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ACTION OF POLYPHOSPHATES IN MEAT PRODUCTS

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Abstract

The action of polyphosphates on meats has been examined in studies using different meats and has been compared with other treatments. The results were monitored by recording cooked yield and observing changes in structure by electron microscopy. Four possible characteristics of polyphosphates are considered: pH, sequestering action, ionic strength and as an ATP (adenosine triphosphate) analogue. In the conditions used in these experiments, the pH-buffering effect of polyphosphates is found as the principal characteristic in meat products.

Some meats were found to be more affected by polyphosphates than others and a hypothesis is developed to explain this. It is proposed that with meats such as pork, which suffer early pH fall post-mortem, there might be a greater association between sarcoplasmic and myofibrillar proteins than with other meats. The association between sarcoplasmic and myofibrillar proteins may be reversed by increasing the pH of the curing brine and this would allow more dispersion of myofibrillar proteins and hence a higher cooked yield.

In most of the meats examined no evidence for specific actomyosin solubilisation by polyphosphates was found except in heart and some higher pH beef meat.

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Introduction

Polyphosphates are widely used in meat products to improve binding properties and are claimed to have antimicrobial properties (Elliot *et al.*, 1964). There is no general agreement on the mechanism for polyphosphate action although there is a well-established synergistic action with sodium chloride and the major effect of polyphosphate is seen when meats are cooked (Mahon, 1961).

Particular properties of polyphosphates have been proposed as the main causes of polyphosphate action. Polyphosphates cover a range of compounds; there is some debate as to whether pyrophosphate or longer chain polyphosphates have most effect. Bendall (1954) noted a similarity between the pyrophosphate structure and that of ATP (adenosine triphosphate) and suggested that pyrophosphate could dissociate actomyosin formed in post-rigor muscle. Kotter (1960) extended this idea and proposed that the dissociated actin and myosin could be further dispersed by salt. Electron microscopic observations on cooked salt and polyphosphate-treated pork, however, reveals that the most resistant regions of sarcomere structure are those in which actomyosin is formed (Lewis and Jewell, 1975; Lewis, 1979, 1981; Rahelic and Milin, 1979).

Some work on the action of polyphosphates on raw meat structures has been reported. Working on myofibrils isolated from rabbit, Offer and Trinick (1983) showed that pyrophosphate can produce dissociation of actomyosin. Knight and Parsons (1984) found that beef myofibrils behaved variably in contact with salt and pyrophosphate solutions. Isolated myofibrils are not necessarily a good model for meat products and to meet this criticism Voyle *et al.* (1984) repeated the earlier approach of Lewis and Jewell (1975) but examining only uncooked pork samples. They interpreted their micrographs as showing dissociation of actomyosin, although they could not explain a loss of material from either side of the Z-disc. Voyle *et al.* (1984) supported their case that only actin filaments were still present by reference to the work of Offer and Trinick (1983), in which SDS electrophoresis was used in conjunction with microscopical observations on isolated myofibrils. The electrophoresis was carried out only on myofibrils extracted at pH 5.5, whereas most commercial polyphosphate brines have a rather higher pH than this (generally pH 7.5 to 9.5), and so some care is needed in extrapolating these results. The electrophoresis showed that myosin was the main protein to show increased solubility in the presence of pyrophosphate. However, troponin and tropomyosin, both thought to be structural components of the thin filaments (Ebashi *et al.*, 1969), were also largely

extracted and it would therefore seem unlikely that uncomplexed thin filaments would remain intact during extraction. Voyle *et al.* (1984) also claimed that the earlier micrographs of Lewis and Jewell (1975) showed evidence of actomyosin dissociation, although they did not offer any alternative interpretation of the micrographs of samples cooked after soaking in salt and polyphosphate brines. These clearly indicate the actomyosin regions as among the most resistant regions of the sarcomere to processing.

In addition to the actomyosin-dissociating capacity of pyrophosphate, Offer and Trinick (1983) also point out that pyrophosphate will depolymerise myosin filaments, especially in the presence of salt. Other properties of polyphosphates claimed to be responsible for their effect on meat are sequestration of metal ions (Hamm, 1960), ionic strength and pH-buffering capacity (Trout and Schmidt, 1984).

In order to test which of these properties produce the structural and functional action of polyphosphates, a range of experiments investigating the effects of meat type, pH (with and without phosphates), sequestering agents, degree of comminution, brine to meat ratio and freezing and thawing have been carried out. This report summarises the findings of the experiments concerning meat type, brine pH and sequestering action.

Methods

Standard Procedure for Soaking and Cooking Meat

Unless stated otherwise, the following method was used to soak and cook the meat pieces.

Cubes of meat, approximately 5 g, were weighed and an equal weight of brine added (4% sodium chloride, 1% sodium pyrophosphate). Samples were stored for 18 hours at 0°–5°C, then the meat pieces blotted to remove excess liquid and reweighed. Small samples from the outside (1 mm) of the block were taken and prepared for electron microscopy. The meat pieces were reweighed then returned to the brine and the vials placed in a beaker, which was immersed in a 75°C water bath for 1 hour. Using a water blank, the temperature was monitored and found to take 25–30 minutes to reach 71°–72°C and did not rise above 73°C. After cooking, the samples were allowed to cool for 1 hour; the meat pieces were removed from the brine, blotted and any gel removed, then weighed. Samples were taken from the outside of the block for electron microscopy. Samples of the raw meat taken prior to soaking were also prepared for electron microscopy. The pH of the brine before and after soaking was recorded. The pH of a salt and pyrophosphate brine fell from about 9.4 to about 6.4 during overnight soaking of meat. Brines were prepared using sodium hydroxide to give a similar buffering capacity to pyrophosphate; in this case, the initial pH of the brine was around 12.1 but this fell during soaking overnight to around 6.5. If the pH of a salt brine was adjusted to pH 9.4 with sodium hydroxide the pH fell to a similar level to that for salt only after overnight soaking.

