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ABSTRACT OF THESIS

The main objective of this work was to study the antioxidant properties of polyphosphates in frozen chickens, which were obtained during supervised factory trials, and to propose reasons for this effect. Previous work has been mainly concerned with why polyphosphates increase the water holding capacity(WHC) of meat.

After a brief review of the types, uses, and effects of polyphosphates in the food industry, their specific use and effects in frozen chicken are discussed. The factors which affect WHC and the lipid and fatty acid compositions of chicken tissues are discussed; as is the mechanism of the autooxidation reaction, and the theories about the mode of action of polyphosphates.

Lipid and fatty acid compositions were determined by chromatographic methods. The degree of autooxidation was determined by the thiobarbituric acid(TBA) test. The distribution of added polyphosphates and the ionic composition of individual muscles were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES). The hydrolysis of polyphosphates was studied using thin layer chromatography(TLC) and phosphorus-31 Fourier transform nuclear magnetic resonance (^{31}P-FTNMR). The reasons for choosing these techniques, and the results of preliminary tests are given.

Polyphosphate treatment was found to result in cooked muscles having reduced TBA numbers and lower levels of calcium and magnesium than untreated muscles; but to have little effect on chill-water uptake, thaw and cooking losses, and lipid and fatty acid composition. Added polyphosphates were found in the pectoralis major and pectoralis minor muscles, but leg muscles contained either none or very small amounts. Polyphosphates were found to undergo considerable hydrolysis immediately on mixing with excised muscle, and during prolonged frozen storage of the whole chicken.

The general conclusions concern the lipid and fatty acid composition of chicken muscles, the autooxidation process, and the scope and reliability of the TBA test.

It was concluded that polyphosphates remove calcium and magnesium from thawed muscle, and that they perhaps associate with phospholipids.

EFFECTS OF POLYPHOSPHATES ON FROZEN CHICKENS

A thesis submitted for the degree of
Doctor of Philosophy

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August 1979

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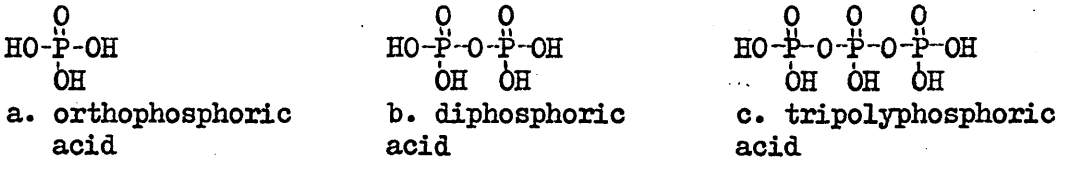
1.1 GENERAL USES OF POLYPHOSPHATES IN THE FOOD INDUSTRY

Polyphosphate is the general term applied to salts of phosphorus oxyacids which contain more than 2 phosphorus atoms. In this thesis, the term will be extended to include diphosphate salts.

The series of phosphorus oxyacids starts with orthophosphoric acid which contains 1 phosphorus atom bound to 1 oxygen atom and 3 hydroxyl groups(Figure Ia). The series continues with the addition of orthophosphate groups, forming P-O-P chains. Ring compounds are possible, but these are relatively unimportant as food additives. The sodium salts of diphosphoric acid(Figure Ib) and tripolyphosphoric acid(Figure Ic) are the most common food additives. However, commercial mixtures may contain higher phosphates in order to exert a specific effect, or to improve the overall solubility of the mixture.

Figure I. STRUCTURE OF ORTHOPHOSPHORIC, DIPHOSPHORIC, AND TRIPOLYPHOSPHORIC ACIDS



a. orthophosphoric acid

b. diphosphoric acid

c. tripolyphosphoric acid

Phosphate salts are used in many foods and food products in order to improve various aspects of their quality[1]. In the meat industry, polyphosphates were first used in the preparation of hams and certain types of sausages, especially frankfurters. Reviews of the earlier uses have been published by Morse[2], and Brotsky and Everson[3] who also list the present day uses of polyphosphates in the meat industry.

In general, polyphosphates benefit hams and other cured meats by stabilising their colour and minimising fluid losses during processing[2]. In sausages and other comminuted meat products, the main benefit of polyphosphate treatment is to aid in emulsification of fats[4,5]. Treated poultry shows a reduction in moisture and cooking losses[6], and the cooked meat is more resistant to oxidative rancidity than the untreated cooked

meat.[7,8] It is the sodium salts of diphosphoric and tripolyphosphoric acids which are the most effective in poultry and other meats.[9]

1.2 APPLICATION OF POLYPHOSPHATES TO CHICKEN

It is generally accepted that incorporation of up to 0.5% (as P_2O_5) by weight of polyphosphates into meat results in beneficial effects, but above this level adverse effects such as deterioration of flavour and appearance may result.[9] Table I shows the maximum levels of phosphate salts allowed in meat products in the USA and EEC countries other than the UK, who have yet to provide such legislation.

Since chickens are usually processed as the whole carcase, early methods of administering polyphosphates involved soaking the eviscerated carcase in a solution of polyphosphates. However, the skin and adipose tissue of chicken act as a barrier to absorption, and the major disadvantage of the soaking technique is the long period of immersion necessary to ensure adequate uptake of phosphate.[10] In fact, early experiments on the polyphosphate treatment of poultry involved soaking in chill solutions for up to 22 hours.[11] Other methods of application include adding to the cook water,[12,13] and post-cook cooling in polyphosphate solutions.[12] Obviously, these 2 methods would be limited to the retail of cooked chicken. A British patent[14] states that addition of solid polyphosphates to the surface of poultry results in adequate absorption and distribution of phosphate throughout the carcase. The scientific literature on the polyphosphates treatment of poultry by injection is scarce. Undoubtedly, much of this work has been carried out by polyphosphate manufacturers and poultry processers and research institutes; and is to be found in the patent literature.[15] However, at least 4 papers have been published which determine the effects of polyphosphate injection into chicken.[12,16,17,18]

Further information about this literature is given in section 1.33.

TABLE 1 LEGISLATION LIMITING THE USE OF PHOSPHATE SALTS IN EEC COUNTRIES AND U.S.A.
(From Iles, 1973[9])

Country	Phosphate salts allowed	Quantity (as% P_2O_5)	Products allowed in	Comments
EEC				
Belgium and Luxembourg	Disodium diphosphate Tetrasodium diphosphate Pentasodium triphosphate (tripolyphosphate)	0.6 max.	Meat products	Salts may be used singly or in combination so that total phosphate (as P_2O_5) does not exceed max., 0.3% being supplied by added phosphate.
	Hexasodium tetraphosphate (tetrapolyphosphate)	0.7 max.		
	Disodium diphosphate Tetrasodium diphosphate Pentasodium triphosphate	0.8 max.	Prepared meats	
France	Sodium or potassium phosphates	0.3 max.	Non-cured charcuterie	
W. Germany	Sodium or potassium diphosphate	0.3 max.	Frankfurters only	
Italy	"Polyphosphates"	0.25 max.	Cooked ham Meat Pies	
		0.4 max.	Cooked sausages	
		Nil	Canned meat, cooked meat products	
Netherlands	Sodium or potassium phosphates	0.5 max.	Meat, meat products	
Denmark	"Polyphosphates"	0.5 max.	Meat products	
U.K.	Salts of phosphoric and polyphosphoric acids			Sodium phosphates may be labelled "emulsify: salts but the chemical name must be used fo: other phosphates
U.S.A.	Di- and monosodium orthophosphates Tetrasodium and acid sodium diphosphates	0.5 max.	Meat products	Incorporation defined as 0.5% max. derived f 5% pickle at 10% pump level. Clear solution: only, allowed.
	Sodium hexametaphosphate Sodium tripolyphosphate			May not be used as extenders and binders in fresh sausages unless already present in uncooked cured pork added to mix.

Following the development of an efficient and reproducible injection unit,[19] this method of polyphosphate treatment is now used commercially for chickens which are to be subsequently frozen. More up-to-date equipment is now available.[20] The chicken is injected into both sides of the breast with a pneumatically operated hand injector. Turkeys over 7kg in weight should also be injected into the thigh muscles.

To ensure distribution of injected polyphosphate throughout the carcase before freezing, it is reported[20] that injection should be carried out after evisceration and before spin-chilling, with a 15 to 30 minute holding period at 0°C to 4°C before freezing. However, Truman and Dickes,[21] found no or very little added phosphorus in the leg muscles of commercially treated chickens, although the treated leg muscles did contain more sodium than the untreated muscles. The phosphorus contents (% wet tissue) of the leg muscles from these treated and untreated chickens were, 0.19 and 0.25, and 0.17 and 0.19 respectively; and the sodium contents, 0.094 and 0.097, and 0.062 and 0.073, respectively. Since only 2 chickens from each treatment were analysed, these findings are inconclusive.

1.3 EFFECT OF POLYPHOSPHATES ON WATER RETENTION AND COOKING LOSS

1.31 THE WATER HOLDING CAPACITY OF MEAT

All meat has an inherent ability to retain its natural moisture, which in chicken comprises about 75% of its weight; and to absorb additional water. This is called the water holding capacity (WHC), and is affected by properties of the meat, including pH and muscle structure;[22,23] and by external processes such as rate of freezing.[24,25] Further details of WHC are given in section 1.611.

A decrease in WHC results in loss of fluid from the meat, and it is this loss which has presented problems to poultry processors. The loss of fluid from fresh whole and cut-up carcases (weep) during storage

and marketing, leads to an unsightly packaged product, as well as presenting problems of weight control. Frozen carcases lose fluid on thawing, which may be considerable and, coupled with the cook loss, may result in a tough dry product. This loss of ability of meat to hold water is caused by protein denaturation and proteolysis, and it is important to remember that both poultry weep and thaw loss result in loss of proteinacious material from the carcase, which may lead to a decrease in the nutritive value.[26]

The pH of the post-rigor muscle is one of the factors which affect these protein changes. The ultimate pH of the muscle after post-mortem glycolysis is about 5.5, and the WHC is at a minimum at this point. This is because this pH is near to the isoelectric point of the myofibrillar proteins which are primarily responsible for the binding of water; and, therefore, at this pH the proteins carry minimum nett charge, and their packing density is thus at its maximum and the hydration at a minimum. Generally, as the muscle pH decreases, the volume of thaw loss and weep are increased.[24] Cooking also produces a decrease in WHC because heat denaturation of tissue proteins leads to loss of fluids.[23] Meat may lose more than 30% of its weight during cooking.

1.32 WATER UPTAKE DURING PROCESSING

Poultry comes into contact with water during the slaughtering and processing stages, and some of this water will be taken up by the carcase. Most of this added water is taken up during the chilling stages, and the amount increases with immersion time, chill water temperature, and with agitation; as well as being dependent on characteristics of the individual bird. Very little water is absorbed through intact skin, and the primary sites of water absorption are through cut and damaged surfaces.[27] Some of the water taken up in this way will be lost on the drip-line following the chill tanks and preceding

the packing table. However, the added water that does remain in the bird is of concern to legislative bodies because of its doubtful hygienic status. The chilling process has recently been the subject of EEC legislation.[28] However, considerable controversy surrounds this legislation because of its economic implications,[29] and doubts as to whether or not hygiene will be improved since the chilling process is not the only stage at which potentially unwholesome water may enter the carcase.[27,30]

A very important aspect of polyphosphate treatment is that the injection process inevitably leads to water being introduced into the carcase, and this has led to consumer and governmental complaints. However, the water content of fresh and processed chicken is subject to the variations discussed above, and providing the volume of polyphosphate injection lies within acceptable limits, the chicken should benefit from such treatment. As yet, the UK has no legislation as to the amount of added water permitted, but in the USA the amount injected is limited to no more than 3% of the weight of the eviscerated carcase. The EEC are expected to enforce standards for the water content of poultry for all member countries.[31] Without legislation the injection process is open to abuse, but a chicken containing a high level of water may infringe other legislation.[32] It is worth mentioning that the most common complaint directed toward polyphosphate treated chickens:that of "selling the consumer water", is entirely misguided, since frozen chickens are less expensive to buy per kg than fresh or untreated chilled chickens.[33]

1.33 CONTROL OF WATER CONTENT BY POLYPHOSPHATES

Most of the published work on the effects of polyphosphates on the moisture content of chickens involves administration of the salts via the chill-water which necessitates long periods of soak(3 to 22 hours). Absorption of up to 10% of the eviscerated carcase weight may

occur during these times,[11] which is too much by present-day standards (see section 1.32). Also, most of this data is of limited use in this discussion since uptake of phosphate is not calculated, and the difference between polyphosphate-treated and untreated samples is not always tested for statistical significance. Added to these problems are many associated with the variables due to test conditions and chicken type, which makes direct comparison of different sets of results very difficult. However, a review of the most useful literature does reveal some consistent trends.

Klose et al,[11] chilled hen and chicken fryer eviscerated carcases for 22 hours in a 5% commercial polyphosphate solution and found a significant decrease in the amount of chill water absorbed due to treatment. Fryer chickens (1 to 1.5kg eviscerated weight) absorbed more water than hens (1.5kg eviscerated weight). Treatment also resulted in a higher mean cooked yield for hens after simmering for 2 hours and fryer chickens after deep-fat frying.

Schermerhorn et al,[6] also found a significant decrease in chill-water uptake due to polyphosphate treatment (6 hour chill in 0,8 and 12% polyphosphate solutions), for eviscerated broiler carcases, which decreased with increasing level of treatment. Although the 4% level of treatment did not result in decreased chill-water absorption, carcases so treated, along with those from the 8 and 12% polyphosphate solutions, did lose significantly less moisture during a 16 hour storage period at 2°C compared to controls. However, this loss did not significantly decrease with increasing level of treatment. All treatment levels resulted in significantly increased cooked yields compared to controls when the carcases were cut up and deep-fat fried, although this effect was largely independant of treatment level. There was little difference in effect between sodium tripolyphosphate and a commercial polyphosphate at any given treatment level.

Schermerhorn and Stadelman,[34] conducted similar experiments to the above using hens, and found the same trends due to treatment on chill-water absorption with little difference in overall effectiveness between sodium tripolyphosphate and a commercial polyphosphate. However, no significant differences between treated and control carcases on moisture loss during storage for 24 hours at 2°C and on cooking loss after baking (heavy hens) or boiling (light hens) were noticed, although treated than controls. It is not possible to compare these results directly with those for mean values tended to be lower for broilers[6] because apart from variations in breed and cooking method, a different statistical criterion was used (5% level of probability for broilers and 1% level for hens). These authors[34] cited Mahon, 1963, who stated that for polyphosphates to be effective in controlling moisture loss, 0.3 to 0.5% added phosphate in the tissue is necessary. Phosphate analyses of the breast muscle showed that light hens absorbed more than the heavy hens which contained less than 0.3% phosphate after each treatment level. Although there was no significant difference in moisture loss results, the chill-water absorption results were statistically significant for light hens when 0.3% or more of phosphate had been absorbed by the breast. However, it was not made clear whether the raw or cooked breast muscles were analysed for phosphate; and, also, these results must be treated with caution since phosphate uptake by breast muscle is unlikely to reflect moisture losses from the whole carcase. Light hens also absorbed more chill-water than heavy hens and had greater cook losses, although this was reported to be due to the degree of cooking rather than the cooking method. This increase in chill-water absorption seems likely to be due to differing skin/fat ratios rather than the increase in phosphate absorption noted for light hens.

