by *C. botulinum*. Refrigeration is the only factor controlling a potential problem with this pathogen. Therefore, research is needed to identify acceptable antibotulinal agents for inclusion in such products should they be temperature abused.

For many years, the processed meat industry has relied on a combination of sodium chloride (NaCl) and sodium nitrite ($NaNO_2$) to prevent botulism in cured meat products. The effectiveness of $NaNO_2$ in delaying botulinal toxin production in meats has been shown to be related to the NaCl content and the pH [6–8]. As the NaCl level is reduced, the safety margin also decreases. In the past few years, there has been a trend to minimize the sodium level in the American diet. This trend concerns food microbiologists in industry, government, and academia.

In 1977, Johnston [Johnston, R.W., personal communication] in the United States Department of Agriculture (USDA), expressed concern about the safety of canned hams made with decreasing levels of NaCl. Canned hams had been recalled due to hard swells and subsequent microbial and chemical analysis indicated the presence of *Clostridium perfringens*, no residual nitrite and a NaCl content of 1.6–1.8%. The USDA concluded that continued marketing of pasteurized canned hams containing insufficient microbial inhibitors constituted a health hazard [Schnurrenberger, L.W., 1977, personal communication]. In 1989, the Food and Drug Administration (FDA) recommended that, at the retail level, meat and poultry products packaged in a reduced oxygen atmosphere must be formulated with at least 120 ppm $NaNO_2$ and have a minimum brine (% salt/% salt + % moisture) × 100 level of 3.5% in order to prevent a botulinal problem [9].

Little work has been published regarding the antimicrobial activity of lactate salts. In 1972, Krol [5] reported decreased growth of lactobacilli, micrococci, and achromobacter bacteria in dry cured, country style ham formulated with sodium lactate. He postulated that this may have been due to a bactericidal effect of sodium lactate.

Correspondence to: M.R. Maas, Oscar Mayer Foods Corporation, Research and Development, PO Box 7188, Madison, Wisconsin 53707, USA.

This paper was presented at The International Conference on the Application of Predictive Microbiology and Computer Modeling Techniques to the Food Industry, April 12–15 1992, Hyatt Regency Hotel, Tampa, FL, USA.

MATERIALS AND METHODS

Model systems using comminuted turkey were used rather than whole turkey breasts in order to prepare small batches that could be uniformly inoculated with spores of *C. botulinum*. Ground turkey was prepared by double grinding raw turkey breasts through a 0.32-cm plate on a Hobart laboratory grinder (model 74142; Hobart Manufacturing Co., Troy, OH).

Uncured turkey breast

Sodium chloride (133.5 g), Kena phosphate (44.5 g; 90% sodium tripolyphosphate–10% sodium metaphosphate; Stauffer Chemical, Westport, CT) and 60% aqueous solution USP grade DL-sodium lactate (0% (Control), 2.0% (378.4 g), 2.5% (402.7 g), 3.0% (450.1 g), or 3.5% (529.7 g); Pfanstiehl, Waukegan, IL) were mixed into ground turkey using a Hobart mixer. Various levels of water were added to compensate for the water added from the aqueous sodium lactate. These included 211.9, 90.9, 60.5, 31.9 and 0 g for 0, 2.0, 2.5, 3.0, and 3.5% sodium lactate, respectively.

For each treatment, the processed turkey was analyzed for moisture, protein, fat, sodium chloride, sodium lactate, sodium phosphate, and pH was determined by procedures of the Association of Official Analytical Chemists [14].

Spores of *C. botulinum* (5 strains of type A (56, 62, 69, 77, and 90) and 5 strains of proteolytic type B (53, 113, 213, 13983, and Lamanna-okra)) were inoculated into the turkey meat preparation and mixed using a Hobart bowl chopper. The final concentration of spores ranged from 2.2–40 per g. Portions (225 g) of this preparation were placed in Cryovac CN 530 (polyolefin and ethylene vinyl alcohol) pouches (O_2 transmission rate of 0.2–0.3 cc O_2/100 in^2/24 h at 21 °C) and vacuum packaged. Since each sample contained 225 g, all samples had a minimum of ca. 500 spores per package.