Meat was obtained by local purchase; in some cases animal and post-mortem details were well documented whilst in other cases the meats were simply purchased 'off the shelf'.

Reagents used to prepare brines were in most cases food grade; in a few cases standard laboratory reagent grade was used.

Preparation for Transmission Electron Microscopy

Small pieces of meat approximately 1 mm^3 were fixed in standard Mirsky's fixative in buffer (a commercial preparation available from National Diagnostics Ltd) for 1 hour, washed several times in distilled water then dehydrated through a graded series of ethanol into absolute ethanol. Samples were infiltrated and embedded in L R White resin and thin sections (approx. 80 nm) were cut on a Reichert Ultramicrotome and collected on copper grids. The sections were post-stained in 7% uranyl acetate in absolute methanol for 2 min, washed in methanol and dried. The grids were coated with a layer of carbon and examined in a JEOL 1200 EX operating at 80 kV. Selected samples were also fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer for 1 hour, washed in water, dehydrated using ethanol and treated as above. Glutaraldehyde- and Mirsky-fixed samples showed essentially similar structures and so Mirsky fixative was used throughout this work.

Sections (1–2 μm) of the resin blocks were also prepared for light microscopy.

Experimental Plan

Effect of Brine pH and Phosphate Type on Pork

All systems were cooked after soaking in 4% salt solution overnight at 5°C at a brine:meat ratio of 1:1. Appropriate additives were added to the brine. Controls containing water only and 4% salt with no additives were also included.

Brine additives investigated were: 1) 1% sodium pyrophosphate ($Na_4P_2O_7$), initial pH 9.4; 2) 1% sodium hexametaphosphate ($NaPO_3)_n$, initial pH 5.9; 3) 1% sodium tetrapolyphosphate ($Na_6P_4O_{13}$), initial pH 7.3; 4) 1% sodium tripolyphosphate ($Na_5P_3O_{10}$), initial pH 8.1; 5) 1% sodium pyrophosphate (initial pH adjusted to pH 5.5); 6) 1% sodium pyrophosphate (initial pH adjusted to pH 6.5); 7) 1% trisodium orthophosphate (Na_3PO_4), initial pH 11.5; 8) 1% disodium orthophosphate ($Na_2H\ PO_4$), initial pH 8.7; 9) 1% monosodium orthophosphate ($NaH_2\ PO_4$), initial pH 4.5; 10) sodium hydroxide to give pH 6.5 in brine after overnight soaking of meat (initial pH 12.1); 11) 0.1M Tris buffer, pH 9.4.

Effect of Calcium Sequestering Agents

Conditions were as above but with the following additives: 12) 1% EDTA; 13) 1% calcium chloride; 14) 1% EDTA plus sodium hydroxide as in 10) above; 15) 1% calcium chloride plus sodium hydroxide as in 10) above.

Effect of Meat Type

Meat samples were soaked in a standard brine containing 4% salt plus 1% sodium pyrophosphate, at a 1:1 brine to meat ratio, overnight at 5°C before cooking. Meats used were:

16)	pork *L. dorsi*	24 h post-mortem	pH 5.5
17)	pork loin	commercial retail	pH 5.6
18)	pork loin	commercial retail	
19)	pork loin	commercial retail	pH 5.8
20)	pork loin	24 h post-mortem	pH 5.7
21)	beef heart	24 h post-mortem	pH 6.2
22)	beef flank	24 h post-mortem	pH 5.7
23)	beef forequarter	24 h post-mortem	pH 5.9
24)	chicken	commercial retail	pH 6.0
25)	cod	commercial retail	pH 6.6.

The quoted pH values were measured on the meats as received.

Effect of Polyphosphates on Beef

23a) Beef forequarter, water only; 23b) beef forequarter, 4% salt only, initial pH adjusted to pH 9.0; 23c) beef forequarter, 4% salt + 1% pyrophosphate; 23d) beef forequarter, 4% salt + 1% pyrophosphate, adjusted to initial pH 6.5; 23e) beef forequarter, 4% salt + 1% pyrophosphate, adjusted to initial pH

5.5; 26a) beef topside, 4% salt only; 26b) beef topside, 4% salt + 1% tripolyphosphate; 26c) beef topside, 4% salt + 1% commercial polyphosphate blend (Fibrosol C700 instant).

Results

Figures 1 and 2 show light micrographs of raw pork and pork cooked after soaking in salt and polyphosphate brine. Figures 3 and 4 show TEM micrographs of raw pork and pork soaked in salt and polyphosphate brine. Figures 5 and 6 show electron micrographs of commercial ham products. Figures 7–16 illustrate the range of structures seen in the meat samples examined along with a subjective evaluation of the type of dispersion, coded A, B, C, D and E. The dispersion types are illustrated diagrammatically in Fig. 17 and are interpreted as follows: A) coagulation of proteins with little dispersion other than some loss of thin filaments on cooking (Figs 7 and 8); B) dispersion of material from H-zone as well as loss of thin filaments; M-line and Z-line generally but not always present (Figs 9 and 10); C) obvious dispersion of H-zone and reduction of M-line; Z-line generally but not always dispersed (Figs 11 and 12); D) extensive dispersion of all regions of sarcomere with only A/I overlap regions visible (Figs 13 and 14); E) dispersion of sarcomeres but leaving Z-lines and M-lines visible; some dissociation of actomyosin in A/I bands (Figs 15 and 16).