Monk et al,[35] investigated the effect of cooking method and post-evisceration chilling in polyphosphate solutions (5 hour chill in 6% commercial phosphate solution) on the cooking losses of hens and broilers. Prior to chilling, each carcase was cut longitudinally in

half and one half treated with polyphosphate and the other used as control. Phosphate analyses of the raw flesh from 2 broilers and 2 hens showed that the broilers had more than 0.3% added phosphate while the hens had less than 0.2%, suggesting greater phosphorus uptake by broilers compared to hens. Cooking in an electronic oven resulted in lower cook losses for all samples than did boiling or pressure cooking methods, which generally resulted in higher cook losses with increasing cooking temperature. Treated broiler samples had significantly higher cook yields than controls after each method of cooking, but there was little difference between treated and control hen samples; which could be a result of insufficient phosphorus uptake.

The effect of post-evisceration chilling in polyphosphate solutions (6 hours in solutions containing 0,4,8, and 10 oz per gallon of a commercial polyphosphate) on the moisture loss from broilers during cutting-up and storage at 2°C for 7 days was investigated by May et al.[36] In this case the lowest level of treatment significantly increased water uptake during chilling compared to all other treatments, but the highest level of treatment significantly decreased water uptake over all other treatments. All groups lost weight during the cutting-up process with treated groups losing significantly more weight than controls. However, treatment significantly decreased weight losses of the cut-up carcases during storage, with all the treated portions being heavier than controls after 7 days. Portion weights increased with the level of treatment, but the portions from the high and intermediate treatments did not differ significantly from one another. Cooked yields were not calculated in this experiment, but Landes,[7] directly treated broiler portions by marination in polyphosphate solutions at 2°C for 12 hours, and found increased cooked yields after baking, for portions from the 12% marination, but not from the 6%. Phosphate analyses of light and dark meat showed that uptake for both types of tissue from the 12%

solution was greater than 0.3%, but this was only true for the white meat from the 6% solution.

The retail benefits of reducing weep from packaged chicken parts are obvious, and more recently an injection process for the polyphosphate treatment of broiler parts suitable for commercial use has been reported.[16] Two different types of multi-needle injection units were compared to overnight marination in a 3% commercial polyphosphate solution at 3°C. Broiler legs were injected with a 6% commercial polyphosphate solution to give an approximately 8% weight increase. All treatments resulted in significantly increased cooked yields after both water cooking and frying, with no significant difference among treatments. However, Farr and May,[12] reported that broiler parts injected with a solution containing 10% sodium chloride and 5% commercial polyphosphate had increased cooked yields compared to other methods of treatment (addition of sodium chloride and/or polyphosphate to the cook water or to the post-cook cooling water). These authors stated that broiler parts so injected had a phosphate uptake near to 0.3%, and that the other 2 methods of treatment resulted in improved cooked yields when 0.2% or more of phosphate was incorporated into the tissue. However, no analytical data was provided to illustrate these claims or to illustrate the well-known synergism between sodium chloride and polyphosphates, which seems likely to account for the superiority of the injection process.

Van Hoof and Daelman,[17] reported the effects of polyphosphate injections at various stages of a commercial processing procedure on the volume of thaw loss collected during 36 hours at +8°C from broiler carcases. They found that broilers which were treated with polyphosphates (presumably after evisceration) by injection into the breast muscle either before, after, or in the absence of spinchilling procedures, had a lower percentage thaw loss than birds similarly treated with injections

of water. Also, the mean thaw loss value of 5 broilers was lower for each polyphosphate treated group than for the group which underwent spinchilling with no injection of water. In contrast, Truman and Dickes,[21] found that the mean thaw loss value of 8 treated chickens was higher than the mean value for untreated birds, but this difference was not statistically significant. However, the amount of thaw loss obtained from frozen meat depends on various factors including temperature of freezing, method of collection, and time and temperature of thawing,[23,24,25] Since the results of Truman and Dickes seemed to have been obtained from chickens purchased from retail outlets, it is probable that processing differences caused such variations in thaw loss volume as to make direct comparison between treated and controls invalid.

Recently, Grey et al[18] have reported results on the effect of commercial polyphosphate injection as practised in the UK (see section 1.2), on the amount of chill water uptake and levels of thaw and cooking losses of broilers. They determined the level of chill water uptake on about 1000 each of treated and untreated broilers during normal factory processing, and found no significant difference due to polyphosphate treatment, (however see section 3.71). Thaw and cooking losses were determined for 48 each of treated and untreated broilers, and there were no significant differences in either value due to treatment. However, the cooked yield of treated broilers was greater than that of untreated broilers, which they calculated was due to the retention of an amount equivalent to 80% of the injected fluid. It appeared that uptake of phosphate by raw pectoralis major muscle was greater than 0.3%. These experiments which involved analysing large numbers of broilers which were obtained during 8 days of normal commercial processing over a period of 13 months, obviously produced a more accurate account of the effects of commercial polyphosphate injection than did the previous 2 experiments. Although no overall statistical differences were found

between treated and untreated broilers, the injection process was found to produce a variable effect on chill water uptake, and also chill water uptake was found to vary according to the day of the experiment.

1.34 SUMMARY

The moisture content of chicken is affected by polyphosphate treatment in the following ways:

a) A reduction in the amount of water taken up during chilling when polyphosphates are added to the chill water, but a variable and statistically insignificant effect on chill water uptake when treatment is by pre-chill injection.

b) A variable effect on thaw and cooking losses; but an overall increase in cooked yield, whether treatment is by soaking in chill solutions of polyphosphates, or by pre-chill injection.

These effects are apparent when at least 0.3% of phosphate has been incorporated into the tissue. Application by injection is more rapid and economically viable than by immersing carcases in polyphosphate solutions. However, the injection process leads to extra water being introduced into the carcase. This is not of great importance when considered in conjunction with the normal movement of water into and out of a carcase during processing and cooking. However, legislative control is required to stop unnecessarily large amounts of water being introduced in this way.

1.4 EFFECT OF POLYPHOSPHATES ON AUTOOXIDATIVE RANCIDITY

Both raw[37,38,39] and cooked[40] meats develop off-odours and off-flavours during storage at refrigerator and frozen temperatures. In fact, Jacobson and Koehler[40] found that cooked chicken and turkey meats deteriorated in flavour after 1 to 4 days of refrigerated storage (4°C) and to a lesser extent during frozen storage (-20°C). Their results of organoleptic and thiobarbituric acid (TBA) tests for light and dark chicken meat appear in Table II.

TABLE II

AVERAGE SENSORY RATINGS[a] AND TBA VALUES[b] FOR COOKED LIGHT AND DARK CHICKEN MEAT STORED FROZEN OR REFRIGERATED FOR 1, 2 OR 3 DAYS
(from Jacobson and Koehler[40])

	Newly Cooked	Frozen			Refrigerated		
		1 day	2 days	3 days	1 day	2 days	3 days
Flavour ratings							
Light Meat	8.6	8.0	7.7	7.7	7.5	6.0	5.4
Dark Meat	8.5	7.7	7.5	7.4	5.9	4.8	4.1
TBA values							
Light Meat	0.80	0.89	0.94	0.88	1.04	1.24	1.48
Dark Meat	1.83	2.10	2.03	1.83	2.08	2.01	2.15

a. Overall flavour rated from 10 to 0, with 10 representing best chicken flavour

b. As mg malonaldehyde per 1000g meat

They found a significant correlation between TBA values and flavour change for turkey meat, but chicken meat was not tested in this way. The TBA test will be discussed in section 2.3 but briefly it is a chemical test performed on the whole meat sample which measures a by-product of oxidation.

Many terms may be used to describe these off-flavours, but generally the meat is said to have become "rancid". This deterioration arises due to breakdown of meat lipids caused by enzymic and oxidative attack.[41] Lipid is the general term applied to those substances which are insoluble in water, but soluble in organic solvents, and in meat refers to hydrocarbons, alcohols, and fatty acids and their derivatives. This thesis will be concerned with lipid deterioration caused by attack of atmospheric oxygen, and this process is called autooxidation. It is the fatty acid components of lipids which undergo autooxidation, unsaturated fatty acids being more susceptible to autooxidation than saturated fatty

acids; and the ease of oxidation increases with the degree of unsaturation. In fact, Enser[42] reports that the relative susceptibility of oleic, linoleic and linolenic acids to oxidation is, 1:12:100. Therefore, it is necessary to consider the fatty acid composition of chicken lipids before discussing their autooxidation and subsequent rancidity.

1.41 LIPID COMPOSITION OF CHICKEN

The fatty acid composition and total lipid content of chicken tissues varies with the type of muscle. An extensive review of post-1960 literature on the lipids of chicken has been published.[43] The total lipid content of light meat (combined values for breast and wing meat) was 1.05gm/100gm muscle, and for dark meat (thigh, drumstick and back tissue) 3.97, for broiler/fryer class of chickens. Higher values were obtained for roaster and stewing hens. The average factor derived from the literature data for calculating the grams of fatty acid per gram of tissue fat for like tissues of all three classes of chicken varied by less than 1%. Light meat contained an average of 0.81gm fatty acid per gram of tissue fat, dark meat 0.86 and skin 0.94. It was established that the distribution of fatty acids within a given type of tissue varied no more among classes of chicken than within a single class. The major fatty acid constituents of chicken tissues were found to be palmitic, oleic and linoleic acids which comprised at least 68% of the total fatty acids in each tissue. The problems associated with obtaining reliable data when conducting literature surveys of this kind were discussed in a separate paper.[44]

The phospholipids of meat tissues are the primary site of auto-oxidation due to their high unsaturated fatty acid content and the fact that they exist in closer contact with tissue catalysts of oxidation than do triglycerides (the major component of neutral lipids).[45] However, Shorland,[39] who studied oxidative development in beef and lamb

during frozen storage, suggested that phospholipids are protected against autooxidation by their close association with muscle proteins; and that neutral lipids play the major role in the development of rancidity during frozen storage of raw meats.

Marion and Woodroof,[46] found that the phospholipids in breast, thigh and skin tissues contained higher levels of 18-carbon saturated and 20-24 carbon unsaturated (3 to 6 double bonds) fatty acids than the triglycerides which contained higher levels of 18-carbon mono- and diunsaturated fatty acids than the phospholipids. Breast muscles contained the highest proportion of phospholipids to neutral lipids, followed by thigh and then skin tissue lipids. Marion and Miller[47] found that an inverse relationship existed between the level of phospholipid and the total lipid content of chicken tissues indicating that higher lipid levels arise primarily from the deposition of neutral lipids.

The lipid composition of chicken changes during post-mortem ageing,[48] frozen storage,[49,50] and cooking;[51,52] Generally, phospholipid content decreases and free fatty acid and triglyceride content increases during frozen storage and to a lesser extent during 48 hour post-mortem ageing. These changes suggest phospholipase activity at freezer temperature and in the early stages of ageing, and in conjunction with protein changes they are important in determining meat tenderness. Cooking with no added fat does not greatly change the fatty acid profiles of tissues,[43] although an increase in linolenic acid due to cooking has been noticed.[51,53]

Factors such as age, sex and diet of chickens affects the lipid composition of their tissues. Edwards et al[54] analysed the entire minced carcase (minus feathers) of broilers at 4,8,12,16 and 20 weeks of age, and found maximum lipid levels at 16 weeks for both males and females. At all ages, females had a greater lipid content than males. The percentage carcase lipid varied with age between 6.7 and 13.1 for males

and between 8.0 and 20.8 for females. The fatty acid composition of the adipose tissue of these birds at 12,16 and 20 weeks of age, showed only small, inconsistent changes with age and between sexes. However, the stearic acid content of males was higher than that of females of the same age. The same inconsistent changes were found in other experiments using male broilers at 4,6,8 and 10 weeks of age; except that the palmitoleic acid content increased with age in both the abdominal adipose tissue[53,55] and breast muscle.[53]

Edwards et al[54] conducted a separate experiment to the above using broilers as previously, but housed in smaller groups in wire-floored battery brooders and growing pens instead of on shavings like the previous group. In this experiment, the weight and lipid content of the carcases at a particular age were higher than for the previous birds, but again the females contained more lipid than the males at each age. Thus, environment also affects the composition of chicken.

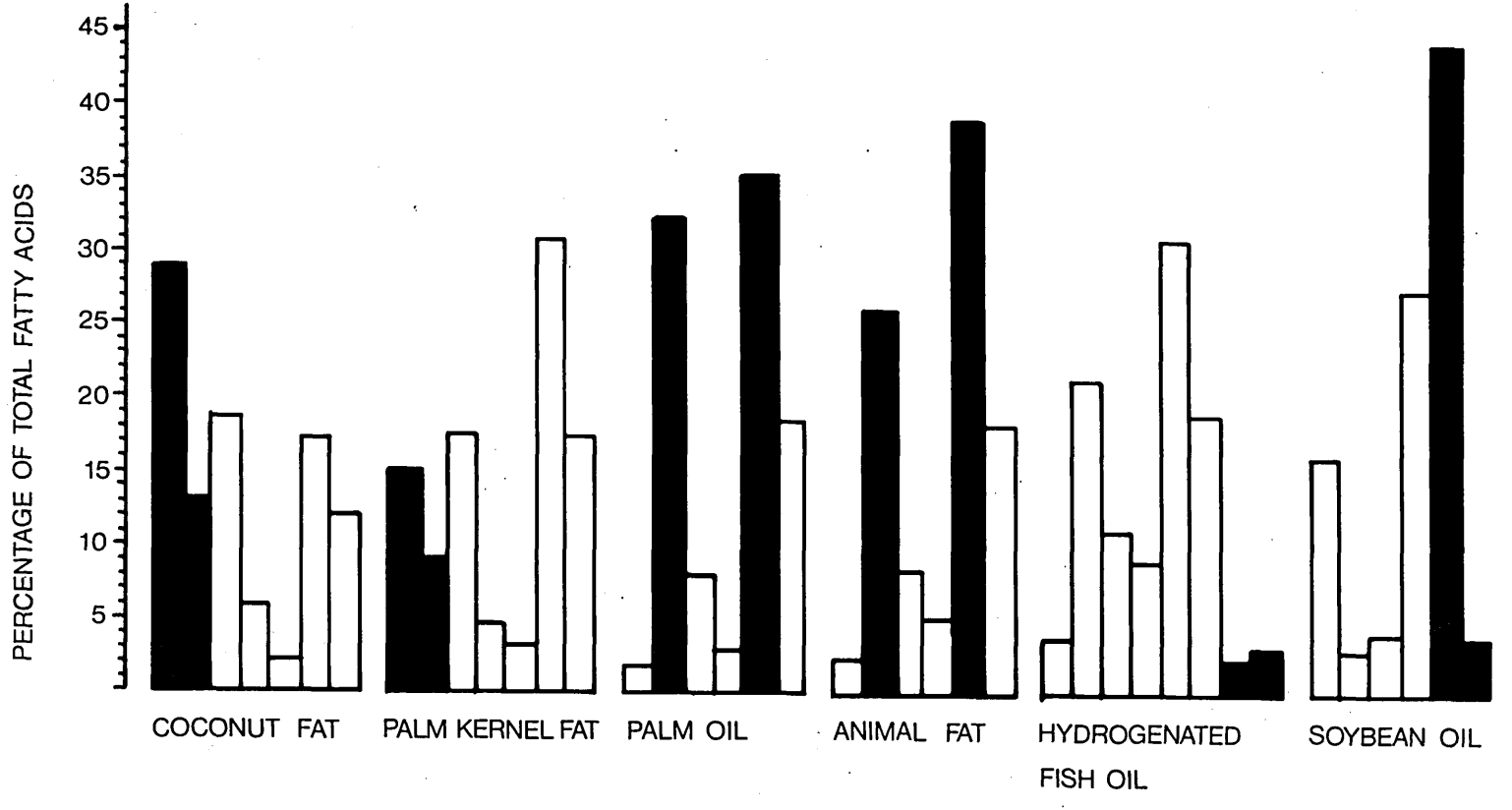
The previous investigations concerned total lipid changes in the whole carcase, but Singh and Essary,[56] found no significant differences due to age or sex in the total lipid content of either breast or thigh muscles of broilers, although the combined age values for each muscle were higher in females than in males. However, it has been reported elsewhere[47] that the total lipid of both breast and thigh muscles of male broilers was higher in 20 week old birds than in 8 week old birds.