The packages were processed up to an internal temperature of 71.1 °C by submersion in 88–93.3 °C water followed by ice chilling for 1 h. This process served to both cook the product and heat shock the spores. Immediately after cooling, *C. botulinum* spore counts were determined on two samples of each treatment by the most probable number (MPN) method using Trypticase-peptone glucose yeast extract broth without trypsin [13].

Incubation, sample selection and preparation were as follows. The inoculated processed turkey was incubated at 27 °C. Five samples per treatment were tested for toxin after 0–10 days of storage. Gassy samples were chosen first for toxin analysis. If no samples exhibited gas, then samples were chosen at random. The entire contents from each package were transferred to a stomacher bag, weighed and an equal weight of gel-phosphate buffer, pH 6.2, was added. Each sample was macerated with a Stomacher (Model 400, Cooke Laboratory Products, Alexandria, VA) for 2 min. After stomaching, the contents of the bag were filtered through cheesecloth into a beaker. The filtrate was centrifuged at 5000 × *g* for 5 min, and the supernatant fraction was tested for botulinal toxin.

For detection of toxin, duplicate ICR albino mice were injected (i.p.) with 0.5 ml of supernatant fluid. Mice were observed for 4 days for symptoms of botulism. If death occurred, two additional mice were challenged with a mixture of sample extract–botulinal antitoxin that was preincubated at 37 °C for 30 min. The botulinal antitoxin preparation (Botulism Antitoxin Trivalent; types A, B and E) was obtained from Connaught Laboratories (Toronto, Ontario, Canada). Unneutralized sample extract was again injected into two more mice as a control. Botulinal toxicity was confirmed if mice receiving antitoxin-neutralized samples survived and mice receiving unneutralized samples died. A treatment was considered toxic the first day toxin was detected.

Three trials were done to evaluate the antibotulinal properties of sodium lactate in cooked turkey breast meat. The effect of sodium lactate on the time until toxin production was analyzed using analysis of variance.

Cured turkey breast

The methods and materials used in the cured turkey breast model system were the same as described above with the following exceptions.

A master batch of meat (118 kg) was prepared containing 0.5% sucrose (549.9 g), 0.5% sodium tripolyphosphate (589.0 g; Stauffer Chemical), 120 ppm $NaNO_2$ (14.16 g), 0.13% sodium chloride (154.84 g), 0.05% sodium erythorbate (57.7 g; Stauffer Chemical) and 20.6% water (24.4 kg). Thirteen individual treatments containing various levels of sodium chloride and 60% aqueous solution USP technical grade L-sodium lactate (Purac Inc., Arlington Heights, IL) were prepared from the master batch in 11.1 kg quantities. In order to ensure that the added water for each treatment was equal, various levels of water were added to compensate for the water added from the 60% aqueous sodium lactate (Table 1). The inoculated samples were held at 4.4 °C for 28 days followed by incubation at 30 °C. Five samples per treatment were tested for toxin at various intervals from 0–56 days of storage at 30 °C.

This study was arranged in a second order central composite statistical design so that maximum information could be extracted from the 13 treatments. In order to make statistical inferences, four replicates of the center point (1.5% NaCl, 1.5% Na lactate) were prepared.

The data to determine the effects of sodium chloride and/or sodium lactate on the time until toxin production were analyzed using an analysis of variance. Prior to statistical analysis, the data were centered and scaled to produce orthogonality between the treatment effects [2]. Backward stepwise multiple regression was used to determine the best model for predicting days to toxicity, only factors that were statistically significant at $P \leq 0.05$ were included. All statistical calculations were performed using Statgraphics statistical package, Vers. 4.0 (Statistical Graphics Corp., Rockville, MD).