The predominant type of dispersion found in the systems examined, along with summaries of the cooked yield and post-soaking pH, are presented in Figs 18, 19 and 20.

It should be noted that a wide range of structures could be found in some of the meat samples and these classifications are a subjective evaluation of the main patterns of dispersion of myofibrillar proteins. Dispersion types B to D are considered as progressively more dispersed manifestations of a single mechanism whilst type E is considered to be a quite different mechanism.

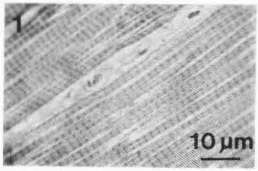

Fig. 1. Phase contrast light microscopy of 2-µm section of raw pork showing myofibrillar striations.

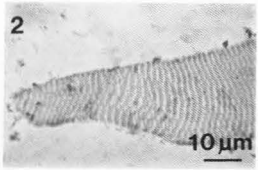

Fig. 2. Phase contrast light microscopy of 2-µm section of pork soaked and cooked in salt and polyphosphate brine, showing twin dark bands in each sarcomere.

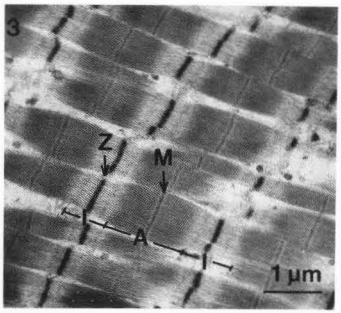

Fig. 3. Electron microscopy of thin section of pork meat showing features of sarcomere structure.

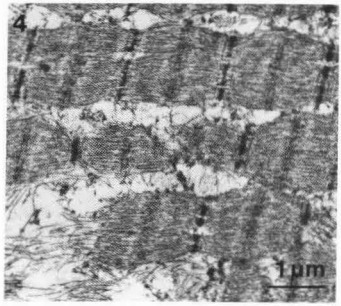

Fig. 4. Electron microscopy of thin section of pork soaked in salt and polyphosphate brine showing some dispersion of proteins from normal sarcomere structure.

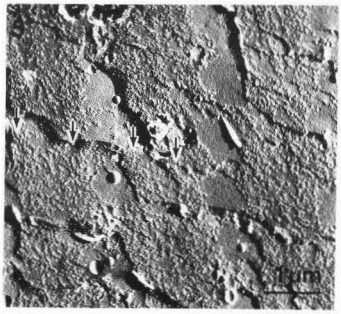

Fig. 5. Electron microscopy of freeze-etched preparation of commercial ham product showing densely packed bands (arrows). (See Lewis and Jewell, 1975, for preparation details.)

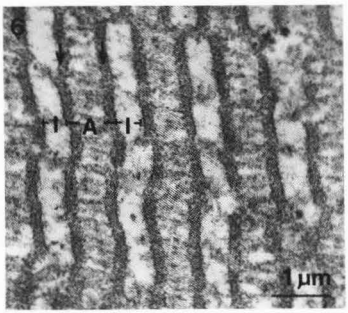

Fig. 6. Electron microscopy of thin section of commercial ham product showing densely staining bands. Similar product to that in Fig. 5. (From Lewis and Jewell, 1975.)

Figures 7–16 are electron micrographs of thin-sectioned preparations of meats cooked after soaking in various brines. Z- and M-lines are indicated on them.

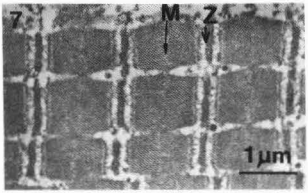

Fig. 7. Beef forequarter soaked and cooked in 4% salt + 1% pyrophosphate adjusted to an initial pH of 6.5; sample 23d. Shows type A dispersion with most myofibrillar proteins heat coagulated in situ. *Sarcomere features readily recognisable.*

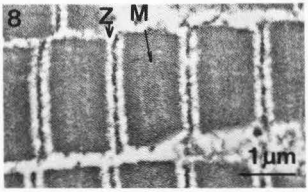

Fig. 8. Pork loin soaked and cooked in 4% salt + 1% monosodium orthophosphate, treatment 9. Shows type A dispersion with only slight extraction of H-zone and readily recognisable sarcomere features.

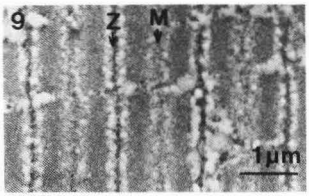

Fig. 9. Pork loin soaked and cooked in 4% salt + 1M sodium hydroxide to give a post-soaking pH of about 6.5, treatment 10. Shows noticeable dispersion in H-zone but with Z- and M-lines still present. Type B dispersion.

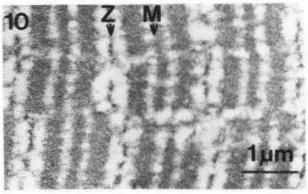

Fig. 10. Pork loin soaked and cooked in 4% salt + 1% sodium hexametaphosphate, treatment 2. Shows marked dispersion of H-zone but Z- and M-lines still present. Type B dispersion.