The effect of diet on the composition of chicken has been studied extensively, in order to obtain optimum growth rates and to produce a bird of acceptable fatness from a flavour and texture point of view. It became clear that the fatty acid composition of poultry lipids was affected by lipid supplements in the diet, and therefore could effect the susceptibility of the bird to autooxidative rancidity. Veen,[57] reported results of feeding broilers diets containing 6% added fats

which were coconut fat, palm kernel oil, palm oil, animal fat, soybean oil and hydrogenated fish oil. It seemed probable that the broilers received the diets until slaughter at 5 to 7 weeks of age, but the exact experimental details were not supplied. Samples of adipose fat were taken from 10 birds from each diet group and mixed, and the main fatty acids determined (Figure II). The characteristic acids of the added fats were clearly recognizable in the adipose fats.

Edwards et al,[54] determined the influence of dietary cottonseed oil, acidulated cottonseed soapstock, beef tallow and poultry fat at levels of 3.25% in starter rations and 5.25% in finisher rations, on carcase composition of broilers. Gross body composition was not influenced by these supplements, but there was a tendency for the birds which received these supplements to have a slightly higher carcase fat content than those on the basal diet. This was to be expected since the supplemented diets had slightly wider calorie:protein ratios than the basal diet. Generally, the whole carcase fat content of all the experimental birds increased with age, and in females the largest increase occurred between the 4th and 5th week and in males between the 6th and 7th week. The fatty acid composition of the carcase adipose tissue from 8 and 9 week old birds showed large differences due to diet. Linoleic acid accounted for more than 50% of the total fatty acids in cottonseed oil and in acidulated cottonseed soapstock, and birds fed these lipids had a much larger linoleic acid content than birds fed the basal diet, or those diets containing other supplements. Similarly, the adipose tissue of birds fed beef tallow contained more stearic and oleic acids than the other birds, since this supplement was very much richer in both these acids than the other fats. Consequently, the isolated adipose tissue from beef tallow fed birds was much firmer to the touch than that from birds fed more unsaturated fats. The fatty acid composition of the total carcase lipids from 8 week old birds

FIGURE II FATTY ACID COMPOSITION OF CHICKEN ADIPOSE TISSUE AS INFLUENCED BY DIETARY FAT SUPPLEMENTS (from Veen[57])



showed similar changes due to diet, but contained more palmitic acid than the adipose tissue lipids.

Jen et al,[55] investigated the effect of corn oil, lard, beef tallow and hydrogenated coconut oil added at a 10% level to the finisher rations (at 4 weeks of age) of broilers, on the fatty acid composition of the abdominal adipose tissue. Again, it was found that the fatty acid composition of the total lipid extracted from the adipose tissue tended to reflect the fatty acid composition of the dietary fats. These fatty acid patterns were incorporated into the tissue within 2 weeks of starting the experimental diets. The neutral lipids of adipose tissues had similar fatty acid patterns to the total lipids from the corresponding tissue, but all neutral lipids had a higher percentage of saturated fatty acids and a lower linoleic acid content than the total lipids. No significant differences in flavour or rancidity (as measured by the thiobarbituric acid test) due to diet were apparent in freshly cooked skin and adipose tissue from 10 week old birds. However, Marion et al,[58] reported a correlation between TBA numbers of raw breast muscle after 12 days' storage at 2°C and the diet which the broilers had received. They reared broilers to 58 days of age on rations differing in protein level (16 or 24%), and in type of supplemental fat (5% of coconut oil, beef tallow, safflower oil, or menhaden oil). The total lipid of the breast muscle was separated into neutral lipids, and the phospholipids, cephalin and lecithin. All the lipid fractions showed fatty acid changes due to the different dietary fats. The neutral lipid fractions exhibited most pronounced changes in the 16- and 18- carbon fatty acids; whilst the phospholipids showed most marked changes in the longer chain fatty acids. The TBA Numbers were generally higher for the muscles from the birds fed the high protein diet, and the highest values were obtained from the muscles of the birds fed menhaden oil, which resulted in higher levels of 20- to 22- carbon polyunsaturated fatty acids than the other dietary

fats. These results were in agreement with those of a similar experiment.[59]

In all the preceding papers, high levels (5% or more) of lipid supplements were added to the rations of experimental birds, but Bartov and Bornstein,[60] determined the minimum levels of dietary vegetable oils which have an effect on the composition of carcase fat. These vegetable oils were supplied by either soybean oil added to the starter rations or derived from the dietary grain (corn or milo). The addition of 0.3% soybean oil or 20% yellow corn or 40% milo, caused marked increases in the degree of unsaturation of abdominal fat as compared to control diets. This increase was caused mainly by an increase in linoleic acid, and resulted in increased susceptibility to oxidation (as determined by the TBA test). Higher levels of dietary oils caused further increases in unsaturation of abdominal fat. These increases decreased the oxidative stability of the abdominal fat when soybean oil or milo was added, but not when corn oil was added. This was thought to be because diets containing corn oil had a higher natural antioxidant (a-tocopherol) content than the other diets, and that this fact could also partly explain the fact that linoleic acid derived from corn oil was deposited into adipose tissue at a significantly higher rate than that derived from milo or soybean oil.

1.411 Summary

The main fatty acids present in chicken tissues are, palmitic, oleic and stearic acids. Phospholipids contain more long chain polyunsaturated fatty acids than neutral lipids, and are thus more susceptible to oxidative rancidity than neutral lipids. The fatty acid composition of carcase lipids tends to reflect the fatty acid composition of lipids present in the diet. Age and sex have little affect on the fatty acid composition of lipids, but have an affect on the total lipid levels, which increase with age, and are higher in females than in males.

1.42 MECHANISM OF AUTOOXIDATION

Autooxidation is a free-radical chain reaction, believed to involve 3 stages:[61]

(1) Initiation This step involves the breakdown of unsaturated fatty acids to form free-radicals,

$$\underset{\text{fat molecule}}{RH} \xrightarrow{\text{initiator}} R^{\bullet} + H^{\bullet}$$

and constitutes the induction period, which is the time that elapses before rancidity becomes organoleptically detectable. Initiators include, light,[62] heat,[63] and heavy metals especially copper and iron.[64,65] Oxidised meat pigments may also act as initiators[66,67,68,69]

(2) Propagation The free-radicals formed during initiation combine with atmospheric oxygen to form peroxide free-radicals, which then act as secondary initiators in the initiation process by reacting with substrate to form more fatty free-radicals and hydroperoxides:

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$$

It is thought that all hydroperoxides are odourless and tasteless,[70] and it is therefore the decomposition products of these hydroperoxides which are responsible for the off-odours and off-flavours of rancid fats. Breakdown of hydroperoxides via the weak O - O bond is the expected mechanism, but thermodynamics predicts degradation rates of 10^2 to 10^5 times slower than actually found at temperatures of 60°C to 100°C.[71] In fact, the degradation process is catalysed or "molecularly assisted" by agents such as, metals, acids, bases,[71] and haem compounds.[72] Transition metals are thought to increase the rate of decomposition of hydroperoxides to free-radicals, by a series of one electron transfer reactions:[61,71]

$$ROOH + M^{2+} \longrightarrow RO^{\bullet} + OH^{-} + M^{3+}$$

$$ROOH + M^{3+} \longrightarrow RO_2^{\bullet} + H^{+} + M^{2+}$$

The free-radicals may then break down according to the following scheme:

$$R_1\text{-}\underset{\underset{O^\bullet}{|}}{CH}\text{-}R_2 \longrightarrow R^\bullet_{1(2)} + R_{2(1)}\bar{C}(=O)H$$

to form aldehydes; and alcohols and hydrocarbons may be formed by the alkyl free-radical gaining $OH^\bullet$ or gaining or losing $H^\bullet$.

However, this simple mechanism does not account for all of the oxidation products found in fat systems; presumably because of the many side reactions which may take place, and many further investigations have been carried out; for example, those by Lillard and Day,[73] Loury,[74] and Michalski and Hammond.[75]

(3) Termination The chain reaction may be terminated by deactivation or destruction of the free-radicals. This may occur when the free-radicals which have accumulated during the propagation stage, react with each other to form inactive non-radicals, thus slowing down the oxidation process:

$$R^\bullet + R^\bullet \longrightarrow RR$$

$$RO^\bullet_2 + RO^\bullet_2 \longrightarrow ROOR + O_2$$

However, the chain reaction may be controlled by antioxidants, of which there are 2 types: those which act as free-radical scavengers, and those which inhibit the production of free-radicals. The general mechanism for the destruction of free-radicals is:[76]

$$RO^\bullet_2 + AH \longrightarrow ROOH + A^\bullet$$

$$\text{or} \quad R^\bullet + AH \longrightarrow RH + A^\bullet$$

where AH is an antioxidant, ROOH is a resonance stabilised hydroperoxide which is incapable of breaking down to form oxidation products, and $A^\bullet$ is a relatively inactive radical. Pokorny[76] gives a detailed account of these reactions. Such antioxidants include synthetic free-radical scavengers such as butylated hydroxyanisole(BHA) and butylated hydroxytoluene(BHT),[77] and a wide range of naturally occurring compounds such as tocopherols.[78]

Prevention of the production of free-radicals is most commonly

achieved by deactivating transition metals, which are the major catalysts of the initiation process. These metal chelating agents include, citric acid, ethylenediaminetetraacetic acid, and phosphoric acid. The metals present as simple fatty acid salts are effectively deactivated, but those present in more complex compounds such as metal porphyrins are not.[61] Often, a combination of antioxidants are used to increase the antioxidant activity i.e. a synergistic mixture.[76,79]

Non-lipidic substances present in meat, such as proteins, may also have an effect on the autooxidation process. Proteins may react with the oxidation products which cause the off-odours and off-flavours, leading to their destruction or modification.[80] Also, metals, in addition to being major catalysts of initiation and hydroperoxide decomposition, may, under certain conditions, terminate the chain reaction.[81]

The addition of anti-oxidants to food substances is controlled by legislation, and the conclusions of the FAAC Report, 1974, on the Antioxidant in Food Regulations, 1966 and 1974, has recently been reviewed.[82]

1.43 INHIBITION OF AUTOOXIDATIVE RANCIDITY BY POLYPHOSPHATES

Polyphosphates, alone, and in synergism with other salts (usually sodium chloride) inhibit oxidative rancidity in cooked meats.[12] They are generally thought to be ineffective in raw meat,[83] but it has been reported that a commercial tripolyphosphate mixture delayed the onset of rancidity in raw turkey breast and leg muscles.[84] Orthophosphate salts are not effective as antioxidants,[8,85] but the other salts commonly used in the poultry industry (di-,tripoly- and hexametaphosphate) are.

Some of the literature reviewed in section 1.33 on the effects of polyphosphates on water retention and cooking losses in chickens, also demonstrated the antioxidant effects of polyphosphates. Only the results of rancidity tests and necessary experimental details will be discussed here, since full details of the experimental designs

were given earlier.

Organoleptic tests, of which one criterion to be judged was rancid flavour, were performed on pre-cooked frozen broiler quarters.[7] With the exception of the initial storage period (0 months), the intensity of rancid flavour was greatest for untreated quarters, the difference being significant at the 6 month storage period.

Organoleptic and thiobarbituric acid(TBA) tests were performed on precooked (water cooked and then fried with or without batter and bread coating) broiler legs during either refrigerated storage for up to 3 days, or frozen storage for up to 9 months.[16] The treated coated fried parts were less rancid than controls after 1 to 3 days refrigerated storage, as determined by both organoleptic and TBA tests (uncoated parts not tested). Frozen parts were only tested organoleptically, and showed decreased rancidity for all treated samples compared to controls. The difference became significant after 3 months storage for uncoated parts, and after 6 months for coated parts. There were no significant differences among types of polyphosphate treatment.

The rate of oxidative deterioration as determined by TBA tests, of precooked broiler halves was significantly reduced due to polyphosphate treatment.[12] In fact, uptake of only 0.04% phosphorus (as P_2O_5) from the post-cook cooling water, resulted in reduced TBA values compared to controls, and further, the TBA values decreased as percentage uptake increased. No effect on cooking losses was evident at these uptake levels (0.04 to 0.15% P_2O_5). In a further experiment, it was found that immersion for only two minutes in a 5% polyphosphate post-cook cooling solution, resulted in reduced TBA values after 24 hour and 7 days refrigerated storage, the TBA values being 3.50,0.49 and 12.50, 3.65 for 24 hour and 7 days storage respectively. No added phosphate was detected in these samples. Unfortunately, organoleptic tests were not performed, and so these surprising results could not be correlated with actual flavour differences. However, Thomson,[86] did find differences

in rancid odour after less than 0.20% phosphate (as P_2O_5) had been incorporated into chicken tissue. A commercial mixture of sodium tripolyphosphate was added to the chill water during commercial processing of fryer chickens which were then cooked and stored for up to 14 days at 4°C. The average degree of off-odour was less than "very slight" for phosphate treated samples from the 1st until the 7th day of storage, and varied between "slightly strong" and "medium strong" in control samples. TBA values varied between 0.7 and 2.6 for treated, and 4.9 and 6.6 for controls during this period. These results therefore, demonstrated the antioxidant effect of relatively low added phosphate levels, by both chemical and organoleptic tests.

It is important to note that there are several types of TBA test, of which two are commonly used.[87,88] The latter is usually carried out in conjunction with a calibration procedure, and the results expressed in terms of TBA Number, which is defined as mg malonaldehyde per 1,000 gm sample (see section 2.3). The results obtained from all the methods not using a calibration procedure, are expressed in terms of TBA Values which have arbitrary units. Values of between 0.5 and 1.0 for both TBA Numbers[88] and Values[87] have been found to correspond to the threshold of rancid flavour.

1.5 OTHER EFFECTS OF POLYPHOSPHATES

1.51 COLOUR

Generally, polyphosphates have a beneficial effect on the colour of all treated meats, because their antioxidant properties result in the inhibition of fading of the natural meat colour which accompanies oxidation.[9]

However, it has been reported that the skin surface of poultry had a bluish cast after immersion in a polyphosphate chill solution,[6,11] which was noticeably different from the natural cream colour of untreated birds. The intensity of the blue colour increased with the concentration

of polyphosphate, and was also detected below the surface of the skin.[6] However, in both cases the colour of the treated cooked products was not noticeably different from that of the controls. This effect on colour could be due to over-application of polyphosphates, but the uptake of phosphorus was not determined in either of these reports.

When high levels (0.5,1.0 and 2.0% w/w) of polyphosphates were incorporated into raw minced chicken meat, the colour of the cooked products darkened (as measured by diffuse reflectance using a colour difference meter) with increasing levels of polyphosphate.[5] There was a highly significant negative correlation between diffuse reflectance values and the level of treatment. The colour was found to be "unacceptable" at the 2% level.