TABLE 1

Levels of sodium chloride, 60% aqueous sodium lactate, and water added to 11.1 kg of the turkey breast preparation master batch

Treatment number	Sodium chloride		Sodium lactate[a]		Added water (g)
	(%)	(g)	(%)	(g)	
1	0.0	0.0	1.5	276.7	110.6
2	0.5	56.7	0.5	92.2	184.4
3	0.5	57.8	2.5	461.1	36.9
4	1.5	169.3	0.0	0.0	221.5
5	1.5	171.4	1.5	276.7	110.6
6	1.5	171.4	1.5	276.7	110.6
7	1.5	171.4	1.5	276.7	110.6
8	1.5	171.4	1.5	276.7	110.6
9	1.5	173.5	3.0	553.3	0.0
10	2.0	229.1	0.0	0.0	221.2
11	2.5	283.6	0.5	92.2	184.4
12	2.5	289.1	2.5	461.1	36.9
13	3.0	343.6	1.5	276.7	110.6

[a] 60% aqueous solution.

Predictive model for refrigerated shelf life

In the studies to develop a predictive model for refrigerated shelf life, the relationship of microbial growth and the refrigerated shelf life of four replicates of uncured sliced turkey breast packaged in a modified atmosphere (50% CO_2, 50% N_2) was determined. Products were collected directly from the packaging line and immediately placed in 4.4 °C storage. Microbial counts were done in triplicate, initially and at appropriate intervals, using APT agar (All Purpose Tween, Difco Laboratories, Detroit, MI) supplemented with 2% sucrose and bromcresol purple indicator.

Non-linear regression was used to determine the best fitting model for predicting refrigerated shelf life. All statistical calculations were performed using Statgraphics statistical package, Vers. 4.0 (Statistical Graphics Corp.).

RESULTS

Uncured turkey breast

The chemical composition and pH of the cooked, uncured ground turkey breast are shown in Table 2. The data presented are averages from all three trials. The turkey meat used in the model system had the same proximate composition, salt content and pH as commercially produced turkey breast products.

The results from all three trials showed similar trends (Fig. 1). As the level of sodium lactate was increased from 2.0 to 3.5%, the production of toxin by *C. botulin* was delayed. The antibotulinal effect of sodium lactate can be predicted by the following mathematical model:

$$\text{Days to toxicity} = 3.13 + 0.39\,(\text{sodium lactate})^2$$

TABLE 2

Chemical composition of cooked, uncured turkey breast

Target sodium lactate (%)	Protein (%)	Moisture (%)	Fat (%)	Salt (%)	Actual sodium lactate (%)	pH
0 (Control)	21.0	68.5	6.2	1.5	0.0	6.29
2.0	21.5	68.3	7.0	1.4	2.0	6.33
2.5	21.5	68.3	6.9	1.4	2.5	6.34
3.0	21.5	68.0	6.2	1.4	3.1	6.33
3.5	20.7	68.1	6.5	1.4	3.4	6.34

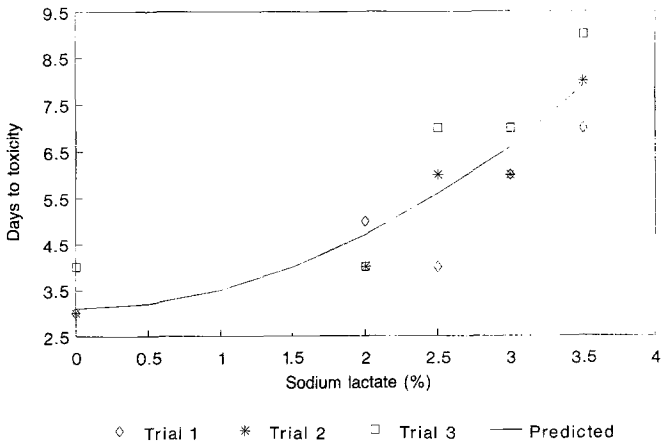

Fig. 1. Effect of different concentrations of sodium lactate on toxin production by *C. botulinum* in cook-in-bag ground turkey held at 27 °C.

The ground turkey meat mixture containing no sodium lactate and held at 27 °C was toxic at day 3. Toxicity was detected in samples containing 2.0% sodium lactate at 4–5 days. Samples containing 2.5 and 3.0% sodium lactate became toxic at 4–5 days and 7 days, respectively. Ground turkey formulated with 3.5% sodium lactate became toxic at 7–8 days.