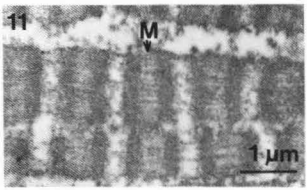

Fig. 11. Pork loin soaked and cooked in 4% salt + 1% sodium pyrophosphate, sample 18. Shows marked dispersion of H-zone and I-band with loss of Z- and M-lines. Type C dispersion.

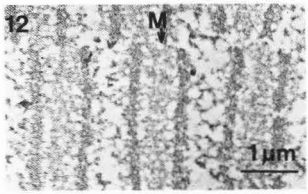

Fig. 12. Pork loin soaked and cooked in 4% salt + 1% disodium orthophosphate. treatment 8. Shows marked dispersion of H- and I-bands and loss of Z- and M-lines. Type C dispersion.

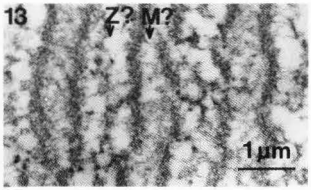

Fig. 13. Pork loin soaked and cooked in 4% salt + 1% trisodium orthophosphate, treatment 7. Shows marked dispersion of H- and I-bands and loss of Z- and M-lines. Type D dispersion.

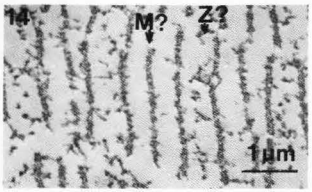

Fig. 14. Pork loin soaked and cooked in 4% salt + 1% sodium pyrophosphate, sample 20. Shows extensive dispersion of all regions of sarcomere except for dark staining bands. Type D dispersion.

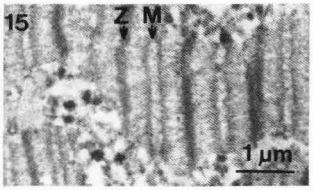

Fig. 15. Beef heart soaked and cooked in 4% salt + 1% sodium pyrophosphate, sample 21. Shows dispersion of A/I overlap regions but Z- and M-lines still recognisable. Type E dispersion.

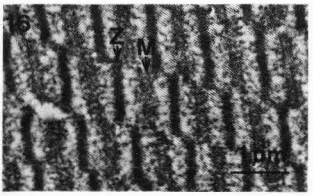

Fig. 16. Beef topside soaked and cooked in 4% salt + 1% sodium tripolyphosphate, sample 26b. Shows dispersion of A/I overlap regions but Z- and M-lines still recognisable. Type E dispersion.

Interpretation

Micrographs can often be difficult to interpret even for experienced microscopists. In the case of meats soaked in salt and polyphosphate solutions the interpretation is open to question. Lewis and Jewell (1975) interpret the presence of fibres of similar thickness to natural myosin and actin filaments as indicating that both types of filament are present in soaked meat, whilst Voyle *et al.* (1984) consider that the thicker filaments are clumped actin filaments. Both views are tenable but in the soaked only samples the filaments are obscured by a colloidal mass of protein, which makes it difficult to decide which of these views is correct.

Interpretation of micrographs must always consider preparation procedures. Voyle *et al.* (1984) and Offer and Trinick (1983) make much of the relatively minor observations by Lewis and Jewell (1975) that light microscopy of processed and raw meat revealed mainly similar structures with only a few cells in processed meats showing differences. A consideration of the preparation procedures may help to explain this. The light microscopy sections were about 10 µm thick, which means that in the thickness of the section about ten myofibrils will lie on top of each other. The thickness of the sections means that the resolution will rarely be adequate to distinguish sufficiently fine detail to show the changes in the sarcomere. Hence only a few cells might be expected to show differences from the normal structure. Interpretation of changes in density in stained sections must also be carried out carefully. Thus when Voyle *et al.* (1984) explain changes in density as being due to swelling or extraction they neglect other possibilities, in particular that the charge on the proteins may have been altered, thereby making it more or less capable of taking up stain. Concentrated salt solutions generally decrease the staining of meat. The problems of variable staining between samples is well known to most microscopists. Figure 9 of Voyle *et al.* (1984) illustrates the problems of uneven staining and points to the dangers of placing too much reliance on changes in density alone.

After consideration of some of the problems of interpretation of micrographs, the present observations can be considered. There is general agreement on the identification of components in the thin-sectioned view of muscle and this is illustrated in Fig. 17. There are various refinements to this simple view of sarcomere structure; in particular, the gap filaments are perhaps the most significant of these. Locker (1984) gives an up-to-date account of the gap filaments and explains their possible location and significance in meat behaviour. Where little dispersion of protein occurs before heating (Type A, Fig. 17) the appearance can be simply interpreted with reference to the raw structure, the principal components being coagulated *in situ*. Type B dispersion of proteins can also be recognised by direct reference to the raw structure. In some cases, it may be difficult to decide which are the remains of M-lines and which are the remains of Z-lines, but often this can be resolved as the I-band is generally more dispersed than the H-zone and in most cases the Z-line is more labile than the M-line.