1.52 FLAVOUR

The most important effect on flavour is the reduction in off-flavours in treated chicken due to the antioxidant properties of polyphosphates. However, the use of all food additives are subject to legislation, of which their effect on the natural food flavour is an important consideration.

A salty flavour is sometimes associated with polyphosphate treatment, but this may be due to high ~~levels of treatment~~ concentrations of polyphosphate. Klose,[11] reported saltiness in hens which had been soaked for 22 hours in a 5% chill solution before cooking, but no saltiness was detected in fryer chickens which received similar treatment for 3 or 22 hours. Levels of phosphorus uptake were not reported. This salty flavour was also noted by Froning,[5] after addition of 0.5,1.0 and 2.0% of polyphosphate into minced chicken meat, but was only found to be objectionable at the 2.0% level. Polyphosphate added to the cook water of chicken parts, was found to produce a salty taste above 4.0% addition.[12]

Landes,[7] reported a baking soda-like flavour in treated broiler quarters, which increased with the level of polyphosphate treatment (12

hour immersion in chill water containing 6.0 or 12.0% polyphosphate), and reduced the over-all flavour ratings for treated parts compared to controls. Chicken and meaty flavours were also reduced by polyphosphate treatment, as was rancid flavour. Uptake of phosphorus (as P_2O_5) appeared to be above 0.5%, and therefore the deterioration in over-all flavour could be attributed to over-application of polyphosphate.

The over-all flavour of white and dark meat from broilers chilled for 6 hours in polyphosphate solutions (4,8 or 10 ounces of polyphosphate per gallon of water), was not significantly different from controls, but treated groups had higher mean flavour scores than controls.[36] This apparent contradiction between these and Landes[7] results for over-all flavour could be due to the different levels of treatment, but it is worth noting differences in organoleptic tests. The term "flavour", used in May's[36] tests, is very vague, and the results probably reflect increased rancid flavour in controls compared to treated samples; especially since the samples were cooked and tested organoleptically after 7 days storage at 2°C. This one case points to the fact that organoleptic results from different sources are not easy to compare.

Cut-up chicken pieces which were marinated in a 5% polyphosphate solution for 12 hours, and then dipped in a mixture containing poultry seasoning and salt before cooking, showed no differences in flavour due to treatment.[89] It is not possible to compare these results with the others quoted here, since the addition of seasonings could have masked any salty flavour due to treatment. Also, the terms used in the organoleptic test were vague.

It is thought that carbonyl compounds are responsible for the "chicken" aroma of cooked chicken, and sulphur containing compounds for the "meaty" aroma.[90] These workers found that polyphosphate (at 1.0 and 2.0% levels in chunks of canned chicken meat which were heat processed at 121°C for 55 min.) caused increases in meaty aroma and in the hydrogen sulphide and methyl mercaptan volatiles, compared to controls.

The 1.0% level of treatment produced better average aroma scores than the control, but the 2.0% level was judged to be less acceptable than the 1.0% level, because of an extremely strong meaty odour. Carbonyl volatiles were reduced due to treatment, but corresponding sensory tests for chicken odour were not performed. TBA values were reduced due to treatment, but there was no difference between treatment levels. Also, when polyphosphates were added to fresh, minced broiler meat prior to TBA tests, no difference due to treatment was apparent. It was therefore assumed that polyphosphates inhibit malonaldehyde (the oxidation by-product measured in the TBA test) production during heat processing, and the reduction in total carbonyls due to treatment was, at least in part, due to the antioxidant properties of polyphosphates. This effect was also thought to account for the improved aroma scores of treated samples compared to controls. Samples of meat and depot fat were cooked under oxidative conditions, and polyphosphates were found to greatly affect the formation of sulphur containing and carbonyl volatiles during cooking. Again, meaty aroma and sulphur containing volatiles increased with treatment, and carbonyls decreased. The reduction in carbonyls was greater in meat than in depot fat, and no sulphur containing volatiles were detected for either control or treated samples of depot fat. The effect of cooking chicken with polyphosphates, on the carbonyl volatiles has been studied in greater detail, using chromatographic techniques.[91] Treatment caused a reduction in dicarbonyls, methyl ketones and 2,4- dienals, but there were no qualitative differences between control and treated volatiles.

1.53 MICROBIAL SPOILAGE

Some evidence exists to show that polyphosphates exert antimicrobial activity in foodstuffs.[92] This survey[92] lists the effects of polyphosphates on various bacteria and the patented claims for the antimicrobial effect of polyphosphates as well as discussing their

specific effect on the keeping quality of foodstuffs, including raw poultry. Generally, the antimicrobial effect of polyphosphates is thought to be due to their metal chelation properties.

Spencer and Smith,[93] found that chicken fryer carcases which were chilled for 6 hours in a solution of a commercial polyphosphate, showed a reduced rate of microbial spoilage, as measured by plate counts, U.V. fluorescence, and off-odour, compared to controls. The shelf life of treated carcases was increased by 1 to 2 days. In more detailed experiments Steinhauer and Banwart,[94] determined the effect of tripolyphosphate and a commercial mixture of polyphosphates on the different spoilage organisms present in poultry. They chilled broiler carcases for 6 hours in 8% solutions of the polyphosphates, and then sampled the breast area of each during storage at 5°C. Treatment did not appear to alter the type of organisms present, but did result in lower average bacterial counts compared to controls. The differences in total and proteolytic counts were not significant, but in one experiment treatment with the commercial mixture of polyphosphates resulted in a significant drop in the number of lipolytic type micro-organisms. Both treatments inhibited odour production, slime formation, and discolouration of the carcases during storage. In fact, after 20 days storage, the treated carcases gave off only a slightly noticeable putrid odour and had maintained their natural bluish colour, whereas, the controls gave off a strong putrid odour after only 16 days of storage, and had turned a yellowish colour. Slime formation was observed over the whole of the control carcases at 16 days of storage, when the treated carcases were just beginning to show slime areas.

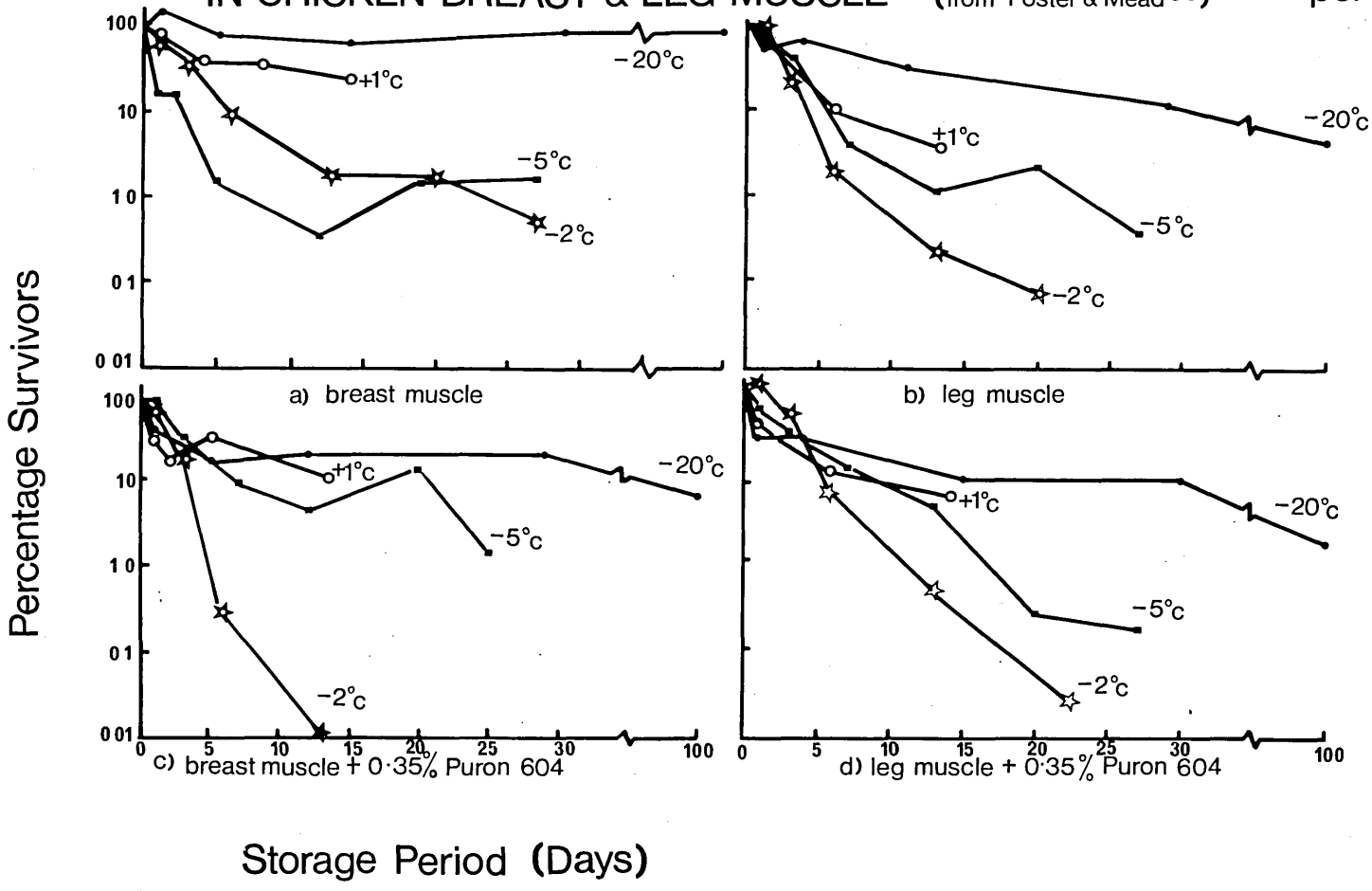
Elliott et al,[95] found that both a commercial polyphosphate and an equivalent mixture of chemically pure polyphosphates inhibited the growth of nonfluorescent pseudomonads in a synthetic medium, but fluorescent strains grew after a short time lag. However, in the mixed

culture of chicken spoilage, only when polyphosphates were present in overwhelming amounts (8% solution), and in intimate and continuous contact with the spoiling surfaces, did they completely prevent growth of the nonfluorescent organisms. Growth of the fluorescent organisms was delayed by both the 3% and 8% solutions of the polyphosphates. Overnight chilling of chicken carcases in the 3% and 8% polyphosphate solutions lengthened their subsequent shelf life 17 and 25% respectively. Chickens held in continuous contact with these solutions during storage at 2.2°C kept 17 and 67% longer respectively. These authors also investigated the mode of action of polyphosphates as antimicrobial agents and concluded that this was not due to pH changes, but rather by chelation of metal ions essential to the growth of bacteria.

All of the above investigations were carried out on whole chicken carcases, but Foster and Mead,[96] investigated the effect of added polyphosphates on the survival of salmonellae in minced chicken breast and leg muscles. The minced muscles from freshly slaughtered chickens held at 1°C overnight, were treated with 0.35% of a commercial polyphosphate, and inoculated with 5 different salmonella cultures. (S.typhimurium, S. agona, S.cerro, S.haardt, S.livingstone). Samples were stored at -20°C,-5°C,-2°C and +1°C, and the resulting trends observed for all 5 strains of salmonella were similar. Consequently only survival data for S.typhimurium were given in detail; and these results are given in Figure III. In the absence of polyphosphates, survival of the test organisms was greater in breast than in leg muscle at all 4 temperatures. Polyphosphates increased the death rate in breast at -2°C and - 20°C, but had little or no effect on leg muscle at any temperature, Type of substrate and pH were found to influence the survival of salmonellae at -2°C, which was very much greater in hydrogen iodide broth at pH 5.8 and 6.4, than in breast muscle at its normal pH of 5.8 and leg muscle at its normal pH of 6.4. Addition of

hydrochloric acid to reduce the pH value of leg to 5.8 increased survival, but raising that of breast to 6.4 by the addition of sodium hydroxide had little effect. Addition of polyphosphates increased the pH of both muscles by 0.4 units, but the difference between treated breast and leg muscles could not be explained solely in terms of pH.

FIGURE III EFFECT OF TEMPERATURE & ADDED POLYPHOSHATE ON THE SURVIVAL OF SALMONELLA TYPHIMURIUM (about 10^6 organisms per gram) IN CHICKEN BREAST & LEG MUSCLE (from Foster & Mead 96)



1.6 MODE OF ACTION OF POLYPHOSPHATES

There is no one hypothesis which fully explains the effects of polyphosphate salts on meat. The action of polyphosphates on water retention in raw meat has been investigated extensively, and the theories put forward to explain this effect have been extended to include cooked meat products, and even to explain the antioxidant properties of these salts. In fact, the antioxidant properties of polyphosphates are not well understood, but in order to study this effect it is necessary to summarise the theories concerning water retention and cooking losses.

1.61 ACTION ON WATER RETENTION

1.611 pH and Ionic Strength

Increases in ionic strength and pH cause the water holding capacity of meat to increase. Generally, this effect is noticed when the pH is greater than the iso-electric point (about pH 5.0) of the major muscle protein, actomyosin. At this pH the WHC is at a minimum due to maximum attraction between proteins, but on increasing the pH and salt concentration this attraction is decreased allowing entry of water. A full account of these effects appear in the review by Hamm.[22]

This review[22] also summarises the current understanding of the state of water in muscles. About 5% of the total water is tightly bound to the myofibrillar protein molecules as hydration water and is hardly influenced by changes in the structure of the proteins during rigor, cooking etc., and does not freeze at normal freezer temperatures. This fraction is usually referred to as "bound" water. The remaining 95% of water is referred to as "free" water, although it is more or less immobilised within the system of myofibrillar proteins. Part of this water may be squeezed from muscle by low pressure, while some is strongly immobilised and can only be expressed with difficulty, but there seems to be a continuous transition between these 2 states. Therefore, calculation of the WHC of a muscle system depends on the

method of measurement of this "free" water. It is this "free" water which is strongly influenced by changes in the spatial molecular structure of the muscle filaments, brought about by changes in pH and ionic strength.

Alkaline polyphosphate salts cause increases in both pH and ionic strength of treated muscles, and therefore, on this basis alone would be expected to increase WHC. However, experimental results[97] show that diphosphate, and to a smaller extent, tripolyphosphate, have a specific effect on WHC, which cannot be explained solely in terms of these two factors, since compared to the longer-chain phosphates, they have neither a high pH nor ionic strength. Also, in practical terms the addition of the recommended amount of polyphosphates to meat (about 0.3% as P_2O_5) is not likely to cause a significant increase in pH.

However, it is worth mentioning the detailed work of Sherman,[98] who investigated the effects of sodium chloride and polyphosphates (tetrasodium diphosphate and a commercial mixture) on the fluid retention of fresh pork at $0^{\circ}C$ and $100^{\circ}C$. He found that fluid retention at both temperatures depended on the solution-meat ratio, and on the method used for its determination. These facts may, in part, account for the apparent contradictions in the literature reviewed by Iles,[9] and in section 1.33. Fluid retention at both temperatures improved with increasing concentrations of salts. At $0^{\circ}C$, fluid retention of the phosphate treated meat after ageing was significantly correlated with the pH of the aged mixtures, which must be above 6.25 in order to improve fluid retention. No such relationship was found for retention of fluid from sodium chloride solutions, where the pH of the aged meat-solutions depended on the original pH of the meat. Fluid retention at $100^{\circ}C$ did not correlate with pH for either salt - or phosphate-meat solutions.

