Food Chemistry **18** (1985) 179–191

Effect of Polyphosphates on the Survival of Pre-stressed *Salmonella typhimurium* Cells in Frozen Chicken Meat

A. Obafemi* & R. Davies

National College of Food Technology, Department of Food Technology, Food Studies Building, University of Reading, Whiteknights, Reading RG6 2AP, Great Britain

ABSTRACT

Six different commercial polyphosphate preparations were evaluated for their lethal efficacies against inoculated pre-stressed stationary phase cells of Salmonella typhimurium *LT_2 in frozen comminuted poultry meat subsequently stored at −18°C for 28 days. At the 0·3% wt/wt concentration used, Puron 6040 was found most potent, achieving approximately 2 log cycle viability reduction, closely followed by Fibrisol N7 and sodium tripolyphosphate with 94·7% and 84·5% viability reductions, respectively. Curaphos 700 instant and tetrasodium pyrophosphate No. 7 both exhibited similar potency magnitudes of approximately 70% viability reduction each, while acid sodium pyrophosphate was the least potent. The polyphosphates slightly altered sample pH values and aided fluid retention without any detectable influence on the a_w value. The findings demonstrate the potential antimicrobial benefits derivable from polyphosphates incorporation coupled with the conferrable textural advantages. It is considered that the observed potencies are influenced by chemical factors including chain length, pH and the subsequent relative stabilities of the polyphosphates in the menstruum system.*

INTRODUCTION

Polyphosphates are known to be widely used in food systems as emulsifiers, buffers, protein–fat stabilisers, dispersants, discoloration

* Present address: Department of Microbiology, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.

Food Chemistry 0308-8146/85/$03·30

inhibitors, rancidity inhibitors, flavour improvers and fluid retainers during the cooking and thawing of frozen products (Ellinger, 1972; Hammence & Kunwardia, 1974). Hammence & Kunwardia (1974) have also reported their use in poultry during normal processing by the injection of 4% polyphosphates solution at a rate up to 6% by body weight, where they are said to improve the quality and consumer acceptance of the processed carcasses. Polyphosphates are further reported to aid water retention and increased tenderness and succulence as well as improving the binding properties of comminute products plus occasional prevention of microbiological spoilage (Ellinger, 1972).

Spencer & Smith (1962) obtained a reduction in the rate of microbiological spoilage on chicken–fryer carcasses when chilled for 6 h in ice–water containing 10 oz of polyphosphate per gallon. The anti-microbial effect was also confirmed by Kraft & Ayres (1961, 1964) who noted a reduction in off-flavour production with the attendant extended shelf-life. Other workers have similarly reported the anti-microbial effect of polyphosphate treatment in poultry including Jimenez *et al.* (1961) and Elliot *et al.* (1964).

Apart from the report of Foster & Mead (1976) there is inadequate information about the potential use of polyphosphates as anti-*Salmonella* agents in poultry meat. In view of the importance of poultry meat as a vehicle of food-borne human salmonellosis (Communicable Disease Surveillance Centre, 1980), coupled with such demonstrable anti-microbial activity of polyphosphates generally, there is a need for evaluating the efficacy of polyphosphates when used in conjunction with freezing as potential poultry meat decontaminants against *Salmonella*. The study was therefore carried out to screen some commercial polyphosphate preparations for demonstrable potency against *Salmonella typhimurium* cells in frozen burger-type poultry comminuted meat and to relate any observed efficacy to the respective chemical nature of such polyphosphates.

MATERIALS AND METHODS

Test organism

Cells of *S. typhimurium* LT_2 were grown in Ozoid tryptone soya broth (TSB) in a 250 ml conical flask at 37 °C with reciprocal shaking at 100 r/min. As determined from the preliminary growth curve, cells were harvested after a 24 h incubation period to obtain stationary phase cells.

The harvesting procedure involved centrifuging at 3000 *g* for 15 min at 24 °C in an MSE Mistral 6 constant temperature centrifuge. Resultant pellets of cells were washed twice with sterile distilled water. Such harvested stationary phase inoculum was pre-stressed by resuspension in a pre-optimised triple stress system containing 5 ppm hypochlorite acidified with 1 % succinic acid at 5 °C. The total stress exposure duration including reharvesting by centrifuging was limited to 20 min. Exploitation of the triple stress system is considered to have a positive potential contribution towards *Salmonella* decontamination in routine poultry processing (Obafemi, 1983). The pre-stressed cells were subsequently washed twice with sterile distilled water prior to the final resuspension and use as inoculum.