The problem of interpretation is more difficult with dispersion types C and D, where there are few reference points other than the periodic dark bands and interpretation is based on indirect observations. In type B dispersion, Z- and M-lines are often dispersed to some extent and so it seems unlikely that they would give rise to a constant periodic structure. Examination of the spaces between the dark bands supports this view. In most cases the nature of these spaces alternates, one space

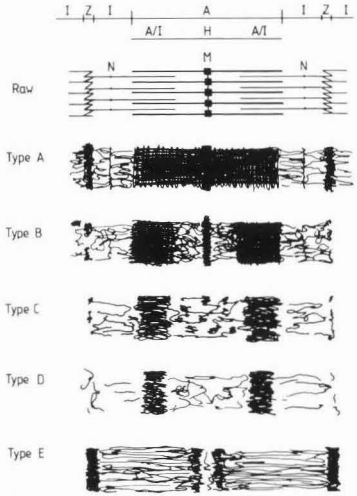

Fig. 17. Sarcomere structure and types of dispersion found in soaked and cooked meats.

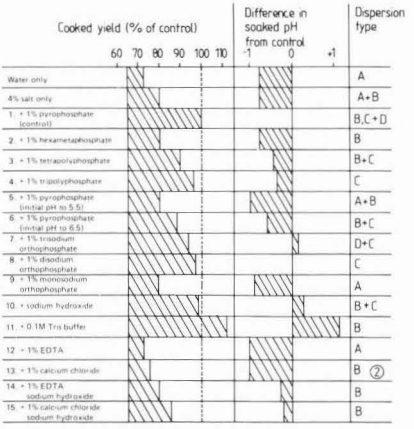

Fig. 18. Cooked yields and pH. Summary of the effect of different soaking brines compared with a control system[1] containing 4% salt + 1% sodium pyrophosphate; pork loin meat.

1 All systems contain 4% salt unless stated; results are combined from several experiments; in each experiment the yields are compared with those of the control for that experiment.

2 M-line extracted.

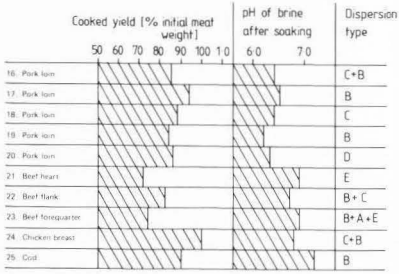

Fig. 19. Comparison of cooked yields, post-soaking brine pH and dispersion type for different meats treated with 4% salt + 1% sodium pyrophosphate brine.

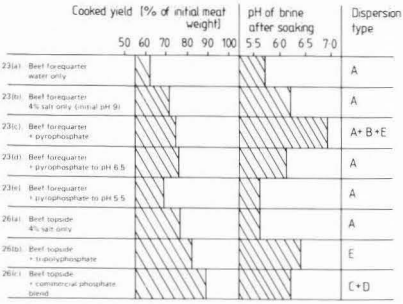

Fig. 20. Cooked yields and pH. Summary of the effect of different soaking brines on beef.

containing few protein aggregates whilst the next has more aggregates and so on. If the dark bands are derived from Z- or M-lines, no basis for this alternating appearance can be found since the space between the M- and Z-line is the same as the space between the Z- and M-lines. Hence it seems more likely that the dark bands originate from a feature which lies between Z- and M-lines. If the dark bands originate from the A/I overlap region, the alternating pattern is readily explained since one space will be from the myosin-rich H-zone and the next space will be from the actin-rich I-band. On this reasoning there seems little doubt as to the region of the sarcomere in which these dark bands form; it is close to the area where the A-band and I-band meet. Identification of the type of proteins which form the dark bands can only be speculative at the present time since no localisation techniques have been reported for cooked salt- and polyphosphate-treated meats, and chemical characterisation is necessarily more disruptive than meat processing. Many proteins have been located within muscle cells and Fig. 21, derived from Maruyama (1985), represents current opinion as to the likely position of various proteins. The main proteins in the A/I overlap regions are actomyosin and connectin (also

A-Band	I-Band		
Myosin	Actin		
M-protein	Troponin		
C-protein	Tropomyosin		
H-protein	Actinins		
F-protein			
I-protein			
X-protein			
	A/I Overlap	N-Line	Z-Disc
	(Actomyosin)	Nebulin	Actinins
	Connectin		Z-protein
	(Titin)		34K-protein
	Paratropomyosin		Z-nin
			ABP (Filamin)

Desmin (skeletin) and vimentin surround and connect myofibrils and vinculin is found near to the sarcolemma.

Fig. 21. Probable location of proteins within sarcomeres (derived from Maruyama, 1985)

referred to as titin) and it would seem likely that these proteins constitute the dark bands. Little is known about the susceptibility of connectin to salt, polyphosphate and heat, although it is reported (Maruyama, 1985) to be extracted by 0.1M phosphate buffer at pH 6.2–7.0 but not at pH 5.5–5.6, which removes denatured actin. It is extracted from fresh muscle along with myosin by 0.6M potassium chloride (Maruyama, 1985). Connectin can interact with myosin and actin and may enhance super-precipitation of actomyosin, although these interactions are dependent on the ionic conditions and it is not clear how the proteins will behave under meat-processing conditions. Work on overstretched and cooked muscle has produced dark bands in the I-band regions of the sarcomere (Locker *et al.*, 1975). These may be formed from connectin, actin and nebulin; however, they are generally more closely spaced and less densely packed than most of the dark bands found in processed meats. Also, in type B dispersion (e.g. Figs 9 and 10), dark bands can be seen to be within the A-bands, and these dark bands are similar in density and spacing to those seen in types C and D dispersion. On this evidence we conclude that the dark bands derive from the A/I overlap and consist mainly of denatured actomyosin and connectin. The remaining sarcomere proteins, in particular unassociated myosin and actin, are probably dispersed through the meat structure in the form of a water-holding gel. The fate of connectin in meats showing type E structure with substantial dispersion of the A/I junctions is unclear. In these samples, the Z-lines are particularly prominent and it may be that connectin, related to the dispersion of actomyosin, precipitates on the Z-lines. A similar type of precipitation is reported in muscle extracted with potassium iodide (Maruyama, 1985).

