5197

Gamma-Irradiation of *Clostridium botulinum* Inoculated Turkey Frankfurters Formulated with Different Chloride Salts and Polyphosphates

SHAI BARBUT, ARTHUR J. MAURER, and DONALD W. THAYER

ABSTRACT

The effects of gamma-irradiation doses (0, 0.5, and 1.0 Mrad) on *C. botulinum* toxin production in turkey frankfurters formulated with three different chloride salts (NaCl, KCl, and $MgCl_2$) at isoionic strength (equal to 2.5% NaCl) and three types of phosphates added to 2.0% NaCl frankfurters were studied. The use of 2.5% NaCl together with 0.5 or 1.0 Mrad was substantially more effective at inhibiting botulinal toxin production when frankfurters were incubated at 27°C than the combination of irradiation with KCl or $MgCl_2$ (40, 9, and 4 days, respectively, when treated with 1 Mrad). Phosphate addition revealed that 0.4% sodium acid pyrophosphate addition was the most inhibitory for botulinal toxin production followed by hexametaphosphate and tripolyphosphate addition.

INTRODUCTION

SODIUM REDUCTION in the North American diet has been recommended as one of the steps to reduce hypertension and symptoms associated with coronary heart diseases and renal failure (Pearson and Wolzak, 1982). Epidemiological studies related to animals and humans, strongly suggest that the consumption of sodium by some consumers should be curtailed to reduce the development of high blood pressure (Sebranek et al., 1983). Processed meat products contribute an average of about 15% to the total estimated dietary sodium chloride (NaCl) intake. However, even though a large portion of the salt in our diet (25–35%) is added at home (IFT, 1980), the consumer seems to be more concerned with the salt content in prepared food items purchased from a store.

Salt added to processed meat products has three major functions: solubilize salt soluble proteins to provide desired texture upon heating, inhibit bacteria growth including pathogens, and provide and enhance flavor (Ingram and Kitchell, 1967). Various ways to reduce sodium content of meat products have been under investigation during the past few years and were reviewed by Maurer (1983) and Terrell (1983) in poultry and red meat products, respectively. Four main approaches were mentioned: reduction of NaCl only; substitution with other chloride salts; addition of other ingredients like phosphates; and/or alteration of processing techniques.

Seman et al. (1980) reported that 50% replacement of the 2.5% NaCl with potassium chloride (KCl) was acceptable in bologna. In addition the replacements of the 1.25% NACl with 50% KCl or 50% magnesium chloride ($MgCl_2$) plus 0.4% phosphate addition was also found acceptable in this type of product. Hand et al. (1982) found that up to 35% of the total 2.5% NaCl in beef/pork or turkey frankfurters can be replaced by KCl or $MgCl_2$ without changing the overall acceptability of the freshly made product; however, after 6 wk of refrigerated storage the two NaCl replacement treatments were inferior to the 2.5% NaCl treatment in overall acceptability.

The information in the literature suggests that NaCl, even though indirectly, still plays a major role in the preservation of various foods including processed meat products. The published research, however, is too limited to provide answers to the large number of questions that may be raised in relation to partial or total replacement of NaCl and its antimicrobial activity in processed foods (Sofos, 1984). The effects of NaCl on inhibiting *C. botulinum*, for example, in processed meat products was demonstrated by Pivnick and Barnett (1965), Tanaka (1982), and Barbut et al. (1986a). They all reported that NaCl concentrations which fall within the acceptable sensory levels had an affect on botulinal toxin development under abused temperature conditions.

The effect of phosphate on improving physical and sensory characteristics of reduced salt meat products was studied by various researchers (Swift and Ellis, 1956; Knipe et al., 1985; Sofos, 1986). It was reported that phosphates, such as tripolyphosphate (TPP), hexametaphosphate (HMP) and sodium acid pyrophosphate (SAPP), can improve the texture and flavor of reduced salt comminuted meat products containing 2.0% or 1.5% NaCl (Seman et al., 1980; Sofos, 1986; Barbut et al., 1987). Phosphate effects were claimed by some to be due to a pH shift or an increase in non-specific ionic strength, whereas others claimed a specific effect where pyrophosphate, for example, was observed to help in dissociating the actomyosin to actin and myosin (Tonomura et al., 1967; Sofos, 1986).