Cured turkey breast

The chemical composition and pH of the 13 treatments of cooked, cured turkey are shown in Table 3. The actual sodium chloride and sodium lactate concentrations were within 0.2% of the target level, except for Treatment 12 in which the actual sodium lactate level was 0.6% below the target level. The ingoing level of sodium nitrite was 120 ppm. After heat processing, the average residual nitrite level was 58 ppm and after 28 days of refrigerated storage the mean level had decreased to 47 ppm nitrite. This decrease is typical for refrigerated meat and poultry cook-in-bag products.

The effect of different combinations of sodium chloride and sodium lactate on botulinal toxin production is shown in Table 4. For an unknown reason Treatment 12 (2.5% sodium chloride/2.5% sodium lactate) did not become toxic

TABLE 3

Chemical composition of cooked, cured turkey breast after 28 days at 4.4 °C

Treatment number	Target sodium chloride (%)	Target sodium lactate (%)	Actual sodium chloride (%)	Actual sodium lactate (%)	Moisture (%)	Protein (%)	Fat (%)	Sodium nitrite (ppm)	Sodium tripoly-phosphate (%)	Sucrose (%)	pH
1	0.0	1.5	0.2	1.5	75.6	23.3	1.7	49	0.3	0.2	6.5
2	0.5	0.5	0.5	0.6	76.4	20.5	1.4	56	0.3	0.3	6.5
3	0.5	2.5	0.6	2.6	75.1	20.4	1.3	43	0.3	0.2	6.5
4	1.5	0.0	1.5	0.0	76.5	21.8	1.3	55	0.3	0.2	6.5
5	1.5	1.5	1.5	1.6	75.7	21.4	0.7	48	0.3	0.2	6.5
6	1.5	1.5	1.5	1.3	75.6	21.1	0.8	33	0.3	0.2	6.5
7	1.5	1.5	1.5	1.5	75.8	20.7	0.6	49	0.3	0.2	6.5
8	1.5	1.5	1.5	1.3	75.6	21.1	0.4	47	0.3	0.3	6.5
9	1.5	3.0	1.4	2.9	75.0	20.8	1.0	45	0.3	0.2	6.5
10	2.0	0.0	2.0	0.0	76.3	21.4	0.8	51	0.3	0.3	6.6
11	2.5	0.5	2.5	0.6	75.7	20.8	0.6	52	0.3	0.3	6.5
12	2.5	2.5	2.5	1.9	74.5	19.8	0.7	45	0.3	0.2	6.5
13	3.0	1.5	3.0	1.5	74.6	21.0	0.8	43	0.3	0.2	6.5

TABLE 4

Botulinal toxin production in cooked, cured turkey breast held at 4.4 °C for 28 days followed by storage at 30 °C

Treatment number	Target sodium chloride (%)	Target sodium lactate (%)	Number of toxic samples at day[a]											
			0	2	4	6	9	12	14	16	22	41	48	56
1	0.0	1.5	0/5	0/5	0/5	1/5	1/5	1/5	0/5	1/1	1/1	–	–	–
2	0.5	0.5	0/5	0/5	4/5	5/5	–	–	–	–	–	–	–	–
3	0.5	2.5	0/5	0/5	0/5	0/5	0/5	3/5	2/5	1/4	–	–	–	–
4	1.5	0.0	0/5	0/5	–/5	3/5	3/5	5/5	5/5	5/5	–	–	–	–
5	1.5	1.5	0/5	0/5	0/5	0/5	2/5	0/5	3/5	2/4	–	–	–	–
6	1.5	1.5	0/5	0/5	0/5	1/5	2/5	3/5	3/5	5/5	–	–	–	–
7	1.5	1.5	0/5	0/5	0/5	1/5	3/5	1/5	3/5	2/5	–	–	–	–
8	1.5	1.5	0/5	0/5	0/5	0/5	0/5	2/5	2/5	3/3	–	–	–	–
9	1.5	3.0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NT[b]	NT	2/2	1/1	–
10	2.0	0.0	0/5	0/5	0/5	3/5	–	5/5	–	–	–	–	–	–
11	2.5	0.5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	NT	NT	1/1	–	–
12	2.5	2.5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	NT	NT	NT	NT	0/2[c]
13	3.0	1.5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	NT	NT	NT	1/1	–

[a] Number of toxic samples.