Chicken comminute system

Freshly killed dressed chicken carcasses, guaranteed to be polyphosphate-free, were obtained from a local farmer. Such carcasses were aseptically dissected to remove chicken flesh devoid of skin and bone components and then minced together aseptically in a previously disinfected Kenwood Chef mixer using the mincer attachment. Subsequently the minced chicken meat was blended together to obtain a homogeneous comminute, by the use of appropriate Kenwood accessories.

From the bulked comminute, 10 g representative samples were weighed out into sterile Sterilin stomacher 400 bags (Sterilin, Teddington, Surrey, UK) to which were added 90 ml sterile phosphate buffer diluents (pH 7) followed by 1 min stomaching in a Colworth Stomacher Type 400 (A. J. Seward, London, UK). One millilitre volumes were pipetted into 9 ml phosphate buffer diluents from which further serial dilutions were made and plated out by surface spread technique on both TSA and TSAD in duplicate. After incubation at 37 °C, counts were made at 24 h and 48 h, to obtain the initial level of background microbial contamination of the comminute.

The remaining comminute was weighed out in sterile stomacher bags, sealed and stored at −18 °C until required.

Polyphosphates: preparation and use

Six different commercial polyphosphate preparations were screened as listed in Table 1 showing brand names, manufacturer and sources from

TABLE 1
Sources of the Different Polyphosphates

Brand name	*Manufacturer*	*Source*
Puron 6040[a]	Albright & Wilson Ltd	Albright & Wilson Ltd
Sodium tripolyphosphate	Albright & Wilson Ltd	Dr P. Gibbs, BFMIRA
Acid sodium pyrophosphate	Fibrisol Ltd	Dr P. Gibbs, BFMIRA
Tetrasodium pyrophosphate	Fibrisol Ltd	Dr P. Gibbs, BFMIRA
Curaphos 700	Fibrisol Ltd	Dr P. Gibbs, BFMIRA
Fibrisol N7	Fibrisol Ltd	Fibrisol Ltd

[a] Supplied as a concentrated stock solution while other polyphosphates were obtained in the powdered form.

which donated. The choice was based on their range of different chemical characteristics in order to determine how such might relate to any exhibited potency in combination with freezing. All polyphosphates were prepared as 5% aqueous solutions and used at 0·3% wt/wt concentration in the comminute samples. This involved pipetting appropriate volumes of freshly prepared polyphosphate solutions into the weighed comminute samples and mixing thoroughly to ensure uniform dispersal. The pH values of the respective polyphosphate solutions were determined as well as values for comminute samples before and after polyphosphate addition using a Vibret Laboratory pH meter model 46A (E.I.L., Richmond, Surrey, UK). The water activity of the respective polyphosphate-containing samples was also determined for 5 g aliquots pre-equilibrated to 23 °C using a calibrated Nova Sina DAL-020 electronic hygrometer (Humitec, Horsham, West Sussex, UK).

Procedure for potency evaluation

Harvested pre-stressed stationary phase cells of the organism were used to inoculate 25 g samples of the polyphosphate-containing comminute samples by pipetting 1 ml of the cell inoculum into such samples giving approx. 10^8 viable cells per g of sample. After manual mixing to ensure even cell distribution in the comminute, the samples were shaped manually to obtain approximately uniform thickness in a burger shape by

the use of external plastic template. Thereafter samples in the container plastic stomacher bags were immersed in liquid Freon bath at −30 °C (Arcton 12, ICI Ltd, London, UK) and frozen at a rate of 20 °C/min achieved at the thermal centre as measured by inserted thermocouples. Some of the representative samples were frozen to −5 °C, interrupted and held at −5 °C/24 h in an ethylene glycol water bath thermostatically maintained by an immersed cooling unit prior to subsequent cooling to −18 °C in liquid Freon. Others were frozen down directly to −18 °C in the liquid Freon without interrupted holding at −5 °C for purposes of comparison.

