13 Antimicrobial activity and functionality of polyphosphates in reduced NaCl comminuted meat products

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INTRODUCTION

Sodium chloride (NaCl) is used in cured meats as a flavoring and antimicrobial agent, and to extract proteins which form a stable emulsion (Schmidt et al., 1981). Cured meats are high in sodium (> 1000 mg/100 g) (Marsh et al., 1980). It has, therefore, been recommended by public health and regulatory authorities that NaCl amounts in food be reduced, at least in the diets of individuals sensitive to hypertension. It has been shown, however, that as NaCl levels are decreased not only water holding capacity is reduced but also product texture and flavor become less acceptable, and the preservative capacity of the product may decrease (Sofos, 1983a; b; c).

In 1983 the United States Department of Agriculture permitted the use of various polyphosphates in all cured meat products. Polyphosphates are effective in improving water-holding capacity of whole muscles when used with NaCl (Shults et al., 1972). In addition, polyphosphates have improved texture of sausages (Puolanne and Terrell, 1983). Studies with meat products, however, are too limited to allow major conclusions on the usefulness of various polyphosphates as antimicrobial inhibitors in combination with reduced NaCl in commercial meat products (Roberts et al., 1981a; b; c; Nelson et al., 1983).

The objectives of this study were to: 1) evaluate the functionality and antimicrobial properties of reduced NaCl (1.25%) comminuted meat products; 2) determine whether various polyphosphates improve functionality and antimicrobial properties of these products; and 3) compare antimicrobial properties of products inoculated before thermal processing in cans and inoculated after thermal processing and packaged under vacuum.

MATERIALS AND METHODS

Ingredients: Common ingredients in all treatments were fresh lean (<5% fat) bull meat and pork (50% lean) trimmings; water (10%); ice (10%), corn syrup solids (0.5%), dextrose (0.5%), white pepper (0.25%), nutmeg (0.0625%), sodium erythorbate (0.03%), and sodium nitrite (0.01%). Treatments were formulated to a final fat content of 30% and included 2.5% NaCl and 1.25% NaCl controls (based on meat block), in addition to six food grade polyphosphate treatments with various molecular chain lengths. Polyphosphates, their chain length and concentrations tested were as follows: Sodium acid pyrophosphate (SAPP) 2, 0.17%; tetrasodium pyrophosphate (TSPP) 2, 0.20%; sodium tripolyphosphate (STPP)3, 0.22%; sodium tetrametaphosphate (TTPP) 5, 0.28%; sodium hexametaphosphate (HMPP)12, 0.33%; and, glassy sodium hexametaphosphate (GHMPP) 21, 0.34%. Each polyphosphate treatment was formulated with 1.25% NaCl. The sum of the ionic strength of 1.25% NaCl + each polyphosphate (provided by FMC Corporation, Philadelphia, PA) treatment was equivalent to 66% of the ionic strength of the 2.5% NaCl treatment. Ionic strength calculations were based on dissociation constants determined by Trout (1984) and average polyphosphate chain length was determined by the method described by Lowenheim (1973).

Product Manufacture: Five kilogram batches (two replicates) were chopped and emulsified in a Meissner model VE, 35 liter vacuum bowl chopper (RMF Steel, Kansas City, MO) to a constant endpoint of 13°C. The emulsions were extruded into 24mm diameter cellulose frankfurter casings, four 303x406 cans (440 g/can), and 25 small (208x108) cans (90 g/can) using an E-Z Pak water powered piston stuffer. Frankfurters were heat processed and smoked to a temperature of 70°C. Large cans (303x406) were left uninoculated, while small cans (208x108) were inoculated (20/treatment) with Clostridium sporogenes spores. Cans (provided by American Can Co., Chicago, IL) were sealed with Dixie can closing machines and thermally processed to 70°C in an open air agitated thermostatically controlled Dixie retort.