[b] NT, not tested because less than 5 samples were left with no visible indications of growth of *C. botulinum*.

[c] *C. botulinum* spore counts of 6.6, 22 spores g^{-1} were present after 56 days.

though it contained viable *C. botulinum* spores even after 41 days of incubation at 30 °C. Treatment 13 which contained a higher level of sodium lactate became toxic after 41 days; therfore, for data analysis purposes, a value of 41 days was assigned to Treatment 12. Using backward stepwise multiple regression techniques, a model was derived to describe the relationship between the time for toxin to be detected and the concentrations of sodium chloride and sodium lactate. The resultant mathematical model represents all the terms that were statistically significant at $P \leq 0.05$ and has a correlation coefficient of 0.917:

$$\text{Days to toxicity} = 1.69 + 4.88(\text{sodium chloride}) - 11.16(\text{sodium lactate}) + 7.23(\text{sodium lactate})^2$$

The response surface plot for this mathematical model is

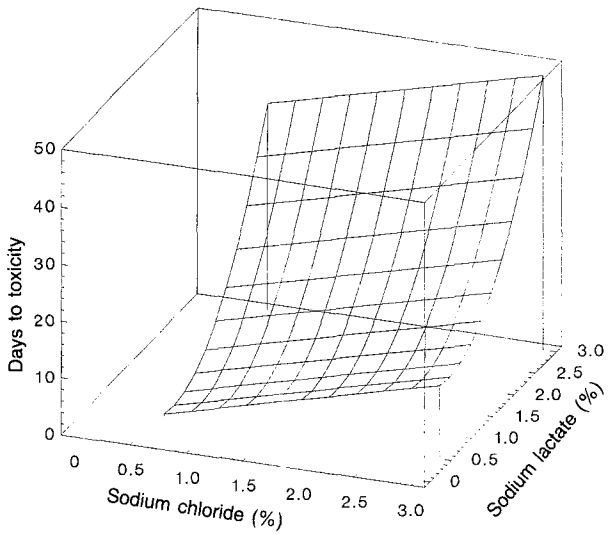

Fig. 2. Response surface plot of botulinal toxin production in cooked, cured turkey breast with varying levels of sodium chloride and sodium lactate held at 30 °C.

shown in Fig. 2. Sodium chloride alone delayed the time before botulinal toxin could be detected by 0.5 days for each 0.1% increase in sodium chloride. The effect of sodium lactate was predicted by a combination of the two lactate terms in the mathematical model. No or little effect was seen before the 1.5% level was attained. Above 1.5% sodium lactate, the antibotulinal effect was greatly enhanced and dependent upon the squared sodium lactate concentration.

Using the mathematical model, the number of days until a sample becomes toxic when temperature abused, can be predicted at any sodium chloride/sodium lactate combination ranging from 0.0% to 3.0%. Combinations that could be used in cured turkey products are illustrated in Table 5. More than 2.0% sodium lactate will greatly increase the margin of safety in low salt (2.0% sodium chloride or less) cured turkey. Levels of sodium lactate approaching 3.0% are needed to insure that the safety of low sodium chloride cured products is not compromised. The 95% confidence limits for the predicted days to toxicity are based on the variation observed in the toxicity results of the four replicates of the center point. This standard deviation was only 2.9 days. Due to the shape of the response surface curve, as the sodium lactate level increases the 95% confidence limits increase.

TABLE 5

Predicted days to toxicity for various NaCl/Na lactate combinations in cooked, cured turkey breast held at 30 °C

Sodium chloride (%)	Sodium lactate (%)	Days to toxicity (± 95% confidence limits)
0.5	2.0	11 ± 4
0.5	2.5	21 ± 5
0.5	3.0	36 ± 11
1.0	2.0	13 ± 2
1.0	2.5	24 ± 4
1.0	3.0	38 ± 10
1.5	2.0	16 ± 2
1.5	2.5	26 ± 3
1.5	3.0	41 ± 9
2.0	2.0	18 ± 2
2.0	2.5	29 ± 3
2.0	3.0	43 ± 10

The model is based on data from samples that had been subjected to temperature abuse after being held refrigerated. This closely resembles the handling practices that cook-in-bag turkey is commonly subjected to in a refrigerated distribution system. Therefore, this model represents a realistic scenario that could be used as a guideline for developing safer low sodium chloride cook-in-bag, cured poultry and meat products.