Finally, in consideration of artefacts it might be asked whether these dark bands could be produced by the preparation processes used for microscopy. This is unlikely since they can be found in samples examined by light microscopy as well as transmission electron microscopy using different fixatives, and also in unfixed freeze-etched preparations.

Discussion

The results raise a number of interesting points for consideration. Taken with the work of Offer and Trinick (1983), they indicate that phosphates can act in two ways on meat structure. Firstly, they can promote the general dispersion of myofibrillar proteins by salt; in this case the A/I overlap regions, rich in actomyosin, would seem to be most resistant to dispersion. Structure types B, C and D represent varying degrees of this type of action. Secondly, they can promote dissociation of actomyosin specifically and so induce the selective dispersion of myosin by salt. Structure type E represents this type of action. Both these mechanisms have been demonstrated and the main point for consideration is under what circumstances each mechanism applies. The following factors seem to be significant: meat type, condition and history; pH of the system; ratio of extracting medium to meat; extent of protein:protein interaction in the meat; and degree of comminution of the meat. There is little doubt that myofibrils freshly isolated from rabbit psoas show dissociation of actomyosin when treated with a great excess of salt and pyrophosphate extraction medium at pH 5.5. Equally, from our studies, it seems unlikely that extensive dissociation of actomyosin is the dominant feature when pork meat is cooked after soaking in salt and pyrophosphate brine. Beef heart generally shows dissociation of actyomyosin; other beef samples have been variable but tend towards limited dispersion of the non-actomyosin regions of the sarcomere. In chicken and cod, the few observations we have made suggest dispersion of the non-actomyosin regions as the main mechanism. One of the features that is likely to be different in these cases is the state of the sarcoplasmic proteins and their interaction with myofibrillar proteins. In the case of isolated myofibrils it may be expected that the sarcoplasmic proteins will have been largely removed and so the polyphosphate can interact directly with the myofibrillar proteins. In the case of pork meat there is a distinct possibility that some post-mortem precipitation of sarcoplasmic proteins may occur around the myofibrils.

Monin and Laborde (1985) have also shown a pH-dependent interaction between myofibrils and sarcoplasmic compounds, which results in higher water-holding capacity at pH values removed from the isoelectric point (i.e. pH 5.5). Thus the interaction between sarcoplasmic and myofibrillar proteins could be reversed by raising (or lowering) the pH. Phosphates are also used in detergents as aids to dispersion by forming soluble complexes, and a similar role in dispersing sarcoplasmic proteins is possible.

Pork has a tendency to produce rapid pH fall post-mortem whilst the meat temperature is high and in extreme cases this can lead to the PSE (pale, soft, exudative) condition, in which large amounts of sarcoplasmic protein are precipitated on to the myofibrillar proteins (Bendall and Wismer-Pedersen, 1962; Scopes and Lawrie, 1963; Voyle, 1979). It is not clear whether any precipitation occurs in non-PSE pork meat but it is possible that it will occur to some extent. Electron micrographs of raw, post-rigor pork generally show some obscuring of the myofilaments (e.g. Fig. 5), compared with freshly excised muscle, which may be caused by sarcoplasmic proteins associating with the myofibrils. In beef, the early pH fall tends to be slower, in extreme cases leading to DFD (dark, firm, dry) meat, where the meat proteins bind their natural water very effectively. It is likely that beef sarcoplasmic proteins will be less prone to associate with the myofibrils. In this respect beef heart had the highest pH and therefore possibly has least association of sarcoplasmic and myofibrillar proteins. In heart, as in isolated myofribrils, actomyosin dissociation is seen as the most noticeable effect of polyphosphate; in both cases it is likely that there is little association between sarcoplasmic and myofibrillar proteins.

Another possible factor in considering the difference

between beef and pork is the chemical structure of the myosin and actin. Differences are thought to exist between muscle types (Fretheim *et al.*, 1985) and probably between species and between heart muscle and voluntary muscle, and these chemical differences may influence the dispersion of myofibrillar proteins by salt and phosphates.

In pork samples, the pH of the brine after soaking appeared to be the predominating feature in determining the cooked yield; where the brine pH was high the cooked yields were also high. This relationship did not apply to other meats; for example, beef heart and beef forequarter both had high pH values in the soaking brine and yet had lower yields than pork meat, which had lower brine pH values. A possible explanation for this can be found by considering the meat pH. Pork pH values were generally lowest and raised pH brines were more effective in increasing the yield in these meats than in beef. It may be that the lower pH in pork meat has been accompanied by some water loss as drip, and that raising the pH allows this loss to be recovered.