As controls, non-polyphosphate containing samples were similarly inoculated and frozen as described. All frozen samples were stored at −18 ± 0·5 °C for 28 days prior to thawing and enumeration.

Enumeration procedure and culture media

Unfrozen representative samples of the respective different types of polyphosphate-containing and polyphosphate-free communities were enumerated after inoculation to determine the actual initial levels of inoculum population to which the final survival would relate. Two hundred and twenty-five millilitres of sterile phosphate buffer (pH 7) was added to the 25 g inoculated samples, stomached for 1 min in a Colworth Stomacher 400 before serial dilutions and plating by the drop count method based on the technique of Miles & Misra (1938). The frozen samples, after storage, were completely thawed ice-free at 37 °C for 5 min before assaying for survival as above. All counts were plated on Oxoid tryptone soya agar (TSA) and concurrently TSA + 0·25 % wt/wt sodium deoxycholate (TSAD). Incubation was carried out at 37 °C prior to counting at 24 h and 48 h. The TSAD was incorporated to enable the detection and estimation of structurally injured survivors that became sensitive to sodium deoxycholate which would be normally tolerated by the healthy cells (Ray & Speck, 1973).

RESULTS AND DISCUSSION

The bacteriological quality of the meat samples was found to be such that the level of background contaminant microflora population, as counted

on TSA, was less than 10^2 colony-forming units (cfu)/g while no growth was obtained on the TSAD plates.

The chemical characteristics of the polyphosphate preparations and their effects on the measured properties of the meat samples are presented in Table 2. The polyphosphates ranged from those that were highly acidic to the strongly alkaline ones and from the short to the relatively longer chain types. Although the majority of the polyphosphates were able to shift the pH of the chicken meat samples slightly from the normal value of 5·9 in the unadulterated form, the final values were comparable. The observed relative stability of the pH values of the meat samples is probably due to the strong buffering capacity of the meat. In effect, therefore, none of the polyphosphates used could be strategically relied upon at the tested levels to substantially alter the sample pH value as a lethal strategy against contaminants. The incorporation of the polyphosphates into the meat samples was also not found to have any effect on the measured water activity values (Table 2). Such polyphosphate-containing samples, however, remained devoid of any exudate while the

TABLE 2
Characteristics of Polyphosphates Evaluated for Potency Against *S. typhimurium* Survival in Frozen Chicken Comminutes

Brand name	*Chain length*[a]	*pH of 5% aqueous solution*[b]	*pH of meat +0·3% wt/wt*[c]	a_w *of meat +0·3% wt/wt*[d]
Puron 6040	*P*2–>*P*4 mixture	6·4	5·9	0·99
Acid sodium pyrophosphate	*P*2	4·3	5·8	0·99
Tetrasodium pyrophosphate	*P*2	10·1	6·4	0·99
Curaphos 700 instant	*P*2–*P*10	8·9	6·0	0·99
Sodium polyphosphate	*P*3	9·2	6·1	0·99
Fibrisol N7	*P*2	6·9	5·9	0·99

[a] No. of phosphate groups per molecule as determined by the manufacturers.
[b] As determined in this study.
[c] As measured when each polyphosphate was added in solution to comminute at 0·3% level.
[d] As measured in Nova Sina equipment at 23 °C.

polyphosphate-free control samples visibly showed some exuded fluid within the container bags, after thawing.

Figure 1 shows the exhibited lethal efficacies of the different polyphosphates when used in conjunction with freezing/storage/thawing stress factors against the survival of inoculated stationary phase cells of *S. typhimurium* in the respective samples. By comparison