Sherman studied the actual absorption of ions by meat proteins and concluded that the ionic strength of the additive solution was important only in so far as it controlled the rate of ion absorption by proteins. The greater the ionic strength the greater the absorption of ions. This theory was extended by later studies,[99] when it was found that at 0°C the influence of alkaline polyphosphates on water retention in fresh pork was related to ionic absorption. Cations were preferentially absorbed, but the effect was very pH dependent with the difference between cation and anion absorption decreasing with increasing pH. Thus, phosphate ion absorption must be operative in some way. However, at 100°C the primary factors governing fluid retention were protein changes related to the influence of heat and the amount of actomyosin solubilised (see section 1.613) at 0°C. Water retention from sodium chloride solutions at both temperatures was linearly related to the concentrations of ions absorbed, with cations and anions being absorbed to approximately the same extent.

Hamm, 1955,1960, and Hellendoorn, 1962 (cited by Iles,[9]) and Bendall,[97] all found that when comparison of various phosphate salts were made at a controlled pH, then diphosphate and tripolyphosphate were more effective in increasing water retention than the others.

The results presented above point to the fact that ions are actually absorbed by meat proteins, and that the effect of added phosphate salts may not be explained solely in terms of ionic strength and pH.

1.612 Chelation of Metal Ions

As we have discussed in the previous section, an increase in ionic strength or pH of meat causes the attraction between proteins to decrease, so allowing water to enter. A similar effect could be envisaged if the calcium and magnesium which bridge muscle proteins

were to be removed. However, if this were true, then removal of these metal ions by any means should result in increased water retention. Hamm and co-workers, 1955-60, 1970-71 (cited by Iles[9]), considered that EDTA and oxalate, both calcium-complexing agents, did increase muscle hydration. However, Sherman[98] found that EDTA had little effect on water retention in pork. Perhaps, as pointed out by Hamm,[100] the large EDTA molecule is prevented from approaching protein-bound metal ions because of steric reasons.

It has also been demonstrated that 60% of the calcium and 20% of the magnesium is firmly bound to muscle proteins and is therefore thought to be unavailable for reaction with added chelating agents.[101] This was demonstrated by dialysis and extraction-centrifuge procedures which were used to determine the amounts of free and bound metals. Only metals not bound to proteins would pass through the dialysis tubing or into the supernatent during centrifuging. Addition of 0.4% polyphosphates (ortho-,di-,tripoly- and glassy phosphates) did not alter the amounts of calcium or magnesium remaining in the meat (L-dorsi beef muscle) after dialysis of both the raw meat and that after heating to 70°C, compared to sodium chloride/meat standards (2.0% sodium chloride). Similar results were obtained by the centrifuging procedure for raw meat.

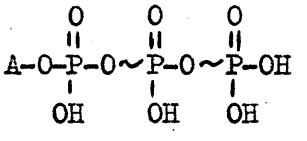
Baldwin and deMan,[102] studied the distribution of calcium and magnesium in muscle (hip of beef) in greater detail than Inklaar[101] by determining a) the total calcium and magnesium in the meat, b) the total in the juice expressed from the meat, c) the total in the ultra-filtrate of the juice i.e. the total soluble calcium and magnesium. The total amounts of protein-bound metals appeared to be similar to those reported by Inklaar. However, the soluble protein-bound calcium increased and the insoluble protein-bound calcium decreased as the chain length of the phosphate additive increased. This suggests that

added phosphates remove calcium from its bound state with insoluble protein. The most effective calcium-complexing salts were sodium tripolyphosphate, sodium tetraphosphate and sodium hexametaphosphate, with sodium diphosphate having some effect and mono- and di-basic sodium phosphate little or no complexing ability. They reported that the magnesium equilibrium was not affected by added phosphates, but their results did show similar trends as for the calcium equilibrium when samples were treated with diphosphate. Thus, tripolyphosphate was found to complex calcium more strongly than magnesium; and diphosphate, magnesium more strongly than calcium; which corresponds to their known relative affinities for these metals (see also section 1.613).

1.613 Analogy with Adenosine Triphosphate

We have seen that pH, ionic strength, and perhaps metal chelation, partly explain the changes in WHC due to added salts. However, it is obvious that certain phosphates exert some specific effect other than these.

Adenosine triphosphate (ATP) is biologically classed as a "high energy compound" because the diphosphate and triphosphate bonds possess a relatively large standard free energy of hydrolysis:



A = Adenosine ~ = high energy bond.

The release of this "stored" energy on hydrolysis of ATP to adenosine diphosphate (ADP) is the key to many biological reactions. The main contractile protein, actomyosin, which is made up of strands of actin and myosin, is thought to gain the energy for dissociation by myosin-activated ATP hydrolysis. The book by Bendall,[103] explores these reactions in great detail. During rigor when ATP can no longer be synthesised, the actin and myosin recombine forcing water out of the myofibrills, which in combination with a drop in pH, leads to a

loss of WHC. Also, uncombined actin and myosin are more soluble than the combined form, thus allowing greater water retention when the proteins are separated. If polyphosphates were to split actomyosin then WHC would increase.

Bendall,[97] was the first to suggest this analogy with ATP, on the basis of the obvious structural similarities between ATP and diphosphate and tripolyphosphate. Yasui et al,[104,105] studied the reactions of added polyphosphates on isolated myosin B (natural actomyosin). They concluded that diphosphate and tripolyphosphate show the specific reaction with myosin B similar to that of ATP, leading to dissociation of actomyosin into myosin A and actin. This reaction depends on high ionic strength and the presence of divalent cations (calcium and magnesium). They proposed that diphosphate and tripolyphosphate showed marked increases in their affinity for actomyosin in the presence of univalent cations (notably, sodium), and a great improvement in their reactivity with actomyosin through the formation of divalent metal - phosphate complexes. Their results suggested that diphosphate was most reactive when combined with magnesium, and tripolyphosphate most reactive when combined with calcium (see also section 1.612). This scheme fits in very well with Bendalls[97] original proposals i.e. that sodium chloride provides the ionic strength component, and diphosphate and tripolyphosphate some other specific component, for reaction with proteins. Also, this scheme indicates that diphosphate and tripolyphosphate do, in fact, behave similarly to ATP. However, these effects of polyphosphates were determined on isolated actomyosin, and the situation in meat is likely to be more complicated.

They further suggested that it was the structural properties of diphosphate which were responsible for this myosin-phosphate reaction, and that tripolyphosphate was effective only after enzymic hydrolysis to diphosphate. Hydrolysis of polyphosphates occurs rapidly

in meat and will be discussed in section 1.64.

Many workers have presented evidence for the strong affinity of diphosphate with myosin (see the reviews by Iles,[9] and Hamm,[100]) but it is by no means certain whether the resulting complex actually leads to dissociation of actomyosin. Chemical tests on the free fluid from meat-salt systems showed that treatment with diphosphate resulted in a greater amount of solubilised actomyosin than treatment with sodium chloride.[98] Also, Bendall,[97] found that the free fluid from muscle treated with diphosphate plus sodium chloride was very viscous and contained actomyosin, when the total ionic strengths of the additives was above 0.40, but when the high ionic strength was due to sodium chloride alone then no such dissolution of actomyosin was found. These tests were not able to show whether dissociation of actomyosin had occurred. However, electron microscopy studies of meat-salt systems showed that polyphosphates are unlikely to actually split actomyosin, although some protein-phosphate reaction had undoubtedly taken place.[106]

1.62 ACTION ON COOKING LOSS

Generally, treated meat loses less fluid on cooking than meat that is not treated. Since phosphates are usually added to raw meat, treatment must result in a change in some property of the raw meat which is extended to the cooked product.

It has been suggested that the actin and myosin which may be solubilised by phosphates, forms a gel on heating, which is capable of retaining water within the meat,[98,99] Alternatively, the dissolved proteins may form a fine, fibrous network on heating, which constitutes a barrier to fluid loss.[4] Shermans work[98,99] on the influence of ion absorption on WHC, showed that at 100°C the fluid retention in lean pork treated with phosphates appeared to be related to the concentration of actomyosin which was dissolved at 0°C during ageing of the solution/

phosphates used. Moreover, when the unretained fluid at 0°C was removed by centrifugation before heating to 100°C, the water content of the heated meat was reduced by 0.1g for every 1mg of soluble nitrogen removed. This relationship was true for both sodium chloride and polyphosphate solutions, and compared to results obtained without salt addition, suggested that these salts reduce the loss of those meat proteins responsible for retention of water at 100°C. There was little difference in the total protein nitrogen in the aqueous extracts from pork treated with either sodium chloride or polyphosphates at concentrations of 0.5 and 1.0%. The amount of actomyosin nitrogen however was greatest in polyphosphate treated extracts.

1.63 ACTION ON AUTOOXIDATIVE RANCIDITY

It has been suggested that polyphosphates act as antioxidants either because they chelate metal ions which are catalysts of oxidation, or the "coagulated" protein as well as preventing fluid loss, also prevents the entry of oxygen.[9] However, the discussion above points to the fact that both of these effects are subject to doubt, and hence no satisfactory explanation of the antioxidant action of polyphosphates has been proposed.

1.64 HYDROLYSIS OF POLYPHOSPHATES

Polyphosphates are readily hydrolysed in solution, usually by an acid catalysed reaction.[107,108,109] In meat products the hydrolysis of added polyphosphates is brought about by muscle phosphatases, and this is a very rapid process.

O'Neill and Richards,[110] used phosphorus-31 nuclear magnetic resonance to detect polyphosphate species in treated chicken muscle. They demonstrated that the hydrolysis of polyphosphates is effectively stopped by addition of EDTA; and that during the thawing process hydrolysis proceeds very rapidly. Jozefowicz et al[111] reported that

very little hydrolysis of polyphosphates occurred in chicken muscle during 6 months storage at -18°C. Presumably, these latter workers halted the hydrolysis by addition of EDTA to the frozen samples, since preliminary work reported by O'Neill and Richards,[110] showed that there were no added polyphosphates in the muscles of commercially treated chickens which had been allowed to thaw.

No other accounts of polyphosphate hydrolysis in chicken muscle have been reported. However, several accounts of polyphosphate hydrolysis in other meats have been published, and these are summarised below.

Mihalyi-Kengyel and Kormendy,[112] found that 40 to 50% of tripolyphosphate hydrolysed immediately after addition to minced pork muscle. During storage at 4°C, the hydrolysis of diphosphate was complete within 3 days, and of tripolyphosphate within 4 days. This rapid hydrolysis has also been reported in fish.[113]

The hydrolysis is thought to be a step-wise reaction:

$$\text{tripolyphosphate} \xrightarrow{k_1} \text{diphosphate} \xrightarrow{k_2} \text{orthophosphate}$$

Sutton,[113] found that k_2 was very much less than k_1 in beef muscle, but that all of the diphosphate had disappeared within 24 hours at 25°C. The hydrolysis rate decreased with temperature, and was dependent on the protein concentration (an approximate measure of the enzyme concentration). The rate of hydrolysis in cured, non-comminuted pork was lower than in the uncured, minced muscle, and it was suggested that non-hydrolysis breakdown reactions occur for diphosphate.[112]

Yasui et al,[104] proposed that diphosphate and tripolyphosphate reacted with salt-free myosin B, especially in the presence of high salt concentrations and divalent cations (calcium and magnesium). Muscle is known to contain sufficient amounts of these ions for reaction to take place. Recently Neraal & Hamm [114,115,116] have published a series of papers on the occurrence of diphosphatases and tripolyphosphatases in beef muscle, and the rate of hydrolysis of added diphosphate and tripolyphosphate.

they found that magnesium ions were primarily responsible for the diphosphatase activity, and calcium ions for the tripolyphosphatase activity. They studied the hydrolysis of diphosphate and tripolyphosphate in beef muscle and found that 0.5% added diphosphate was broken down within 2 to 12 hours,[116] whereas 0.5% tripolyphosphate in the same muscle took only 9 to 19 minutes.[115] Van Hoof,[117] mentioned earlier work by Neraal and Hamm in which they found that diphosphatase activity was higher in pre-rigor beef muscle (pH optimum 7.0) than in post-rigor muscle, whereas, tripolyphosphatase activity increased during the first 2 or 3 days post-mortem (pH optimum 5.7). He confirmed their findings that tripolyphosphatase was more sensitive to heat denaturation than diphosphatase, by his results obtained with pork muscle. Heating to an internal temperature of 72°C did not inhibit further hydrolysis of diphosphate during storage of pork muscle at 4°C, whereas it completely inhibited the further hydrolysis of residual tripolyphosphate under the same conditions. Tripolyphosphatase activity was again found to be greater than diphosphatase activity, and increased with the time post-mortem. In fact, addition of 0.3% tripolyphosphate (expressed as P_2O_5) at 1 hour post-mortem resulted in complete hydrolysis within 5 hours, whereas addition at 48 hours post-mortem resulted in complete hydrolysis in only 30 minutes. Diphosphate added 1 hour post-mortem was 50% hydrolysed in 5 minutes, and completely hydrolysed within 5 to 24 hours, whereas complete hydrolysis occurred within 9 to 72 hours after addition at 48 hours post-mortem. Hydrolysis of both diphosphate and tripolyphosphate was higher in meat of normal quality (pH 6.0), than in PSE meat quality (pH 5.5).

Awad,[118] also found that appreciable hydrolysis of polyphosphates took place immediately after addition to meat and continued with time. Also, when the meat was heated to destroy phosphatases before treatment, some initial hydrolysis still took place, which was independent of the amount of polyphosphate added and did not increase

with time. Thus some factors other than enzymic hydrolysis, such as pH and ionic environment, must also bring about polyphosphate hydrolysis.

The effects of added polyphosphates are apparent even after prolonged storage and cooking (see sections 1.33 and 1.43), when the polyphosphates have undoubtedly been hydrolysed to orthophosphate which has little or no effect.[8,85] Therefore the changes in treated meat which bring about these effects on moisture losses and oxidative rancidity, must depend on some irreversible reaction which takes place soon after treatment.

2. METHODS

2.1 STATISTICAL ANALYSES

Statistically significant differences between polyphosphate treated and untreated samples, were determined, according to the value of the Student's t Test statistic:

$$t = \frac{\sqrt{n}\left(\bar{x}_u - \bar{x}_p\right)}{\sqrt{s_u^2 + s_p^2}}$$ where n is the number of polyphosphate treated samples (which equalled the number of untreated samples); $\bar{x}_u$ and $\bar{x}_p$ are the mean values of untreated and polyphosphate treated samples, respectively; and s_u^2 and s_p^2 are the variances ($s^2 = \frac{\sum(x - \bar{x})^2}{n - 1}$) of the results for untreated and treated samples, respectively. Standard deviations (s) were calculated from $\sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$. The calculated value of t was checked for significance at 95% or higher probability level, from statistical tables, using the two-tail criterion, and 2n - 2 degrees of freedom.

Correlation coefficients (r) between 2 sets of results obtained from the same sample were calculated as follows:

$$r = \frac{n\sum x_1 x_2 - (\sum x_1)(\sum x_2)}{\sqrt{n\sum x_1^2 - (\sum x_1)^2)\ (n\sum x_2^2 - (\sum x_2)^2)}}$$ where n is the number of samples and x_1 and x_2 are the results of the two experiments. Statistical significance at 95% or higher probability level was determined for n - 2 degrees of freedom from statistical tables.

For both of these tests, when a result was statistically significant at the 90% probability level, this was taken as statistically invalid for sample sizes of 3 or 6. In these cases, the results were said to follow a trend, or to approach significance.