Predictive model for refrigerated shelf life

The microbial counts of lactic acid spoilage bacteria throughout the refrigerated shelf life of uncured, sliced turkey breast and the predicted growth curve with 95% confidence limits are illustrated in Fig. 3. This product is visually and/or organoleptically spoiled when the microbial count attains a level of log 7.5 CFU g^{-1}. The actual shelf life of the four trials was 46, 44, 43, and 56 days. The mathematical model based on the four trials predicts the termination of shelf life at 37 days. Using the 95% upper confidence limit, the shelf life of this product would be terminated after only 24 days. The lower confidence limit is useful since it never reaches a log count of 7.5 CFU g^{-1}. This predictive model would only be useful to determine a very conservative estimate of shelf life of this product.

The predictive curve was generated using the following mathematical model:

$$\text{Log Count} = a - b(1 - 3^{(-c(\text{absolute value of}(d\text{-time})))})$$

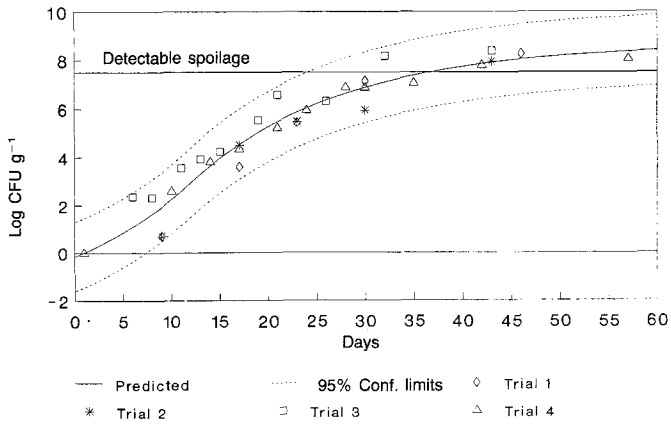

Fig. 3. Predicted growth and 95% confidence limits derived from microbial counts from four shelf life trials of sliced uncured turkey breast at 4.4 °C.

where a = log count at midpoint of the log phase of growth, b = maximum log count − log count at midpoint of the log phase of growth, c = slope of log phase of growth curve and d = time at the midpoint of the log phase of growth.

Care must be used when predicted growth curves are used to predict the shelf life of refrigerated meat and/or poultry products because the shape of the predicted growth curves is highly dependent upon the initial load of *potential* spoilage bacteria. In this case, psychrotrophic lactic acid bacteria that can grow in the presence of 1.7% sodium chloride, 2.5% sodium lactate and under modified atmosphere (50% CO_2/50% N_2) conditions.

DISCUSSION

The model systems used in the *C. botulinum* studies were designed to match as closely as possible, the production of vacuum-packaged, cook-in-bag turkey products. This model system deviates from the commercial product in two major aspects. The first is that the inoculum level of *C. botulinum*, which ranged from 2.2–40 spores g^{-1} is substantially higher than the contamination level normally found in fresh and processed meat. In several studies the concentration of naturally occurring *C. botulinum* spores ranged from 0.00004 to 0.00167 g^{-1} in raw [3] and processed meats [1,4,12]. Skjelkvale and Tjabert [11] detected a low level of mesophilic clostridia species from semipreserved meat products but no *C. botulinum*. This first factor gives the study a 'worse than normal' character.

Secondly, turkey breasts were ground then ingredients and spores were thoroughly mixed with the meat to ensure uniform distribution. Under production conditions, the sodium chloride, sodium phosphate, and sodium lactate would be prepared as a brine solution and pumped into whole raw turkey breast. Since sodium lactate demonstrated antibotulinal activity under these conditions, it is likely that sodium lactate will be at least as effective in delaying botulinal toxin production in turkey meat processed under standard manufacturing conditions.