Test Organism and Inoculum: A stock culture of C. sporogenes P.A. 3679 was obtained from the National Food Processors Association. Spore suspensions were prepared according to the method of Santo Goldoni et al. (1980). The raw emulsions in the 208x108 cans (20/treatment) were inoculated with a syringe with 2 ml of heat shocked (80°C, 15 min) spore suspension (100 spores/g, target inoculum). Five small cans, which served as uninoculated controls, were injected with 2 ml of sterile distilled water. Cooked product in the uninoculated large (303x406) cans was sliced and inoculated with C. sporogenes spores (65/g, target inoculum) by spreading the inoculum with a glass rod on the slices. Slices (3/nylon package) were inoculated with 0.4 ml of suspension and vacuum packaged to -0.8 bar (20 packages/treatment).

Microbiological Evaluation: Inoculated cans and vacuum packages were stored at 27°C and monitored daily for gas production (swelling). Total mesophilic anaerobic and aerobic counts, and product pH were determined for inoculated canned and vacuum packaged products. Thirty grams of product were blended with 270 ml of sterile 0.1% peptone diluent and serial dilutions were made in 0.1% peptone for microbial analyses. These initial blends were also used for pH determinations. Trypticase soy agar (TSA, BBL) in Lee tubes (Ogg et al., 1979) was used to determine total mesophilic anaerobic counts and total anaerobic spore counts (37°C, 24 hr). APT agar (BBL) was used to determine total mesophilic aerobic plate counts after incubation at 37°C for 48 hrs.

Frankfurter Quality: Evaluation of uninoculated frankfurters included consumer sensory taste panel (ten untrained panelists evaluated color, texture, flavor and overall acceptability on a 9-point hedonic scale, 9-like extremely, 1-dislike extremely), and objective evaluation of product texture with a single blade cell modified Warner-Bratzler meat shear, model 2000 (G. R. Electric Manufacturing Co., Manhattan, KS). Texture (shear force) was also determined on canned uninoculated product using 25 mm plugs of cooked product.

Emulsion Stability and Losses: Frankfurter smokehouse yield and canned product cook yield were expressed as the percentage of the difference between product weight before thermal processing and product weight after processing. The method of Townsend et al. (1968) as modified by Sofos (1983b) was used to evaluate emulsion stability. Moisture, fat, protein, and NaCl contents of canned products were determined according to standard procedures (AOAC, 1975).

Experimental Design and Statistical Analysis: A randomized complete block design with two replicates was used in this study. Each replicate represented one block that was prepared and processed on one day. Data were analyzed by analysis of variance, and when F values were significant, Fischer's Least Significant Difference was used to separate statistical differences between treatment means (Snedecor and Cochran, 1971).

RESULTS AND DISCUSSION

Emulsion Stability and Cook Yield: Emulsion losses in cans, tubes and frankfurters were significantly (P<0.05) higher in the reduced (1.25%) NaCl product compared to the regular (2.5%) NaCl product (Table 1). Losses in cans and tubes (2.5% NaCl) were less dramatic than in frankfurters. This was because cans and tubes prevented product dehydration which happens in smokehouse cooked frankfurters. The lower losses of 1.25% NaCl frankfurters compared to 1.25% NaCl product in cans and tubes can be explained if we consider that fat caps entraped by the casings were included in yield determinations. The high losses of reduced (1.25%) NaCl products were (to various degrees) prevented by the different polyphosphates tested (with the exception of GHMPP which increased losses in frankfurters). The effect of HMPP on cook yield and emulsion stability was intermediate between that of the ineffective GHMPP and the other four highly effective phosphates (Table 1). Since all reduced NaCl (1.25%)/polyphosphate treatments were formulated to a constant ionic strength, differences in emulsion stability may be attributed to pH differences or other unknown factors (Trout, 1982; Hamm, 1970; Hellendorn, 1962).

Table 1. Cook yield (%), emulsion losses (%) and brine (%) in the cooked (70°C) comminuted meat product (two replicates).