2.2 CHICKEN SAMPLES

Batches of 12 polyphosphate treated and 12 untreated frozen broiler chickens were obtained direct from the factory, and transported to the laboratory packed in solid carbon dioxide. They were stored in a

freezer at - 18°C or -20°C until required for analysis.

Batch A were obtained from Eastwoods, Newark. Factory trials were supervised by Dr. R.C. Osner of Sheffield City Polytechnic. (SCP). The carcases were treated after evisceration and before spin-chilling with a 4% solution of Puron 604 by injection into both sides of the breast muscle using an automatic injection device. The level of injection varied between 7.9% and 12.4% of the eviscerated carcase weight. All of the chickens were weighed after spin-chilling and drip to calculate the uptake of chill water.

Batch B were obtained from J.P. Wood & Sons, Craven Arms, by arrangement with Albright and Wilson Ltd., Oldbury (A & W). Factory trials were supervised by Mr. J. Bennett (A & W), Dr. Osner (SCP) and the author. The carcases were treated after evisceration and before spin-chilling with a 5% solution of Puron 604 (supplied by A & W), by injection into both sides of the breast muscle using an automatic injection device. The level of injection varied between 4.2% and 6.9%.

Batch C were obtained as for Batch B, with Mr. R. Krakowicz (A & W) and the author supervising factory trials. The carcases were treated after evisceration and before spin-chilling with a 5% solution of Puron 604 injected by hand using a syringe, into both sides of the breast muscle. The level of injection varied between 4.8% and 5.6%. The uptake of chill water was calculated for both treated and untreated chickens.

2.3 THIOBARBITURIC ACID TEST TO ASSESS THE DEGREE OF RANCIDITY

2.31 CHOICE OF METHOD

Generally, the methods of determining the degree of rancidity may be divided into those that measure some property related to protein breakdown, and those that are related to fat spoilage. In this work, the extent of fat spoilage after frozen storage and during holding was to be determined, and the results of polyphosphate-treated and untreated

samples compared.

A review of the possible methods for assessing the spoilage of meat has been published by Pearson.[119] The thiobarbituric acid test (TBA test) was considered to be the best method of assessing the extent of fat spoilage in this research because it measures oxidation in the whole meat sample, and not just the extractable fat, as do the other methods related to fat spoilage (e.g. peroxide test). The test depends upon the reaction of 2-thiobarbituric acid with a 3- carbon product of autooxidation thought to be malonaldehyde, to give a red pigment which is determined spectroscopically. Malonaldehyde was originally thought to be an end-product of oxidation, but this is now known to be untrue. In fact, Tarladgis and Watts,[120] found that during the controlled oxidation of pure, unsaturated fatty acids, malonaldehyde production closely followed oxygen uptake, and reached a peak at the same time as oxygen uptake started to decline. Oxygen availability seemed to be a limiting factor for the destruction of malonaldehyde as well as for the oxidation of the fatty acids. However, this would only seem to limit the reliability of this test for very rancid samples, and for samples during the very early stages of autooxidation when there is not a sufficient quantity of malonaldehyde for reaction.

The malonaldehyde is distilled from an acidic meat slurry and the distillate heated with an acidic TBA solution to develop the red colour. Acid/heat treatment was thought to be necessary to liberate malonaldehyde from the test sample and for the condensation of malonaldehyde with TBA.[88] However, Tarladgis et al,[121] found that the TBA reagent itself broke down during acid/heat treatment to yield at least one compound absorbing at the same wave length as the TBA/malonaldehyde complex, and a method omitting acid/heat treatment was developed.[122] However, providing that a TBA/acid blank solution is prepared along with the meat distillate/TBA solution, any such breakdown products should not interfere with quantitative results. The

test may be quantified by using 1, 1, 3, 3, tetraethoxypropane (TEP) which hydolyses on acid/heat treatment to yield malonaldehyde, and the results are expressed as TBA Numbers, which are a measure of the mg of malonaldehyde per 1000g sample. TBA Numbers have been correlated with odour ratings,[40,86,88] and with levels of fatty acids in various lipid fractions of chicken pectoralis major muscles[58] (see section 3.25).

The intensity of the red colour is very dependent on the actual test conditions e.g. pH of the meat slurry, time of distillation and the quantity of distillate collected.[88] Various different methods have been proposed,[87,88,122,123] but the one of Tarladgis et al[88] has been the most widely used and was chosen for this research. It has been favourably compared to other meat spoilage tests by Pearson.[124]

2.32 APPARATUS AND REAGENTS

The apparatus is shown in Figure 1V. The heating mantle was obtained from Electrothermal. The time taken to collect 50 cm^3 distillate did not vary by more than 2 minutes between the 6 heating mantles when the controls were at maximum. The distillation glass ware was standard quick-fit.

A 100cm^3 capacity homogenisor was used (M.S.E.), andoptical densities were measured using a Unicam SP 500 spectrophotometer.

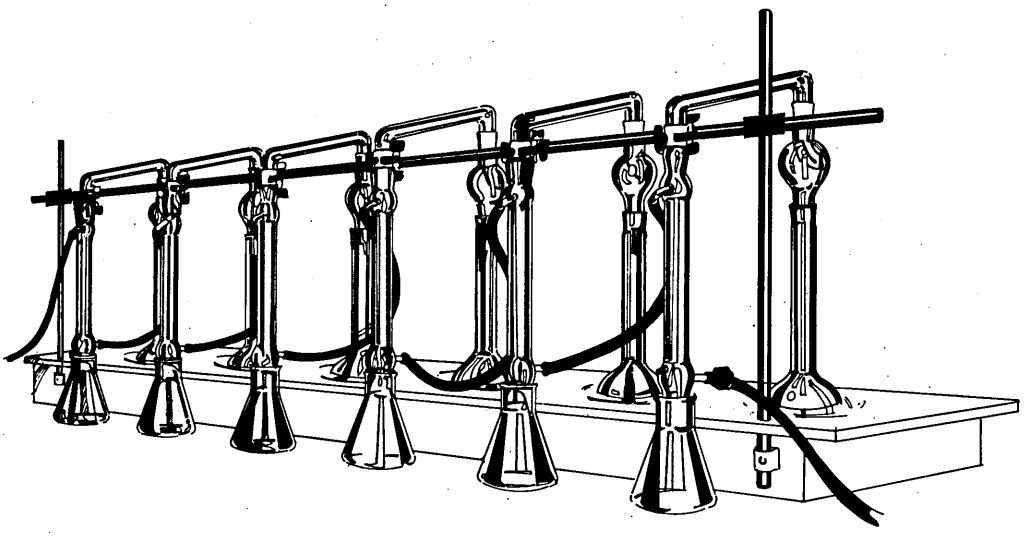
The stock solution of 1,1,3,3, tetraethoxypropane (TEP) (BDH Chemicals Ltd.) was 1×10^{-3} M in distilled water; and the solution of thiobarbituric acid (TBA) (BDH Chemicals Ltd.) was 0.02 M in 90% glacial acetic acid. Antifoam B was obtained from Sigma Chemical Co. Ltd.

2.33 PROCEDURE

Ten grams of muscle were homogenised with 50cm^3 of distilled water. The mixture was quantitatively transferred to a distillation flask with 47.5cm^3 of distilled water, and 2.5cm^3 of hydrochloric acid (4 mol. dm^{-3}) added to bring the pH to 1.5. A few drops of Antifoam B

FIGURE IV

DISTILLATION APPARATUS USED IN THE TBA TEST



were put onto the neck of the flask, and glass beads added to prevent bumping. The distillation was continued until $50cm^3$ of distillate were collected. Five cm^3 of distillate were pipetted into a $50cm^3$ glass boiling tube, and $5cm^3$ of TBA reagent added. The tube was stoppered and the contents mixed and immersed in a boiling water bath for exactly 35min. A distilled water/TBA reagent blank was prepared and heated along with the sample tubes.

After heating, the tubes were cooled in tap water for exactly 10min, and then the optical density (O.D.) of the solutions was determined against the blank at 532nm using 10mm cuvettes. Sometimes only 5g of muscle were tested, and the results were treated accordingly.

2.34 CALIBRATION

A malonaldehyde standard curve was constructed by making appropriate dilutions of the stock TEP solution and determining the red TBA/malonaldehyde complex by the method described for testing the meat distillate. The results are plotted in Figure V, and show that Beers Law was obeyed over the entire range of test concentrations.

The percentage recovery of malonaldehyde was determined by following the distillation procedure for various standard solutions and comparing the results of these distillations (Figure VI) with those of the standard curve (Figure V) as follows:

$$\% \text{ recovery} = \frac{\text{O.D. distillate}}{\text{O.D. original solution}} \times 100$$

But, $50cm^3$ distillate collected from $100cm^3$ solution, therefore, the distillate value must be divided by 2:

$$= \frac{\text{O.D. distillate} \div 2}{\text{O.D. original solution}} \times 100$$

$$= \frac{\text{slope Figure VI} \div 2}{\text{slope Figure V}} \times 100$$

$$= \frac{(1.98 \times 10^7) \div 2}{1.38 \times 10^7} \times 100$$

$$\% \text{ recovery} = 71.6\%$$

STANDARD CURVE FOR TBA TEST

FIGURE V

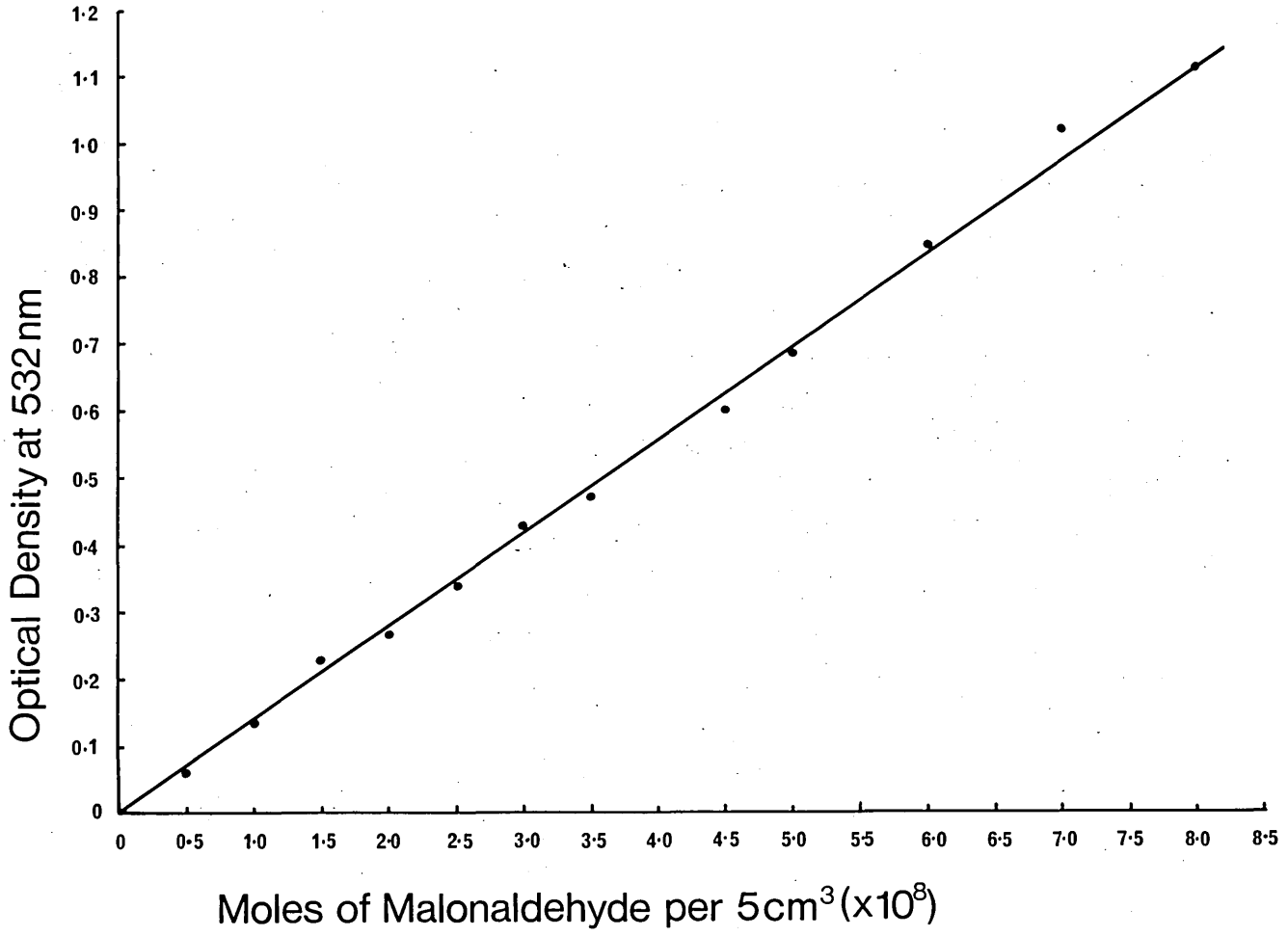
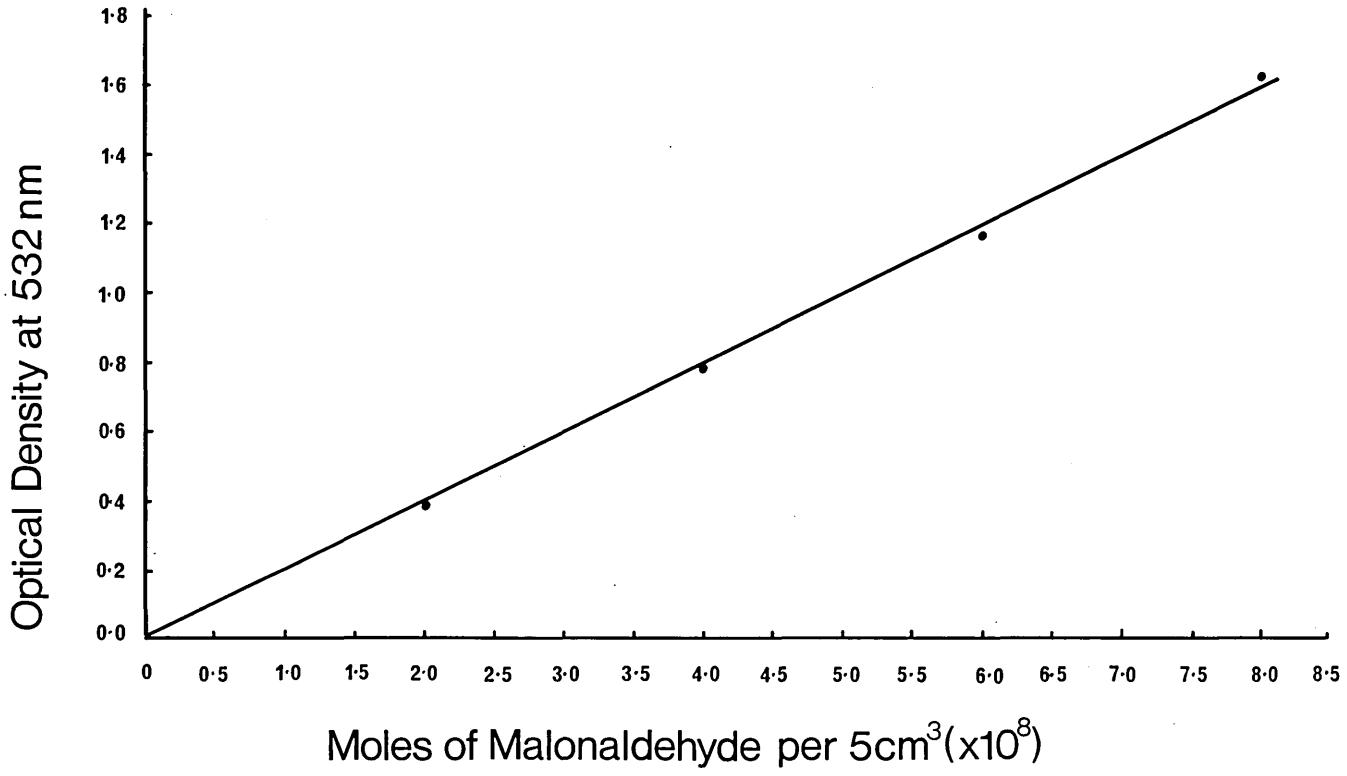