Tompkin (1984) in his review on phosphates stated that "the antimicrobial potential of phosphates has not been fully explored and only limited information is available." Nelson et al. (1983) showed that 0.4% SAPP was more effective than TPP and HMP at the same pH when used together with 0.26% potassium sorbate and 40 ppm nitrite to inhibit *C. botulinum* in chicken frankfurters. Barbut et al. (1986b) showed that in turkey meat emulsions SAPP was more effective in inhibiting botulinal toxin production when it was compared to HMP and TPP, however, without sorbate.

The use of food irradiation is a promising way to help preserve heat sensitive foods. A scientific status summary by the Institute of Food Technologists (IFT, 1983) reviewed the efforts made in this area during the last three decades. Radiation can be used to replace some food preservatives. Wierbicki and Brynjolfsson (1979), for example, reported that gamma-irradiation was used successfully to substitute for most of the nitrite in ham, bacon and corned beef. They reported that those products were found to be indistinguishable in flavor from commercially produced counterparts. Radiation dose effects expressed as D-values for *C. botulinum* inactivation was summarized by Kreiger et al. (1983).

The objectives of this study were to investigate the antibotulinal potential of gamma-irradiation in doses up to 1 Mrad when NaCl was totally replaced by two other chloride salts and when three polyphosphates were used in a reduced salt product.

MATERIAL & METHODS

Treatments

Eighteen frankfurters treatments (Table 1) were prepared. Three different chloride salts (NaCl, KCl and $MgCl_2$) at equal ionic strengths (IS) equivalent to 2.5% NaCl, and three types of polyphosphates TPP, HMP, and SAPP added to 2.0% NaCl frankfurters were compared in

Author Barbut is with the Dept. of Animal Science, Univ. of Guelph, Guelph, Ontario Canada N1G 2W1. Author Maurer is with the Dept. of Poultry Science, Univ. of Wisconsin, Madison, WI 53706. Author Thayer is with the ARS, USDA, Eastern Regional Research Center, Philadelphia, PA 19118.

Table 1—Effects of radiation dose on C. botulinum toxin production in turkey frankfurters formulated with different chloride salts and polyphosphates

Trt. no.	Trial	Salt Type	Salt IS[a]	Salt %	PO_4[b] 0.4%	Dose Mrad	pH	Days at 27°C: 1	2	3	4	5	6	7	8	9	10	11	12	13	15	20	30	40	50
								(No. of toxic samples out of 3)																	
1	A	NaCl	0.42	2.5		0	6.53	—[f]	—	—	—	3													
	B						6.52	—	—	—	3														
2	A					0.5	6.55	—	—	—	—	—	—	—	—		—		—		—	—	—	—	—
	B						6.50	—	—	—	—	—	—	—	—		—		—		—	—	—	2	
3	A					1.0	6.55	—	—	—	—	—	—	—	—		—		—		—	—	—	—	—
	B						6.52	—	—	—	—	—	—	—	—		—		—		—	—	—	—	
4	A	KCl	0.42	3.13		0	6.60	—	—	1	2	3													
5	A					0.5	6.59	—	—	—	3														
6	A					1.0	6.58	—	—	—	—	—	—	—	—	1	3								
7	A	$MgCl_2$	0.42	1.33		0	6.43	—	—	—	3														
8	A					0.5	6.45	—	—	—	—	3													
9	A					1.0	6.45	—	—	—	1	1	3												
10	A	NaCl		2.0	TPP[c]	0	6.71	—	—	—	3														
11	A				TPP	0.5	6.71	—	—	—	3														
12	A				TPP	1.0	6.73	—	—	—	—	—	1	1	1	1	1	3							
13	A	NaCl		2.0	HMP[d]	0	6.52	—	—	—	3														
14	A				HMP	0.5	6.54	—	—	—	—	1	—	—	—	—	—	—	3						
15	A				HMP	1.0	6.55	—	—	—	—	—	—	—	—	—	—	—	8	3					
16	A	NaCl		2.0	SAPP[e]	0	6.30	—	—	—	—	—	—	—	—	—	3								
17	A				SAPP	0.5	6.32	—	—	—	—	—	—	—	—	—	—		—		—	—	—	1	—
18	A				SAPP	1.0	6.31	—	—	—	—	—	—	—	—	—	—		—		—	—	—	—	—

[a] IS = Ionic strength.
[b] PO_4 = Phosphate.
[c] TPP = Tripolyphosphate.
[d] HMP = Hexametaphosphate.
[e] SAPP = Sodium acid pyrophosphate.
[f] No toxin sample out of the three checked was found.

an irradiation experiment. Only the 2.5% NaCl treatment, which represents the most commonly used NaCl level in this type of product (Maurer, 1983; Sofos, 1983), was tested twice in two different trials.