Parameter	NaCl (%) and Polyphosphate type (%)								LSD (0.05)
	2.5		1.25						
	-	-	SAPP	TSPP	STPP	TTPP	HMPP	GHMPP	
Frankfurter yield	93.6	90.7	92.9	92.9	92.5	93.0	91.6	84.3	2.8
Can yield (wt. %)	98.2	89.9	98.5	99.9	99.9	99.3	93.9	92.1	3.1
Tube loss (wt. %)	5.4	28.7	6.3	4.1	3.5	7.4	18.8	25.3	4.9
Brine (% w/v)	3.60	2.01	2.08	1.99	1.97	2.05	1.95	1.99	-

Proximate Composition: Moisture (50.5-54.1%), fat (31.2-33.7%), and protein (11.9-13.7%) were not significantly different (P>0.05) among NaCl controls (2.5%, 1.25%) and reduced NaCl/polyphosphate treatments (data not shown). Generally, low yielding treatments had slightly lower moisture contents and slightly increased fat and protein contents (i.e. reduced NaCl, HMPP, and GHMPP). Sodium chloride and brine (% NaCl, w/v, in the water phase) contents among the reduced NaCl (1.25%) and reduced NaCl/polyphosphate treatments were similar (1.00-1.14% NaCl; 1.95-2.08% brine, Table 1). Therefore, products of similar composition were obtained and valid comparisons among treatments could be made.

Sensory Evaluation: Analysis of variance of frankfurter texture, flavor, and overall acceptability, evaluated by a consumer taste panel, did not show significant (P>0.05) treatment effects (Table 2). This was probably due to the large variation among replicates and individual panelists. However, general observations indicate that the reduced NaCl (1.25%) control was disliked by panelists and the addition of polyphosphates did improve acceptability.

Table 2. Sensory evaluation scores (9 - point hedonic scale: 9 - like extremely, 1 - dislike extremely) and shear force (kg) of frankfurters (two replicates).

Parameter	NaCl (%) and Polyphosphate type (%)								LSD (0.05)
	2.5		1.25						
	-	-	SAPP	TSPP	STPP	TTPP	HMPP	GHMPP	
Color	5.9	4.7	5.3	5.3	6.7	6.2	6.1	5.8	0.5
Texture	5.8	3.3	5.2	5.9	6.8	6.0	5.5	5.3	NS
Flavor	6.0	3.9	5.0	5.6	5.6	5.7	5.5	5.4	NS
Acceptability	5.8	3.7	5.1	5.5	6.0	5.7	5.7	5.3	NS
Shearforce	0.86	0.53	0.92	1.20	1.07	0.93	0.67	0.47	0.25

Instrumental Texture: Shear force results (Table 2) follow the general pattern established in smokehouse and canned cook yield. SAPP, TSPP, STPP, and TTPP significantly (P<0.05) improved texture measurements (0.9-1.2 kg) over the reduced NaCl control treatment (0.5 kg) and were comparable to the 2.5% NaCl control products (0.86 kg). The two long-chain polyphosphates (HMPP and GHMPP) in combination with reduced NaCl did not show any significant difference (P>0.05) from the reduced NaCl control treatment. Therefore, HMPP and GHMPP would not be considered to be effective in partially replacing NaCl, while SAPP, TSPP, STPP, and TTPP would be effective replacements.

Product pH: The raw and cooked emulsion pH values (Table 3) of both NaCl control treatments were similar (6.03 and 6.05, 6.22 and 6.21, respectively). Addition of SAPP decreased raw emulsion pH to 5.99, while all other polyphosphate treatments increased product pH by 0.1 to 0.4 units (P<0.05). These results are consistent with those of Shults et al. (1972); and Trout (1982), who reported that the order of pH increase by polyphosphates was pyrophosphate > tripolyphosphate > hexametaphosphate. With storage at 27°C, packaged product pH (Table 3) decreased as products spoiled. Initial pH values and changes during storage of canned products (data not shown) were similar to those presented above for packaged products, with the exception that pH decreases with storage of canned product were less pronounced and were followed by an eventual increase in pH as spoilage progressed.

Table 3. pH of vacuum packaged product inoculated after thermal processing with Clostridium sporogenes and stored at 27°C (two replicates).