FIGURE VI

GRAPH TO CALCULATE THE PERCENTAGE RECOVERY OF MALONALDEHYDE IN THE TBA TEST



The TBA Number (mg malonaldehyde per 1000g sample) is calculated by multiplying the O.D. of the test solution by a constant, K; which is calculated from the standard curves as follows:

$$K = \frac{\text{concentration in moles per } 5cm^3 \text{ solution}}{\text{O.D.}} \times \text{Molecular Weight of malonaldehyde} \times \frac{10^7}{\text{Weight of sample}} \times \frac{100}{\% \text{ recovery}}$$

$$= \frac{1}{\text{slope Figure V}} \times 72 \times \frac{10^7}{10g} \times \frac{100}{71.6}$$

$$\underline{K = 7.2}$$

2.4 LIPID ANALYSES

2.41 CHOICE OF METHODS

The following general scheme was adopted:

EXTRACTION OF TOTAL LIPID
↓
SEPARATION INTO NEUTRAL LIPID
AND PHOSPHOLIPID USING COLUMN CHROMATOGRAPHY
↓
IDENTIFICATION OF LIPID CLASSES
BY THIN LAYER CHROMATOGRAPHIC(TLC)
PROCEDURES
↓
METHYLATION OF THE FATTY
ACIDS OF EACH LIPID CLASS
↓
DETERMINATION OF THE FATTY
ACID COMPOSITION OF EACH LIPID
CLASS BY GAS LIQUID CHROMATOGRAPHY(GLC)

There are a wide variety of methods and techniques to choose from in the field of lipid analysis, because of recent advances, especially in the field of chromatography and computer-assisted techniques (see for example Perkins et al.[125]). The book by Kates,[126] on the laboratory techniques of lipidology, provided much of the general information required for the successful handling of lipid materials. The methods were chosen after a study of the methods in general use in the scientific literature, and after examining the original research papers. All other things being equal, the criteria governing the choice of method

were, speed, availibility of apparatus and reagents, and previous experience of the techniques involved.

2.42 EXTRACTION OF TOTAL LIPID

2.421 Moisture Determination

The moisture content of muscles was determined in order to calculate the solvent to water ratio in the lipid extraction procedure, by drying duplicate samples to constant weight in a vacuum oven at 95°C and 100 mm mercury pressure.

2.422 Procedure

The procedure of Bligh and Dyer,[127] was employed. Samples of wet muscle between 15g and 100g were taken and homogenised, in either a Waring blender or Ato mix, with chloroform and methanol for 2 min. The quantities of solvent used were such that the proportions of chloroform: methanol: water were 1:2:0.8. A further volume of chloroform was added and the mixture homogenised for 30 sec, before adding an equal volume of distilled water and blending for 30 sec. The proportion of solvents to water was now 2:2:1.8. The homogenate was then filtered through a Whatman No.1 filter paper on a Coors No. 3 Buchner funnel with slight suction applied, and the filtrate transferred to a measuring cylinder. The blender jar was rinsed with 1 volume of chloroform which was filtered as before and the filtrate added to that in the measuring cylinder.

The proportions of solvents were calculated to ensure complete phase separation, and any cloudiness at this stage always disappeared on standing, to leave a slightly turbid upper aqueous layer, and a clear yellow chloroform layer which contained the total lipid.

The volume of the chloroform layer was recorded and the aqueous layer carefully removed, along with the first few cm^3 of the chloroform layer in order to avoid contamination of the lipid extract with non-lipid material. An aliquot of the chloroform extract was taken and evaporated to dryness under a stream of nitrogen on a water bath at 50°C, and the

residue dried over phosphorus pentoxide in a vacuum desiccator.

The percentage total lipid in the sample was calculated as follows:

$$\% \text{ total lipid} = \frac{\text{Weight lipid in aliquot} \times \text{Volume chloroform layer}}{\text{Volume aliquot}} \times \frac{100}{\text{sample weight}}$$

The remaining chloroform extract was dried over anhydrous sodium sulphate, filtered, and evaporated as before to a suitable volume which was then stored over nitrogen at $-18^{\circ}C$ until required for further analysis.

It was not possible to extract the total lipid from cook-out juices by this method because of the small sample size (about $5cm^3$), and uncertain water content. In fact, addition of chloroform and methanol resulted in emulsions which were very difficult to separate. Instead, the juices were shaken 3 times with small amounts of chloroform, sometimes with added anhydrous sodium sulphate to inhibit emulsification. The extracts were filtered through anhydrous sodium sulphate and evaporated on a rotary evaporator to a suitable volume ready for transmethylation (section 2.442).

2.43 SEPARATION OF TOTAL LIPID INTO NEUTRAL LIPID AND PHOSPHOLIPID BY COLUMN CHROMATOGRAPHY

2.431 Preparation of Adsorbent

The procedure of Carroll,[128] was followed. Thirty grams of 60 to 100 mesh Florisil (Fisons) were mixed with $90cm^3$ of concentrated hydrochloric acid and heated on a boiling water bath for 3 hours. The hot supernatant liquid was decanted off and the residue washed with $20cm^3$ of concentrated hydrochloric acid, and then heated overnight with another $90cm^3$ of concentrated hydrochloric acid. The hot supernatant was again decanted off, and the residue washed with water, first by decantation and then on a Buchner funnel, until the washings were neutral to pH paper. The washed residue was then sucked dry on a Buchner funnel, transferred to a glass dish, and heated for 24 hours in an oven at $120^{\circ}C$. The dry

Florisil was then heated overnight with 90cm^3 of concentrated hydrochloric acid, and the hot supernatant decanted off. The residue was washed with water until neutral as before, and then with 40cm^3 each of methanol, methanol: chloroform (1:1), chloroform, and finally, ether. The residue was allowed to air dry, before activating by heating overnight at 120°C. The acid-washed Florisil was stored in a stoppered flask at room temperature until required.

2.432 Preparation of Column and Elution Procedure

Ten grams of acid-washed Florisil were slurried in 20cm^3 of chloroform and added to a 1 x 30cm glass column, to give a column height of 25cm. About 100mg of total lipid in 5cm^3 of chloroform were added to the top of the column by a Pasteur pipette.

The lipid classes were then eluted according to the scheme of Carroll,[128] by the sequential addition of 75cm^3 of each of the following solvents: chloroform, chloroform: methanol (95:5), chloroform: methanol (90:10), chloroform: methanol (75:25), chloroform: methanol (50:50), methanol. However, no lipid was detected (by TLC, see section 2.443) in fractions 2 and 3, and when both fractions were combined and evaporated to dryness, only about 2mg of material were collected. According to Carroll, these 2 fractions contain small amounts of lipid tentatively identified as phosphatidic acids and polyglycerophosphatides, and also possibly some neutral lipid.

Since a clean separation of neutral lipids and phospholipids was required with no further separation of the 2 classes, a modified elution pattern was employed using 75cm^3 each of chloroform, chloroform: methanol (90:10), and methanol. It was shown by TLC that all of the neutral lipid was contained in the first fraction, and only phospholipids in the third fraction. Sometimes, a small amount of lipid was present after evaporation of the second fraction but this was always discarded since it could not be positively assigned to either lipid class by TLC.

However, recoveries of material were usually greater than 90% when calculated in terms of the weight of lipid eluted in fractions 1 and 2 and that put on the column. Figure V11 shows a typical elution pattern.

The neutral lipid and phospholipid fractions were evaporated to dryness on a rotary evaporator and dried to constant weight in a vacuum desiccator containing anhydrous phosphorus pentoxide. The weight percentages of neutral lipid and phospholipid were calculated, and the dried lipids were dissolved in chloroform and stored over nitrogen at -18°C until required.

2.433 Identification of Lipids by Thin Layer Chromatography

Preliminary investigations into this technique involved choosing the most suitable types of TLC plates, solvent systems, and detection techniques. Commercially prepared pre-scored glass plates coated with silica gel G were used (Anachem). Most identification work was performed on plates measuring 2.5 cm x 10 cm. The solvent system used for neutral lipids was, petroleum ether : ethyl ether : acetic acid (90 : 10 : 1);[129] and for phospholipids, chloroform : methanol : acetic acid : water (25 : 15 : 4 : 2).[130] The neutral lipids were detected by putting the dry developed plates into a beaker containing a few iodine crystals, when the lipids appeared as yellow-brown spots after a few minutes; and the phospholipids by spraying with molybdenum blue solution[131] which caused blue spots to appear almost immediately.

The procedure followed was to use drawn-out melting point tubes to spot the solutions of lipid material onto the plates which were allowed to dry and then put into a beaker lined with filter paper and containing about 5 mm of solvent. The plates were taken out when the solvent was within 1 cm of the top of the plate, and allowed to dry in air, when the lipid components were detected by either iodine or molybdenum blue.

Pure lipid standards (Sigma Chemical Co.Ltd.) were run alongside the samples on the TLC plates in order to identify the lipid

FIGURE VII ELUTION PATTERN FOR SEPARATION OF TOTAL LIPID BY FLORISIL COLUMN CHROMATOGRAPHY

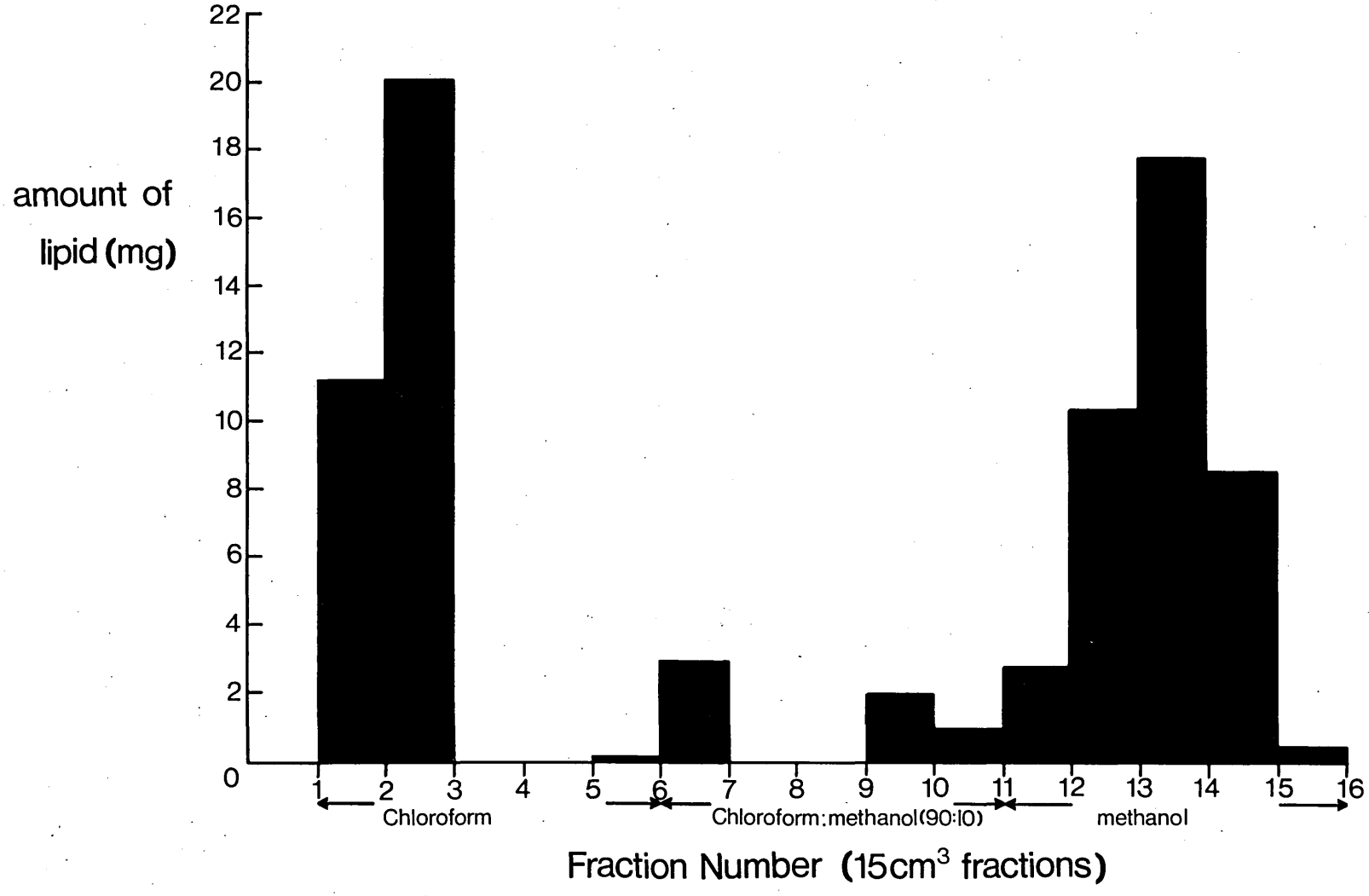
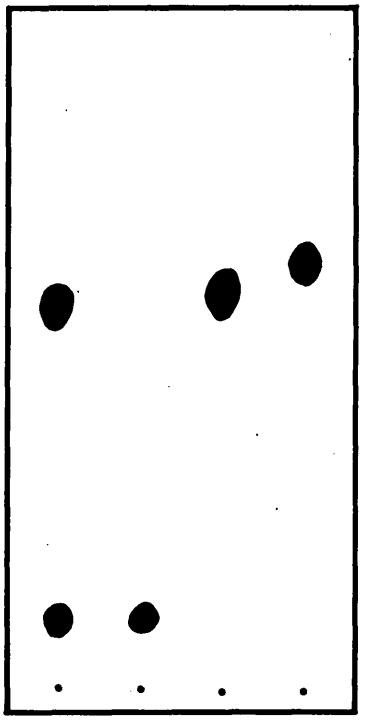


FIGURE VIII

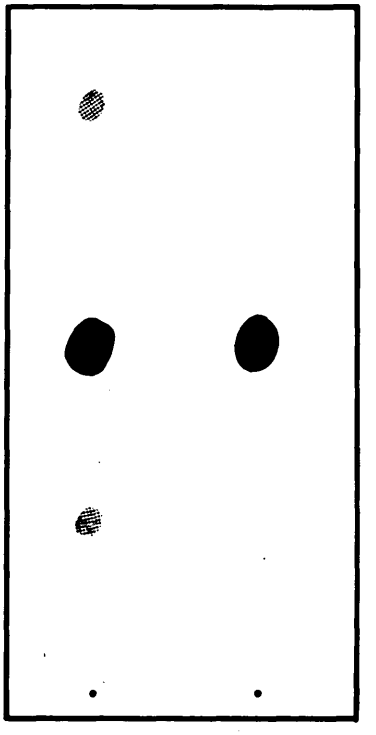
IDENTIFICATION OF LIPIDS BY TLC



1 2 3 4

Neutral Lipid System

1 Chicken neutral lipid
2 Cholesterol
3 Glycerol tripalmitate
4 Stearic acid



1 2

Phospholipid System

1 Chicken phospholipid
2 95% egg lecithin
(phosphatidyl choline)

components. Figure VIII shows some typical results.