Ingredients and product manufacturing

Mechanically deboned turkey meat (MDTM) with 14.0% protein, 13.5% fat, 70.5% moisture and 1.2% ash (AOAC, 1975) was used. The common ingredients in the frankfurters were 2.0% corn syrup solids, 1.0% dextrose, 0.25% white pepper, 0.07% nutmeg, 0.05% sodium erythorbate, 0.015% sodium nitrite and 0.5% liquid smoke (Milwaukee Seasoning Laboratories, Germantown, WI). NaCl (Columbus Chemical, Inc., Columbus, WI), KCl, $MgCl_2$ (Fisher Scientific) TPP, HMP, and SAPP (Stauffer Chemical, Inc., Washington, PA) concentrations varied among treatments. Product manufacturing, *C. botulinum* inoculation and pH measurements of the cooked products are described in a previous publication (Barbut et al., 1986b).

Spore inoculum

The *C. botulinum* spore inoculum used was a composite of equal numbers of five strains each of type A and B (56A, 62A, 69A, 77A, 90A, 53B, 113B, 213B, 13983B, and Lamanna — akra B). The inoculum concentration was 4.5–5.0 x 10^2 spores/g of frankfurter emulsion. All raw batters were inoculated with the same volume of spore suspension.

Irradiation

Frankfurters were vacuum packaged individually followed by washing the packages with 400 ppm chlorine solution and ethanol. The frankfurters were kept in a cooler and irradiated on the following day at doses of either 0.5 or 1.0 Mrad at a temperature of 2 ± 1°C (by immersing the packages in slushed ice and water) in a self-contained, Cesium-137 irradiator with a strength of 132,000 Ci at the Eastern Regional Research Center, USDA, Philadelphia, PA. A dose rate of 10 krad per minute was used in the experiment. Ferrous sulfate/cupric sulfate ($FeSO_4$/$CuSO_4$) dosimeters were used to monitor the absorbed doses.

Toxicity testing and spores enumeration

Botulinal toxicity presence using the mouse bioassay test (three packages sampled/treatment/observation), confirmatory testing and spore enumeration by the 5-tube most probable number (MPN) method are also described in previous publication (Barbut et al., 1986b).

RESULTS & DISCUSSION

Chloride salts

The antibotulinal effects of the three chloride salts (NaCl, KCl, $MgCl_2$) added at the same IS of 0.42 (equivalent to 2.5% NaCl) coupled with irradiation treatments are presented in Table 1. The results clearly showed that NaCl was the most inhibitory salt to *C. botulinum* toxin production after exposure to 0.5 or 1.0 Mrad of gamma-irradiation. This was a significant finding considering the trend on the market today for sodium reduction/replacement in processed food products. Both KCl and $MgCl_2$ have been reported in the literature to have the potential to partially replace NaCl in meat products (Seman et al., 1980; Hand et al., 1982).

In the nonirradiated treatments NaCl delayed toxin production by at least one day more than either KCl or $MgCl_2$. The results are in agreement with Barbut et al. (1986a) who reported NaCl to be the most inhibitory salt for *C. botulinum* toxin production in poultry frankfurters when NaCl, KCl and $MgCl_2$ were compared. It was shown in that study (Barbut et al., 1986a) that at higher concentrations two of the salts were even more inhibiting; at an IS of 0.68 (equivalent to 4.0% NaCl), the salts were ranked as NaCl > KCl >> $MgCl_2$ and delayed toxin production up to 30, 16 and 3 days, respectively.

At the 2.5% NaCl level, 0.5 Mrad was sufficient to inhibit toxin production up to 40 days in the second trial and at least up to 50 days (experiment was terminated) in the first trial. Under the conditions provided for *C. botulinum* growth in this experiment (27°C, anaerobic environment, heat shock due to cooking and high inoculation levels), a one month delay in toxin production can be considered very effective. These results represent a delay in toxin production of at least 10 fold (in both trials a and b) due to the 0.5 Mrad treatment. At the 1.0 Mrad level, no toxin was detected throughout the entire experiment when 2.5% NaCl was used.

Total replacement of NaCl with KCl coupled with irradiation treatment was not as effective in delaying botulinal toxin production. Exposure of KCl containing frankfurters to 0.5 Mrad delayed toxin production by one day as compared to the non-irradiated sample. Application of 1.0 Mrad to the KCl containing frankfurters delayed toxin production up to the 9th day, which represents a threefold improvement. This improvement was not as effective as the irradiated NaCl treatment where toxin production was inhibited for a minimum of 40 days when 1.0 Mrad was used.