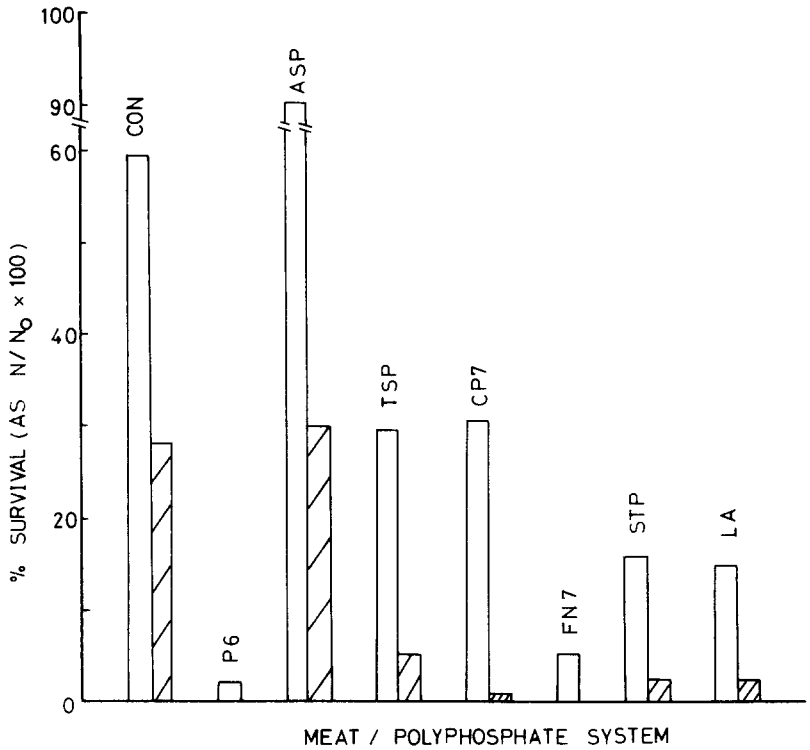

Fig. 1. The influence of polyphosphate type on the survival of inoculated stationary phase cells of *S. typhimurium* LT_2 in polyphosphate/chicken meat system subjected to defined identical freezing/storage/thawing regime. Polyphosphate (0·3 %) containing 25 g chicken meat samples were uniformly inoculated with harvested pre-stressed stationary phase cells of *S. typhimurium* LT_2. Freezing was carried out at 20 °C/min in liquid Freon bath directly to −18 °C and stored at $-18 \pm 0{\cdot}5$ °C for 28 days before thawing at 37 °C for 5 min, then enumerating the survivors. Open columns = counts on TSA plates. Cross-hatched columns = counts on TSAD (TSA + 0·25 % wt/vol sodium deoxycholate). CON = control polyphosphate-free sample; P6 = Puron 6040 polyphosphate containing sample; ASP = acid sodium pyrophosphate containing sample; TSP = trisodium pyrophosphate containing sample; CP7 = Curaphos 700 instant containing sample; FN7 = Fibrisol N7 containing sample and STP = sodium tripolyphosphate containing sample.

with the polyphosphate-free controls, the beneficial effect of the use of the polyphosphates in achieving survival reduction of inoculated cells in the frozen meat samples becomes evident (in addition to any textural advantages). In most cases, except for acid sodium pyrophosphate, the level of survival was less than 30%, with Puron 6040 emerging as the most potent of all (permitting approximately 2% survival only). This was followed by Fibrisol N7 and sodium tripolyphosphate.

In Fig. 2, showing the survival levels obtained when samples were held at −5 °C for 24 h in the course of freezing to −18 °C, the results obtained