Days of Storage (27°C)	NaCl (%) and Polyphosphate type (%)								LSD (0.05)
	2.5		1.25						
	-	-	SAPP	TSPP	STPP	TTPP	HMPP	GHMPP	
Raw	6.03	6.05	5.99	6.44	6.35	6.20	6.11	6.16	0.05
Cooked	6.22	6.21	6.06	6.48	6.45	6.32	6.31	6.33	0.33
3	5.97	5.63	6.01	5.78	5.88	5.89	6.04	6.11	0.33
5	5.30	5.29	5.28	5.45	5.54	5.58	5.74	5.60	0.33
9	5.12	4.58	5.14	5.52	5.12	5.14	5.24	5.38	0.33

Microbiological Growth: Actual anaerobic spore counts of canned meat product inoculated with C. sporogenes before thermal processing averaged 76/g, while vacuum packaged meat product inoculated after thermal processing averaged 10/g (data not shown). As expected, after thermal processing

anaerobic counts of canned product decreased (Table 4) by more than one log cycle (3.22-3.66 to 1.39-1.96). Differences between treatment combinations on a given day were not (P>0.05) significant, although individual treatment counts increased significantly (P<0.05) after 5 days of storage at 27°C. This indicates that at an abuse temperature of 27°C, mesophilic anaerobes grew rapidly and reached maximum growth within 5 days of storage at 27°C, irrespective of treatment. Aerobic and anaerobic counts for vacuum packaged product (data not shown) changed similarly to the above anaerobic counts for canned product.

Table 4. Anaerobic counts (log CFU/g) of canned product inoculated before thermal processing with Clostridium sporogenes spores (76/g) and stored at 27°C[a] (two replicates).

Days of Storage (27°C)	NaCl (%) and Polyphosphate type (%)								
	2.5	1.25							LSD
	-	-	SAPP	TSPP	STPP	TTPP	HMPP	GHMPP	(0.05)
Raw	3.44	3.26	3.22	3.61	3.66	3.55	3.27	3.42	NS
Cooked	1.94	1.72	1.70	1.53	1.69	1.39	1.53	1.96	2.34
3	3.68	3.57	3.09	4.98	4.98	4.98	3.69	3.98	2.34
5	7.48	7.66	5.97	8.06	7.75	7.48	7.88	7.37	2.34
9	7.40	*	7.36	*	*	*	6.97	7.21	NS

*Not determined because treatment was spoiled.

Gas Production: Although bacterial counts among NaCl controls (2.5%, 1.25%) and reduced NaCl/polyphosphate treatment combinations did not differ in canned and vacuum packaged products, gas production and can and package swelling did show differences among treatments. The data of Figure 1 indicate that the 2.5% NaCl control and reduced NaCl (1.25%)/SAPP combination were the most effective in delaying formation of gas in canned product. The two long-chain polyphosphates (HMPP and GHMPP) were next in effectiveness in delaying gas production. All other polyphosphates (TSPP, STPP, and TTPP) were not effective. These general observations also held true for gas production in vacuum packaged product inoculated after thermal processing (Fig. 2). The primary difference between canned and packaged product was that gas production overall occured more rapidly in product packaged in pouches (Table 5).

Table 5. Number of days (27°C) to first gas swell of inoculated vacuum packages and cans, and uninoculated cans (two replicates).

Product	NaCl (%) and Polyphosphate type (%)							
	2.5	1.25						
	-	-	SAPP	TSPP	STPP	TTPP	HMPP	GHMPP
	Days to First Gas							
	Cans							
Inoculated	9.5[a]	3.5[b]	8.0[a]	4.5[b]	4.0[b]	3.5[b]	5.5[b]	5.5[b]
Uninoculated	27[a]	5.5[b]	10.5[b]	4.5[b]	4.5[b]	4.0[b]	5.5[b]	5.5[b]
	Packages							
Inoculated	6.0[a]	3.0[b]	5.0[a]	3.0[b]	3.5[b]	3.0[b]	5.5[a]	5.0[a]

a,b Means with different superscripts in the same row are significant (P<0.05).

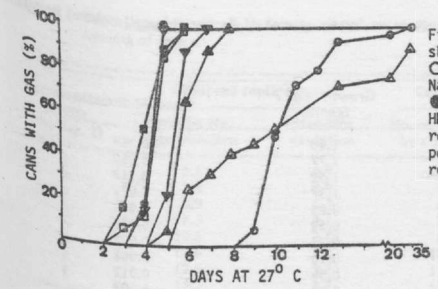

Figure 1-Cans (%) showing gas swells. ○2.5% NaCl;□1.25% NaCl;△SAPP;▽TSPP; ●STPP;■TTPP;▲ HMPP;▼GHMPP (two replicates; 15 cans per treatment and replicate).