No marked improvement over the nonirradiated treatment was observed when the $MgCl_2$ frankfurters were irradiated with 0.5 or 1.0 Mrad. Heinis et al. (1977) and Ma-lin and Beuchat (1980) reported that the presence of magnesium ions in media and/or foods actually improved recovery of heat- or cold-injured *V. parahaemolyticus*. Similarly, Hughes and Hurst (1976) showed that Mg^{++} was required for repair of heat injured *S. aureus*.

Phosphates

Three types of phosphates which are commonly used in the meat industry were added to the 20% reduced NaCl poultry frankfurter (2.0% NaCl) and evaluated for their antibotulinal effect. Comparing the non-irradiated treatments indicated that only SAPP was effective in delaying toxin production. Both the TPP and HMP treatments became toxic after the 4th day, whereas the SAPP treatment inhibited toxin production until the 10th day. This is in agreement with previously reported results (Barbut et al., 1986b). Nelson et al. (1983) reported a similar trend when comparing the three phosphates in the presence of sorbate.

Exposure of the reduced salt frankfurters containing TPP to 0.5 Mrad did not improve the antibotulinal effect; exposure to 1.0 Mrad did delay toxin detection by 2 days as compared to the non-irradiated control (6 vs 4 days) and all the TPP samples were toxic by the 11th day. In frankfurters containing 2% NaCl + HMP and treated with 0.5% Mrad, one toxic sample was found on the 5th day and all samples were toxic by the 12th day; with 1.0 Mrad all HMP containing frankfurters were toxic by the 13th day.

The SAPP treatment was more effective than either the TPP or the HMP treatments in delaying toxin production. SAPP containing frankfurters treated with 0.5 Mrad delayed toxin production up to 40 days compared to 10 days without irradiation. No toxic samples were found when the SAPP treatment was exposed to 1.0 Mrad. Inhibition of botulinum toxin production in the 2% NaCl + 0.4% SAPP treated with 1.0 Mrad was equivalent to 2.5% NaCl treated with 1.0 Mrad.

Additional information obtained in this study dealing with estimating the number of survived spores (in few of the treatments) by the MPN method suggested that *C. botulinum* spores were inactivated or injured due to gamma-irradiation exposure, and their ability to grow/recover mainly depended on the type salt present in the media. Neither the 0.5 Mrad nor the 1.0 Mrad treatment was sufficient to totally inactivate all the C. botulinum spores. The lowest number recorded was 10 spores/g after exposure to 1 Mrad in the 2.5% NaCl treatment, suggesting that in each of the treatments there were still enough viable spores that had the potential to grow. Sublethal damage, as a result of heat, chemical or irradiation, will results in spores which are more sensitive than uninjured spores to selective agents such as: NaCl (Chowdhury et al., 1976), antibiotics (Rowley et al., 1983) and nitrites (Roberts and Ingram, 1966). In this study it was observed that *C. botulinum* spores were inactivated or injured by exposure to gamma-irradiation, and their ability to recover and produce toxin was directly related to the type of salt in the meat system. NaCl at a concentration of 2.5% exhibited the best *C. botulinum* inactivation when coupled with irradiation, followed by KCl and $MgCl_2$. When phosphates were added to the reduced NaCl treatments, only SAPP demonstrated a marked antibotulinal activity which was further enhanced when coupled with gamma-irradiation treatment.

REFERENCES

AOAC. 1975. "Official Methods of Analysis," 12th ed. Association of Official Analytical Chemists, Washington, DC.

Barbut, S., Tanaka, N., and Maurer, A.J. 1986a. Effects of varying levels of chloride salts on *Clostridium botulinum* toxin production in turkey frankfurters. J. Food Sci. 51: 1129.

Barbut, S., Tanaka, N., and Maurer, A.J. 1986b. Effects of sodium chloride reduction and polyphosphate addition on *Clostridium botulinum* toxin production in turkey frankfurters. J. Food Sci. 51: 1136.

Barbut, S., Maurer, A.J., and Lindsay, R.C. 1987. Effects of reduced sodium chloride and added phosphates on physical and sensory properties of turkey frankfurters. J. Food Sci. Submitted.

Chowdhury, M.S., Rowley, D.B., Anellis, A., and Levinson, H.S. 1976. Influence of postirradiation incubation temperature on recovery of radiation-injured *Clostridium botulinum* 62A spores. Appl. Environ. Microbiol. 32: 172.

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.