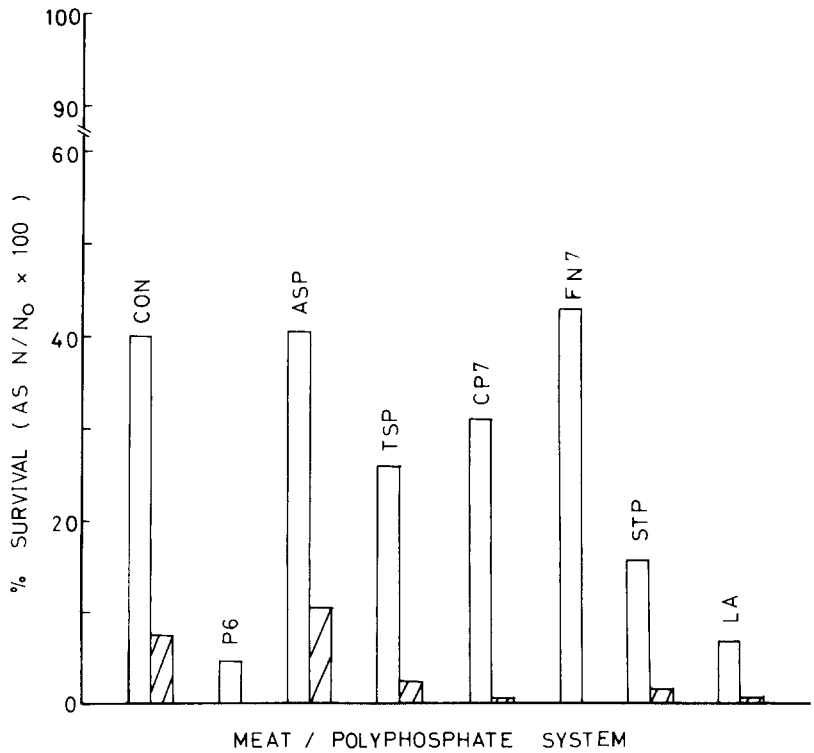

Fig. 2. The relative potencies of the different incorporated polyphosphates against the survival of *S. typhimurium* LT_2 cells inoculated and frozen in chicken meat subjected to additional lethal holding at −5 °C for 24 h. Polyphosphate (0·3%)-containing 25 g meat samples were uniformly inoculated with harvested pre-stressed stationary phase cells of *S. typhimurium* LT_2. Inoculated samples were frozen in liquid Freon bath to −5 °C, held at −5 °C for 24 h before cooling down to −18 °C followed by 28 days storage at −18 ± 0·5 °C. Thawing was carried out at 37 °C for 5 min before counting on TSA and TSAD (TSA + 0·25% sodium deoxycholate). (Key as Fig. 1)

clearly show that the holding effect generally did not enhance the lethal efficacy of the polyphosphates except that of the acid sodium pyrophosphate, while the potency of Fibrisol N7 was actually reduced. These observations are evident by comparing survival levels obtained in polyphosphate-free control samples with polyphosphate-containing ones, as well as by comparing the respective survival levels in Fig. 2 with those obtained in Fig. 1. Therefore, for practical purposes, such sample holding at $-5\,^{\circ}C$ has no lethal benefit, expecially when used in conjunction with most polyphosphates tested, although it is found advantageous in reducing cell survival from 60% to 40% in polyphosphate-free control samples.

The observed increased potency of Puron 6040 may have to do with the increased chain length characteristics. It has long been speculated that the mode of antimicrobial activity of polyphosphates is connected with their ability to chelate essential ions and substances required for the maintenance of microbial structural integrity (Hamilton-Miller, 1965). Batra (1965) also found that the ability of various polyphosphates to sequestrate metal ions increased with increasing chain length. In addition, according to Ellinger (1972), increasing chain length of polyphosphates is usually within limits, accompanied by increased anti-microbial activity. From the foregoing, it therefore seems likely that Puron 6040 is more potent by virtue of its chain length. This probably enables it to chelate vital ions needed for structural integrity leaving the cells weakened to the injurious effects of freezing and frozen storage. Long chain polyphosphates are also known to be capable of undergoing polyelectrolyte interactions with proteins (Ellinger, 1972) and this could further hamper the meat system from offering adequate protection against freezing damage of the cells, via the component $-NH_2$, $-OH$ groups suspected for cryoprotective H— bonding (Ray & Speck, 1973).

Similar reasons could have accounted for the observed slightly less potent results of sodium tripolyphosphate because Puron 6040 contains higher phosphates with chain lengths greater than three ($P > 3$) while sodium tripolyphosphate does not contain such. On the basis of chain length, it would have been expected that Curaphos 700 instant, with chain length $P2$–$P10$ should have shown comparable potency with Puron 6040 but this was not found to be so. Rather 99·8% of the survivors in the system involving the use of Curaphos 700 instant were rendered injured and sensitive to deoxycholate implying a great deal of structural injury. The apparent discrepancy could be a reflection of the relative stabilities of

the different polyphosphates with long chains in the comminute system and, in addition or alternatively, a reflection of the actual composition of the respective polyphosphates as higher phosphates, apart from the differences in pH. Hammence & Kunwardia (1974) showed that long chain polyphosphates could be broken down into simpler phosphate units in the form of ortho- and pyro-phosphates. However, the factors governing the rate of breakdown were not elucidated.