Our results indicate that sodium lactate delays the production of botulinal toxin and this effect is concentration dependent. Although the specific mode of action or mechanism of lactate in the delay of botulinal toxin production is unknown, we propose two possible mechanisms. One, the presence of high levels of lactate ion may shift the pyruvate reduction to lactate reaction closer to its thermodynamic equilibrium, thereby inhibiting a major anaerobic energy metabolism pathway that is essential for growth. Two, in *C. botulinum*, lactate efflux from the bacterial cell may be coupled to ATP generation from proton transfer across cell membranes as demonstrated with *Streptococcus fecalis* by Simpson et al. [10]. A high level of extracellular lactate may inhibit this mechanism.

When developing predictive models for use in commercial food safety applications, it is imperative that model food systems are utilized as the test vehicle rather than media studies. This is necessary because model food systems incorporate many of the intrinsic and extrinsic factors that may influence growth in the product that may not be built into a media test system. Also, the use of a predictive model based on a model food test system will be easier to sell to non-microbiologists who must have confidence in the development method before it will be accepted as a product development tool.

The same premise applies for the development of predictive models for refrigerated shelf life. Here the preferred test vehicle would be the naturally contaminated product rather than a model food system or a media system that has been inoculated with a monoculture of spoilage bacteria.

ACKNOWLEDGEMENTS

I thank Mike Doyle, Kathy Glass and Pat Ford, Food Research Institute, University of Wisconsin for use of their facilities. I also thank Dave Brauch and Tom Jalinski for their statistical consultation.

REFERENCES

1 Abrahamsson, K. and H. Riemann. 1971. Prevalence of *Clostridium botulinum* in semipreserved meat products. Appl. Microbiol. 21: 543–544.

2 Cochran, W.G. and G.M. Cox. 1957. Experimental Designs, 2nd edn, John Wiley and Sons, New York.

3 Greenberg, R.A., R.B. Tompkin, B.O. Bladel, R.S. Kittaka and A. Anellis. 1966. Incidence of mesophilic *Clostridium* spores in raw pork, beef, and chicken in processing plants in the United States and Canada. Appl. Microbiol. 14: 789–793.

4 Insalata, N.F., S.J. Witzeman, G.J. Fredericks and F.C.A. Sunza. 1969. Incidence study of spores of *Clostridium botulinum* in convenience foods. Appl. Microbiol. 17: 542–544.

5 Krol, B. 1972. Meat products. Voedingsmiddelentechnologie 3: 157–158.

6 Pivnick, H. and H. Barnett. 1965. Effect of salt and temperature on toxinogenesis by *Clostridium botulinum* in perishable cooked meats vacuum-packed in air-impermeable plastic pouches. Food Technol. 19: 140–143.

7 Pivnick, H. and H. Bird. 1965. Toxinogenesis by *Clostridium botulinum* types A and E in perishable cooked meats vacuum-packed in plastic pouches. Food Technol. 19: 132–140.

8 Riemann, H., W.H. Lee and C. Genigeorgis. 1972. Control of *Clostridium botulinum* and *staphylococcus aureus* in semi preserved meat products. J. Milk Food Technol. 35: 514–523.

9 Rothschild, L., Jr. (ed.) 1989. FDA not planning regulations on refrigerated foods. Food Chem. News, July 10 1989: 3–5.

10 Simpson, S.J., R. Vink, A.F. Egan and P.F. Rogers. 1983. Lactate efflux stimulates [$^{32}P_i$]ATP exchange in *Streptococcus faecalis* membrane vesicles. FEMS Microbiol. Lett. 19: 111–114.

11 Skjelkvale, R.L.H. and L.B. Tjaberg. 1974. Incidence of clostridia in meat products. Nord. Vet. Med. 216: 387–389.

12 Taclindo, C., Jr, T. Midura, G.S. Nygaard and H.L. Bodily. 1967. Examination of prepared foods in plastic packages for *Clostridium botulinum*. Appl. Microbiol. 15: 426–430.

13 U.S. Food and Drug Administration. 1984. Bacteriological Analytical Manual, 6th edn, Association of Analytical Chemists, Arlington, VA.

14 Williams, S. (ed.) 1984. Official Methods of Analysis of the Association of Official Analytical Chemists, 14th edn, Association of Official Analytical Chemists, Inc., Arlington, VA.