Heinis, J.J., Beuchat, L.R., and Jones, W.K. 1977. Growth of heat-injured *Vibrio parahaemolyticus* in media supplemented with various cations. Appl. Environ. Microbiol. 33: 1079.

Hughes, A. and Hurst, A. 1976. Magnesium requirement of *Staphylococcus aureus* for repair from sublethal heat injury. Can. J. Microbiol. 22: 1202.

IFT. 1980. Dietary salt; a scientific status summary by the IFT Expert Panel on Food Safety and Nutrition and the Committee on Public Information. Food Technol. 34(1): 85.

IFT. 1983. Radiation preservation of foods. Food Technol. 37(2): 55.

Ingram, M. and Kitchell, A.G. 1967. Salt as a preservative for foods. J. Food Technol. 2: 1.

Knipe, C.L., Olson, D.G., and Rust, R.E. 1985. Effects of selected inorganic phosphates, phosphate levels and reduced sodium chloride levels on protein solubility, stability and pH of meat emulsions. J. Food Sci. 50: 1010.

Kreiger, R.A., Snyder, O.P., and Pflug, I.J. 1983. *Clostridium botulinum* ionizing radiation D-value determination using a micro food sample system. J. Food Sci. 48: 141.

Ma-lin, C.F.A. and Beuchat, L.R. 1980. Recovery of chill-stressed *Vibrio parahaemolyticus* from oysters with enrichment broths supplemented with magnesium and iron salts. Appl. Environ. Microbiol. 39: 179.

Maurer, A.J. 1983. Reduced sodium usage in poultry muscle foods. Food Technol. 37(7): 60.

Nelson, K.A., Busta, F.F., Sofas, J., and Wagner, M.K. 1983. Effects of polyphosphates in combination with nitrite-sorbate or sorbate on *Clostridium botulinum* growth and toxin production in chicken frankfurter emulsions. J. Food Prot. 46: 846.

Pearson, A.M. and Wolzak, A.M. 1982. Salt: Its use in animal products — a human dilemma. J. Anim. Sci. 54: 1263.

Pivnick, H. and Barnett, H. 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(7): 1164.

Roberts, T.A. and Ingram, M. 1966. The effects of sodium chloride, potassium nitrate and sodium nitrite on the recovery of heated bacterial spores. J. Food Technol. 1: 147.

Rowley, D.B., Firstenberg-Eden, R., and Shattuck, G.E. 1983. Radiation-injured *C. botulinum* type E spores: outgrowth and repair. J. Food Sci. 48: 1829.

Sebranek, J.G., Olson, D.G., Whiting, R.C., Benedict, R.C., Rust, R.E., Kraft, A.A., and Woychik, J.H. 1983. Physiological role of dietary sodium in human health and implications of sodium reduction in muscle foods. Food Technol. 37(7): 51.

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.

Sofos, J.N. 1983. Effects of reduced salt (NaCl) level on sensory and instrumental evaluation of frankfurters. J. Food Sci. 48: 1692.

Sofos, J.N. 1984. Antimicrobial effects of sodium and other ions in foods: a review. J. Food Safety 6: 45.

Sofos, J.N. 1986. Use of phosphate in low-sodium meat products. Food Technol. 40(9): 52.

Swift, C.E. and Ellis, R. 1956. The action of phosphates in sausage products. 1. Factors affecting the water retention of phosphate treated ground meat. Food Technol. 10: 546.

Tanaka, N. 1982. Private communication. Food Research Institute, Madison, WI.

Terrell, R.N. 1983. Reducing the sodium content of processed meats. Food Technol. 37(7): 66.

Tompkin, R.B. 1984. Indirect antimicrobial effects in foods: Phosphates. J. Food Safety 6: 13.

Tonomura, Y., Imamura, K., Ikelhara, M., Uno, H., and Harada, F. 1967. Interaction between synthetic ATP analogues and actomyosin systems. J. Biochem. 61: 460.

Wierbicki, E. and Brynjolfsson, A. 1979. The use of irradiation to reduce or eliminate nitrite in cured meats. Presented at the 25th European Meeting Meat Research Workers, Budapest, August 27-31.

Ms received 1/9/87; revised 4/8/87; accepted 4/25/87.

Research supported by the College of Agricultural and Life Sciences, and the University-Industry Research Program, Univ. of Wisconsin, Madison, WI 53706, and the ARS, USDA, Philadelphia, PA 19118.

We express our appreciation to the Food Research Institute, University of Wisconsin-Madison for *C. botulinum* toxin testing facilities and to the Wisconsin Turkey Federation for donating meat and materials.