It is not clear whether the products of the polyphosphate hydrolysis are the critical lethal agents or the polymeric forms. Even if it were the hydrolysed subunits (e.g. ortho- and/or pyro-phosphates) more of the subunits could be derived from the polymers (weight for weight) depending upon what proportion of the longer chain polyphosphates like Puron 6040 actually exist as the $P > 3$ form. The rates of the relative breakdown to these supposedly 'active' units would also be expected to influence potency when comparing two long chain polyphosphates. However, it seems very unlikely that the simpler subunits (ortho- and pyro-phosphates) are the active agents. This suggestion is based on evidence from the water activity data as further explained.

It is known that polyphosphates are capable of binding water by undergoing ionisation such that they then form hydrated complexes with water molecules ((Ellinger, 1972). In addition it is known that ionisation decreases as chain length increases (Ellinger, 1972). Hence it would be expected that the shorter chain polyphosphates should then bind more water as refected in the water activity, a_w. The fact that the measured a_w values were not different rules out a_w effect as the probable mode of anti-microbial action due to the polyphosphates. It also lends support to the fact that it is the chain length of the existent polyphosphates that is probably the determinant factor in potency. However, the relative stability of the polyphosphate in this long chain form would then also influence potency. It is therefore most probable that Curaphos 700 instant becomes more rapidly broken down than Puron 6040 and in addition, or alternatively, the concentration of the higher phosphates proportion in the Curaphos 700 instant is such that it causes pronounced injury effect but not high enough to be efficiently lethal.

Further evidence to support the postulate that the exhibited potency is probably affected by the relative stability is found in the data from Table 2 and Fig. 1 combined. For example, a comparison of the exhibited potencies of acid sodium pyrophosphate and Fibrisol N7, having survival levels of approximately 90 % and 5 %, respectively, indicates that the only

characteristic difference is in their pH values, since both have a chain length $P = 2$. However, in the course of attaining an equilibrium pH value of 5·8, in samples containing acid sodium pyrophosphate of an original pH value of 4·3, it is very probable that any lethality potential by virtue of the hydrogen ion concentration (International Commission on Microbiological Specifications for Foods, 1980) would have been lost due to inactivation by the buffering action of the meat system. By contrast, Fibrisol N7, also having $P = 2$, has an initial pH value of 6·9 that is closer to the final value of pH 5·9 and consequently would be expected to be more stable and thus reflect the obtained higher potency. Similar reasons might additionally account for the observed anomaly when the potency levels exhibited by Curaphos 700 instant and Puron 6040 are compared as shown in Fig. 1.

Thus from the foregoing, it would appear that the critical factors influencing polyphosphate potency include the original chain length, the existent chain length in the menstruum system, and the subsequent

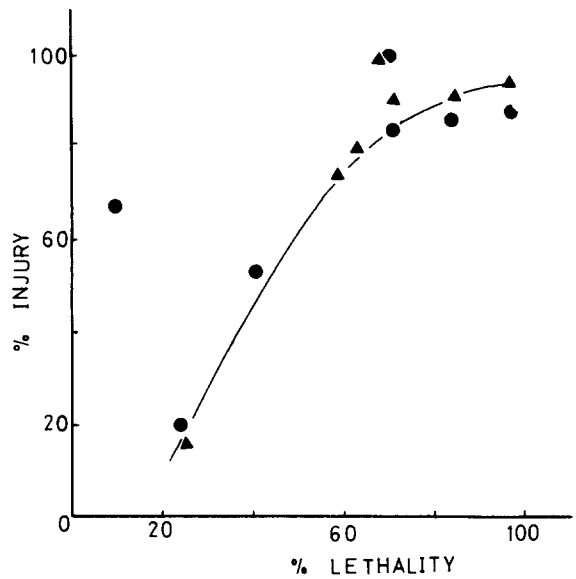

Fig. 3. The manifested interrelationship between the injury magnitude of survivor cells due to deoxycholate injury and the achieved lethal effectiveness in the different polyphosphate containing samples. The lethal efficacy achieved by the different polyphosphates was plotted against the corresponding magnitude of deoxycholate sensitivity among survivors. ●—● samples frozen directly to −18 °C with no holding at −5 °C. ▲—▲ samples held at −5 °C for 24 h in the course of freezing to −18 °C.

relative molecular stabilities in terms of chain length and hydrogen ion concentration.