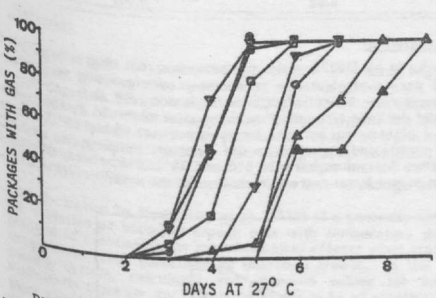

Figure 2-Vacuum packages (%) showing gas swells.○2.5% NaCl; □1.25% NaCl;△SAPP; ▽TSPP;●STPP;■ TTPP;▲HMPP;▼GHMPP (two replicates; 15 packages per treatment and replicate).

Days to First and 50% Gas: Only 3.0 to 5.5 days were required for the first gas swell to occur in the reduced NaCl control and in most reduced NaCl/polyphosphate (TSPP, STPP, TTPP, HMPP, and GHMPP) treatments (Table 5). Differences among these treatments were not significant (P>0.05), even though the first gas swell for HMPP and GHMPP occured in 5.0-5.5 days. The 2.5% NaCl control and SAPP/low NaCl combination required 6.0-7.5 and 5-8 days of storage at 27°C respectively, for the first gas swell to occur. In terms of the number of days to 50% gas swells, the same trends were apparent (data not shown). These results agree with those found by Robach (1979) who investigated the effect of curing ingredients (nitrite, sorbic acid, polyphosphates) on cured comminuted pork inoculated with C. sporogenes (1000 spores/g). Other researchers (Nelson et al., 1983; Wagner and Busta, 1983) have also shown similar patterns of polyphosphate effectiveness in delaying outgrowth of Clostridium botulinum. The results of Roberts et al. (1981a; b; c) were with commercial polyphosphate blends and do not apply directly to this study. Therefore, SAPP and possibly HMPP or GHMPP in combination with reduced NaCl (1.25%) would provide additional preservative capacity in a reduced NaCl product.

Inoculated vs. Uninoculated Product: Comparing the number of days to first gas swells in inoculated and uninoculated cans, inoculation substantially reduced shelf-life in the 2.5% NaCl treatment, but did not appreciably change the already very short shelf-life of the reduced NaCl (1.25) and low salt/polyphosphate combination treatments (Table 5). This indicates that with reduced NaCl (1.25%) the preservative capacity was reduced to such a low level that microorganisms (natural contamination) that survived the pasteurization treatment were sufficient to cause spoilage and gas swells in a very short time.

CONCLUSIONS

Reduced NaCl (1.25%) levels in comminuted meat products resulted in reduced functionality and antimicrobial properties. Products formulated with reduced (1.25%) NaCl in combination with each of four polyphosphates (SAPP, TSPP, STPP, or TTPP) had improved functionality, while those formulated with HMPP and, especially, GHMPP were of relatively low functionality.

SAPP, and to some extent HMPP and GHMPP improved antimicrobial properties of reduced NaCl (1.25%) comminuted meat products inoculated with C. sporogenes spores. The other polyphosphates (TSPP, STPP, TTPP) did not improve the greatly reduced antimicrobial activity of the low NaCl (1.25%) meat product.

Product pH was decreased by SAPP (6.06-6.08) while all other polyphosphates increased product pH (6.31-6.48). Further research is needed to determine whether antimicrobial properties of SAPP are due to pH declines in the product and/or due to a specific polyphosphate effect.

Antimicrobial effects were similar in products inoculated in cans before thermal processing (70°C), and in similar products inoculated after thermal processing (vacuum packages). Gas production, however, appeared sooner and pH decreases were more rapid and dramatic in vacuum packaged product.

Among the 1.25% NaCl control and 1.25% NaCl/polyphosphate treatments, excluding SAPP, very little difference was noted between the number of days to first gas swell for inoculated and uninoculated cans; thus, indicating that with reduced NaCl (1.25%) the preservative capacity was reduced to such a low level that microorganisms surviving the pasteurization treatment were sufficient to cause swelling in uninoculated cans in a very short time (4-5 days). Furthermore, only when the preservative capacity was increased either by 2.5% NaCl or SAPP was a difference noted in the number of days to first gas swell between inoculated and uninoculated cans.

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