Non-heart beef muscles were more variable in their behaviour than pork; beef flank behaved like some pork samples; beef forequarter was fairly resistant to dispersion of myofibrillar proteins by both salt and phosphates; and beef topside seemed to be able to react in the same way as pork meat or heart. A partial explanation may lie in the post-mortem pH values of the meats; beef heart had the highest pH value – about 6.2 – whilst beef flank had a post-rigor pH value similar to that found in most of the pork samples, i.e. about 5.6. Hence it seems that for beef and pork polyphosphates are most effective in meats having lower pH values. A similar observation was made earlier for meats comminuted with fat (reported by Lewis, 1986). In general terms, meat having a pH value below about 5.9 will show improved cooking yields if the pH of the brine is raised to about 6.1 or more. For meats with a pH greater than 6 the effect of raising the pH or adding phosphates seems to be marginal in terms of cooked yield. The limited observations on chicken and cod suggest that they do not fit directly into the above hypothesis. However, it may be that the general principle applies but that the critical pH values are different. Further work would help to elucidate this.

These observations indicate that pH is the main effect of polyphosphates and that raising brine pH can be most effective in meats which have a lower pH. The work also indicates something of the importance of the other characteristics of polyphosphates which may play a role in meat products.

It seems clear that the sequestering action of polyphosphates is not of great importance in pork since the addition of EDTA and calcium chloride lowered the cooked yield compared with systems in which they were absent.

The series of experiments including 1% pyrophosphate at different pH values indicates that the presence of pyrophosphate (as an ATP analogue) has only a minor effect. Pyrophosphate- and tripolyphosphate-containing systems gave only marginally higher yields than other systems at the same pH.

In these experiments the ionic strength effect of polyphosphates did not seem very significant. At first sight this contradicts the conclusions of Trout and Schmidt (1984), who claim that both pH and ionic strength are significant features of polyphosphate action. In our experiments the level of salt was fairly high (about 2% overall) whilst Trout and Schmidt were considering low-salt products, and at lower salt levels the ionic strength of polyphosphates may be more important. However, previous work (Lewis and Jewell, 1975), using constant ionic strength brines, indicated that both sodium chloride and polyphosphate were necessary to produce optimum effect. This suggests that the chloride ion also has a specific effect and indicates that salt may be only partially replaced by polyphosphates.

A curious feature of meat behaviour on processing is the variable appearance of the M- and Z-lines. This has been observed in our own work and in that of Voyle *et al.* (1984).

Two small observations in this work may help to explain this phenomenon. Tris buffer produced quite extensive dispersion of myofibrillar proteins but M- and Z-lines could generally be recognised; addition of calcium chloride reduced general myofibrillar dispersion but removed the M-line. Hence it seems that M-line behaviour may be linked to calcium availability whilst Z-line dispersion may be a specific feature of polyphosphates. Previous work (Lewis, 1981) also showed that pork cooked in polyphosphate solution without salt showed extraction of the Z-lines. It is also known that Z-lines in different fibre types have different structures (Gauthier, 1970) and this may be a source of variation. In particular, the dense staining of Z-lines in processed heart probably reflects the different structure of the Z-line in heart. Connectin may also associate around the Z-line and the availability of connectin may depend on the extent to which actin and myosin are dispersed.

Overall, our findings point to pH as the predominant characteristic of polyphosphates in meat products. In our view this is linked to the association between sarcoplasmic myofibrillar and cytoskeletal proteins. Where rapid pH fall occurs post-mortem, we consider that some precipitation of sarcoplasmic proteins may occur; this might be reversed by a high pH brine which would thereby improve cooked yield. If this hypothesis is correct then meat pH can be considered as a principal factor in understanding the variability of meat behaviour.

The nature of the interactions of sarcoplasmic and myofibrillar proteins with the cytoskeletal proteins, in particular connectin, and the influence of pH on these interactions is largely unknown. The next step in understanding polyphosphate and pH effects in meat may well depend on the unravelling of these interactions. It must be emphasised that other differences such as connective tissue organisation, biochemical characteristics of muscle fibres and differences in chemical structure of myosin and actin may also have some bearing on meat behaviour. Control of these features is, however, rather more in the area of genetics, whilst pH adjustment is an operation which can be undertaken in a meat factory.

Conclusions

1. Some meats, generally those with low pH values, are more likely to produce increased yields in the presence of polyphosphates than others.
2. Where polyphosphates are effective in increasing yield, their ability to maintain a high pH seems to be the most important characteristic. Other alkaline materials – sodium hydroxide, trisodium orthophosphate and Tris buffer – are also effective in increasing yields if present at high enough levels.
3. Increased yields are generally associated with increased dispersion of myofibrillar proteins.
4. In most meats the A/I band overlap region is the most resistant to salt and polyphosphate, although evidence for actomyosin dissociation is seen in beef heart muscle and sometimes in other beef muscles.
5. The sequestering action and ATP analogue properties of polyphosphates seem to be of less significance in explaining

the behaviour of polyphosphates in meats. The contribution to ionic strength of polyphosphates has little effect in the presence of an overall level of 2% salt.

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Discussion with Reviewers

R.J. Carroll: Are any data available on the changes in the water-holding capacity of the uncooked product resulting from the various brine treatments?

Authors: Weight changes on soaking and cooking were recorded and selected samples were examined by electron microscopy. In our view the microscopy of most of the soaked samples shows dispersion of protein from the H-zone, although the presence of generally dispersed proteins makes interpretation difficult and we have concentrated on the cooked samples. Some examples of soaked yields are as follows: pork in water 101%, pork in 4% salt 107%, pork in 4% salt plus 1% polyphosphate 116%, pork in 4% salt plus 1% trisodium orthophosphate 119%, pork in 4% salt plus 1% pyrophosphate (initial pH to 5.5) 111%, beef heart in 4% salt plus 1% pyrophosphate 105%, and beef forequarter in 4% salt plus 1% pyrophosphate 116%. Note that these figures are results of single experiments and have not been averaged against the controls in the same way as the results in Fig. 18.