Furthermore, results obtained from preliminary experiments had shown that exposure of cells of the test organism to the polyphosphate solutions always resulted in most cells (at least > 99 %) becoming injured and sensitive to deoxycholate on TSAD plates. However, such level of injury was not usually obtained in the polyphosphate-containing meat samples obviously by virtue of the protective nature of the meat. Nevertheless, in Fig. 3, there is an indication that the level of injury and lethality may, within limits, be interrelated as shown. Since the index of injury measured by the deoxycholate only involves the structural type (Ray & Speck, 1973), with the added suggestion that such may be induced by polyphosphate chelation of vital structural ions (Hamilton-Miller, 1965), it also seems very probable that some of the earlier-mentioned critical factors like chain length of the polyphosphates may be responsible for the observed injury. Consequently, it would not be unexpected that there could be such positive interrelationships between injury and lethality as was obtained here, since similar critical factors are at play in mediating both cellular manifestations.

ACKNOWLEDGEMENTS

The authors express their appreciation to Dr P. Gibbs of the Leatherhead Research Association, Leatherhead, Surrey, Fibrisol Limited, London, and Albright and Wilson Warley, Worcestershire, for the kind donation of the polyphosphate samples used in the study.

REFERENCES

Batra, S. C. (1965). The ionization behaviour of some poly(meta)phosphates used in food products. *J. Food Sci.* **30**, 441–4.

Communicable Disease Surveillance Centre, Public Health Laboratory Service (1980). Surveillance of food poisoning and *Salmonella* infections in England and Wales: 1970–9. *B. Med. J.* **281**, 817–8.

Ellinger, R. H. (1972). Polyphosphates in food processing. In: *CRC Handbook of Food Additives*, (Furia, T. E. (Ed.)). CRC Press, Boca Raton.

Elliot, R. P., Straka, R. P. & Garibaldi, J. A. (1964). Polyphosphate inhibition of growth of pseudomonads from poultry meat. *Appl. Microbiol.* **12**, 517–22.

Foster, R. D. & Mead, G. C. (1976). Effect of temperature and added polyphosphate on the survival of salmonellae in poultry meat during storage. *J. appl. Bact.* **41**, 505–10.

Hamilton-Miller, J. M. T. (1965). Effect of EDTA upon bacterial permeability to benzylpenicillin. *Biochem. Biophys. Res. Comm.* **20**, 688–91.

Hammence, J. H. & Kunwardia, G. H. (1974). Polyphosphates and moisture in meat products. *J. Asoc. Pub. Anal.* **12**, 85–92.

International Commission on Microbiological Specifications for Foods (1980). pH and acidity. In: *Microbial Ecology of Foods. Vol. 1. Factors affecting life and death of microorganisms.* International Commission on Microbiological Specifications for Foods. Academic Press, New York.

Jimenez, A. V., Fernandez, R. P. & Girauta, M. E. (1961). Utilization of antibiotic and chelating substances in the treatment and storage of poultry under refrigeration. In: *Symp Substances Etrangeres Aliments Madrid* pp. 530–7. *Chem. Abstr. 59*: 3256e. Cited by Ellinger, R. H. (1972). Polyphosphates in food processing. In: *CRC Handbook of Food Additives* (Ruria, T. E. (Ed.)). CRC Press, Boca Ratun.

Kraft, A. A. & Ayres, J. C. (1961). Production of fluorescence on packaged chicken. *Appl. Microbiol.* **9**, 549–51.

Kraft, A. A. & Ayres, J. C. (1964). Development of microorganisms and fluorescence on poultry dipped in water containing iron. *J. Food Sci.* **29**, 218–23.

Miles, A. A. & Misra, S. S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg. (Camb.)* **38**, 732–49.

Obafemi, A. (1983). *The survival of* Salmonella typhimurium *in processed frozen poultry.* Ph.D. Thesis. University of Reading.

Ray, B. & Speck, M. L. (1973). Freeze-injury in bacteria. Chemical Rubber Company Critical Reviews. *Clin. Lab. Sci.* **4**, 161–213.

Spencer, J. V. & Smith, L. E. (1962). The effect of chilling chicken Fryers in a solution of polyphosphate upon moisture uptake, microbial spoilage tenderness, juiciness and flavour. *Poultry Sci.* **41**, 1685.