S. Cohen and J. Smith: Many researchers indicate that sodium chloride and some phosphates swell the meat microstructure while mechanical action may be required for protein extraction. Would the lack of the mechanical action in your work help to explain the variability you observed in actomyosin dissociation? (Ref. Schmidt GR. (1984). Processing effects on meat product microstructure. Food Microstructure 4, 33–39.)

G.R. Schmidt: Would the application of mechanical energy to meat during its incubation with solutions of different composition have an effect on the amount of structural change taking place?

Authors: Our experience of examining tumbled meats shows that different meats behave variably with respect to actomyosin dissociation even when mechanically treated. This variability seems to depend on intrinsic structural features such as degree of contraction and nature of interstitial connective tissue. Even comminuted meats have shown variable responses to salt solutions. In general, mechanical treatment allows changes to occur more rapidly, but in some cases we have observed that the effect of tumbling in salt-only brines is similar to that of soaking in salt and polyphosphate brine.

S. Cohen and J. Smith: Do you believe that the cook yield data are based on a large enough sample (5 g that is sampled prior to cooking) to represent industrial processing adequately? Additionally, did the length of cooking (1 h at 75°C) possibly negate some yield potential due to over-cooking?

C.A. Voyle: How many samples of each meat type were exposed to the treatment described?

Authors: At least two samples of each meat were used in each treatment. In the case of control samples (i.e. 4% salt plus 1% pyrophosphate), up to twelve samples of each meat were used: the good agreement between these samples leads us to believe that our samples give a reasonable indication of commercial meat performance. There is also a similarity between the structure of our samples and of commercial cooked, cured pork products. Other work carried out in our laboratories suggests that the model system gives a reasonable indication of meat behaviour at pilot-plant scale.

The earlier work of Lewis and Jewell (1975) examined cooking at different temperatures and heating rates. From this work we consider that our process gives a reasonable approximation to a pasteurised product. Higher temperatures (about 90°C and above) produce much greater changes in the connective tissue with consequent changes in cooked yield and texture.

The presentation of the results as bar charts rather than as numbers is intended to avoid giving a false impression of precision.

C.A. Voyle: You have referred to 'dispersion' of protein rather than extraction or solubilisation. What do you think is the difference, if any?

G.R. Schmidt: Would it have been possible to analyse the incubating media for the presence of proteins extracted from the I-band?

Authors: The reference to 'dispersion' is quite deliberate as it describes the effect that we see, i.e. proteins are dispersed from the ordered positions that they occupy in the native meat structure. Extraction would imply complete removal of the protein from the meat and we do not know whether this happens. Solubilisation is more difficult to define, but to us suggests that individual molecules go into solution; we have no way of telling whether the dispersion occurs at a molecular level or at a multi-molecular level. We consider that dispersion covers all these possibilities.

It would probably be possible to analyse the brines for I-band proteins, although we think that there could be problems with the salt and polyphosphate present. Since we are considering dispersion rather than extraction, it may well be that analysing for I-band material would not give too much additional information. However, we consider that our findings and recent work on cytoskeletal proteins open up many possibilities for obtaining a better understanding of meat behaviour and we believe that these will require biochemical and chemical techniques in addition to microscopy.

G.R. Schmidt: Does the fact that the lowest salt concentration utilised in this experiment was 2% preclude making conclusive statements about the role of ionic strength on the changes in microstructure of meat?

Authors: Previous work (Lewis and Jewell, 1975) considers the effect of ionic strength in a little more detail. From this work we conclude that the best salt levels for cooked yield fall between 2% and 5% overall in our system (estimated ionic strength 0.45 to 1.0). At a constant ionic strength (estimated as 0.56), changing the ratio of salt to tripolyphosphate markedly altered the type of structural change observed.

From our past and present work we conclude that the ionic strength of polyphosphates is of minor importance in the presence of 2% salt. Tripolyphosphate present at 0.5% overall in the absence of salt tends to disperse the Z-line on cooking but leaves the other major myofibrillar structures intact and is less effective in improving cooked yield than a mixture of salt and polyphosphates. Tripolyphosphate alone at approximately 1% overall appears to disperse Z-lines and much I-band material, possibly dissociating actomyosin.

G.R. Schmidt: Would knowing more about the origin of the meat have been of any value in interpreting the microstructural changes that take place during incubation with the various solutions?

Authors: Undoubtedly a systematic study of the behaviour of meat from different origins would be of great value. However, to be of real value, it would have to cover a much wider selection of meats and as such was beyond the scope of this project.

G.R. Schmidt: Is there an optimal pH for causing structural changes in muscle?

Authors: Structural changes can occur at all pH values and 'optimal pH' depends on the nature of the product required. For most meat products the balance between maintaining structural integrity and obtaining good yield will probably require a pH around 6.0 to 6.5, although this may vary for different meats and microbiological considerations may dictate a lower pH for some products.

Reviewer V: Have you any thoughts on the interaction of meat pH and brine pH during soaking?

Authors: In general, the pH of the soaking brine changes to become closer to the initial pH of the meat during soaking. The extent to which this happens depends largely upon the relative buffering capacities of the meat and brine, although enzymic hydrolysis of polyphosphate may also affect the final pH. In our view it is the buffering capacity of the brine additives rather than their initial pH which is the most important characteristic. On cooking, the pH changed slightly, typically by 0.1 pH unit in either direction.