2.44 METHYLATION OF LIPIDS

2.441 Choice of Method

Fatty acids are too polar to be easily separated by GLC procedures, and it is necessary to prepare their methyl esters which may be separated successfully by GLC. A modification of the transmethylation procedure of Peisker,[132] which was partially developed for this research by Mrs. Thomas,[133] was used. This method was chosen because it does not require the use of dangerous materials (as do some others) and it is the least time-consuming of all methods.

2.442 Procedure

About 30 mg lipid and 1 cm^3 methylating reagent (chloroform : methanol : concentrated sulphuric acid 100 : 100 : 1) were added to the reaction tube and the apparatus assembled as in Figure 1X and inserted into an aluminium heating block. After reaction at 190°C for 40 minutes, the device was cooled under running water before carefully releasing the pressure. A small piece of zinc was added to the reaction mixture to neutralise any unreacted acid, and the excess solvent evaporated off under a stream of nitrogen on a warm water bath. The product was washed with 1cm^3 distilled water and extracted 3 times with petroleum ether. The ether extracts were dried over anhydrous sodium sulphate and the mixture of methyl esters evaporated to a suitable volume ready for direct injection onto the GLC column. If the esters were to be stored before GLC analysis, the petroleum ether was evaporated off and the esters redissolved in chloroform and stored under nitrogen at -18°C.

2.443 Determination of Percentage Methylation

Pure fatty acids underwent complete methylation in 10 min. (as determined by TLC), but chicken lipid required a longer reaction time. The optimum reaction time was determined by analysing chicken lipid mixtures after 20,30 and 40 min. reaction. The fatty acid composition of

METHYLATING DEVICE

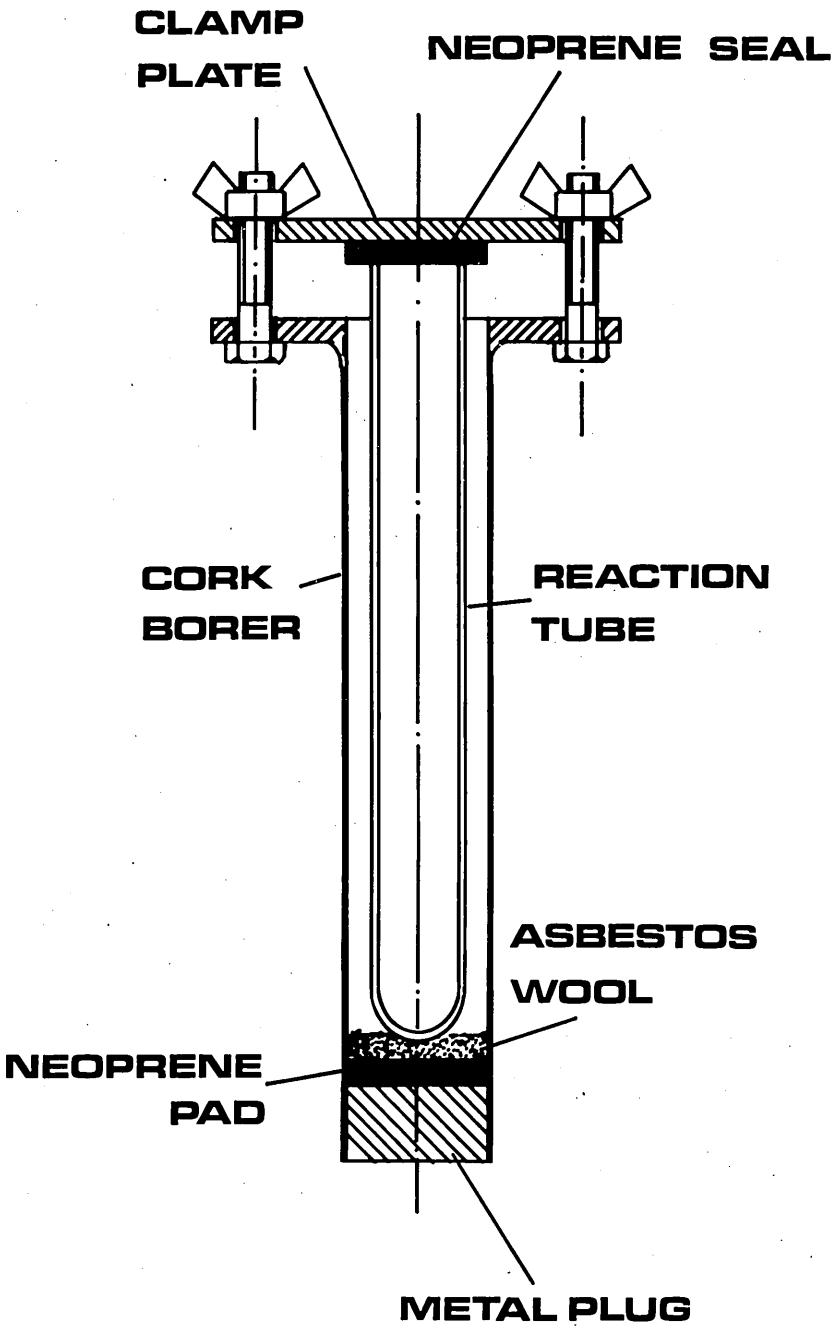


FIGURE IX

the mixtures was quantatively determined by GLC (section 2.4543), and the degree of methylation was 16.8%, 76.4% and 85.8% respectively. TLC results of samples after 45 and 50 min. reaction indicated that methylation did not proceed further, and therefore 40 min. was chosen as the optimum time. The fatty acid composition of the reaction mixtures after 20,30 and 40 min., (Table III) were similar, indicating that incomplete methylation did not occur at the expense of any one acid.

2.45 GAS LIQUID CHROMATOGRAPHIC ANALYSIS OF FATTY ACID METHYL ESTERS

2.451 Nomenclature of Fatty Acids

A convenient manner of referring to a fatty acid is to write its number of carbon atoms plus its number of double bonds e.g. stearic acid has 18 carbon atoms and no double bonds, thus: C18:0 or just 18:0. Well-known fatty acids continue to be called by their "common" name, in preference to their systematic name (according to IUPAC conventions), and these, along with structural formulae and short-hand notations appear in Table IV.

2.452 Operating Conditions

A considerable amount of preliminary work was carried out to establish the most suitable type of column adsorbent and operating conditions for fatty acid analysis. This preliminary work was started by Mrs. Thomas,[133]

A Pye Unicam GCV Gas Liquid Chromatograph was used throughout for all of the results presented here, although earlier work was carried out on a Pye 105 chromatograph.

Diethyleneglycol succinate (DEGS) was chosen as column adsorbent with 60 - 80 mesh chromosorb W as the stationary phase and nitrogen at 50 cm^3 per min. as the carrier gas. Glass columns (5 feet by $\frac{1}{4}$ inch) of 15% DEGS were purchased from Pye Unicam Ltd., Cambridge, as required, according to the column efficiency as determined by the resolution (R), which is a measure of the degree of separation of 2 peaks, according to

TABLE III

EFFECT OF METHYLATION REACTION TIME ON THE FATTY ACID COMPOSITION[a] OF CHICKEN LIPID.

FATTY ACID[b]	REACTION TIME (minutes)		
	20	30	40
C14:0	1.1	1.2	1.2
C16:0	22.6	23.6	23.9
C16:1	9.3	8.7	9.0
C18:0	7.7	5.8	6.2
C18:1	39.2	42.6	43.7
C18:2	14.1	12.4	12.5
C18:3	1.7	2.0	1.9
C20:4	2.2	2.0	0.8
C_B	2.1	2.0	0.8

a. Values expressed as a % of total fatty acids

b. See sections 2.451 and 2.453 for explanation of symbols.

TABLE IV

COMMON NAME, STRUCTURAL FORMULA, AND SHORT-HAND NOTATION OF FATTY ACIDS PRESENT IN CHICKEN LIPID

COMMON NAME	STRUCTURAL FORMULA	SHORT-HAND NOTATION
Myristic acid	$H_3C-(CH_2)_{12}-COOH$	C14:0
Palmitic acid	$H_3C-(CH_2)_{14}-COOH$	C16:0
Palmitoleic acid	$H_3C-(CH_2)_5-CH=CH-(CH_2)_7-COOH$	C16:1
Stearic acid	$H_3C-(CH_2)_{16}-COOH$	C18:0
Oleic acid	$H_3C-(CH_2)_7-CH=CH-(CH_2)_7-COOH$	C18:1
Linoleic acid	$H_3C-(CH_2)_4-(CH=CH-CH_2)_2-(CH_2)_6-COOH$	C18:2
Linolenic acid	$H_3C-(CH_2)_4-(CH=CH-CH_2)_3-(CH_2)_3-COOH$	C18:3
Arachidic acid	$H_3C-(CH_2)_{18}-COOH$	C20:0
Arachidonic acid	$H_3C-(CH_2)_4-(CH=CH-CH_2)_4-(CH_2)_2-COOH$	C20:4

the following equation:

$$R = \frac{2(t_2 - t_1)}{W_1 + W_2}$$, where t_2 and t_1 are the retention times of C18:1 and C18:0, and W_1 and W_2 their baseline widths. A resolution of 1.0 is required for good quantitative results.

The column temperature used was either 180°C, 185°C or 190°C, depending on the age of the particular column. This was because column adsorbent is slowly eluted with time, which decreases retention time and hence R; and decreasing the temperature off-sets this change. When a new column was used and/or the column temperature changed, a calibration mixture was injected onto the column and the fatty acid composition of the mixture determined to see if the change in conditions altered the analysis (see section 2.4543).

A flame ionisation detector (FID) was employed using a hydrogen (at 55cm^3 per min) and air (oxygen 71% and nitrogen 19%) flame at 220°C. Briefly, this type of detector depends on measuring the potential difference caused by ionisation of column eluents in the flame. This current is amplified and the signal recorded. A Philips dual channel recorder was used. The attenuation setting was varied between $2x10^2$ and $64x10^2$, to give peaks of 20 to 60% full-scale deflection.

Samples were injected onto the column using syringes (S.G.E) of either 1μl or 10μl capacity. The temperature of the injection port heater was 190°C.

2.453 Qualitative Analysis

Peaks were identified by comparing their retention time with those of pure fatty acid methyl esters (either obtained from Sigma or prepared from fatty acids by the transmethylation procedure in section 2.442). The retention time of a particular compound is related to its boiling point, with volatile compounds being eluted before compounds of higher boiling point. In these experiments using a DEGS column, the

order of elution of fatty acid methyl esters followed the number of carbon atoms, except for C18:3 which was eluted after C20:0. Where fatty acid standards were not available, the peaks were tentatively identified from a study of the literature. Fatty acid methyl esters of C20:4 and C22:1 standards had the same retention time, but this peak was assigned to C20:4 in the chicken lipids after a study of the literature. The peaks identified only as C_A and C_B are likely to arise from high carbon number (poly)unsaturated fatty acids.

A typical chromatogram of chicken muscle fatty acids is illustrated in Figure X.

2.454 Quantitative Analysis

2.4541 Measurement of Peak Areas

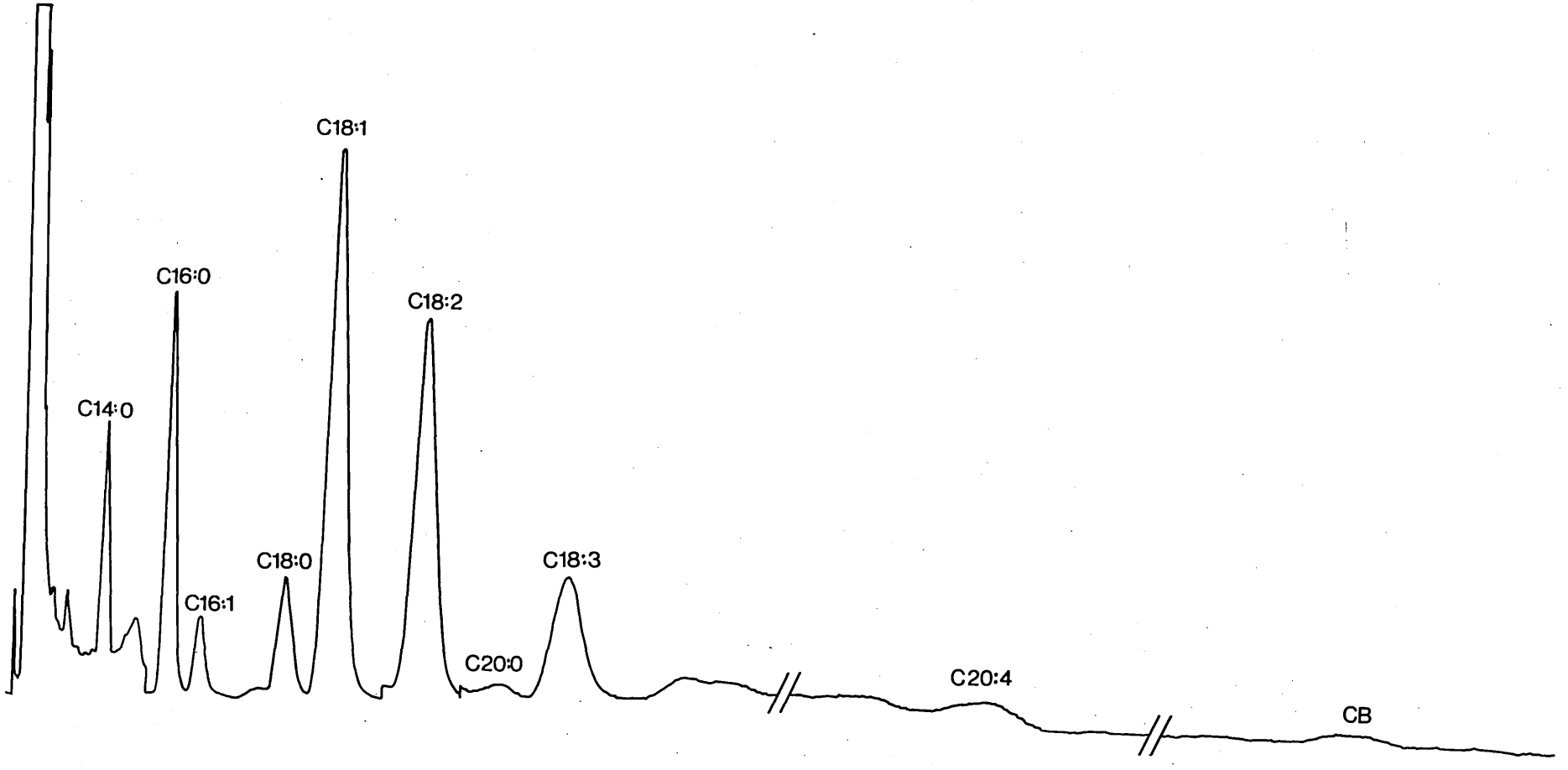
When a flame ionisation detector is employed, the area under any peak is proportional to the amount of compound present. During preliminary investigations, peak areas were measured by determining the product of the peak height and the width of the peak at half height. Reproducible results were obtained, but the method was very time-consuming and involved the measurement of very small distances, especially the peak widths of the earlier sharp peaks. Therefore, the quicker method of determining the product of peak height and retention time was used. An advantage of this method is that it involved only 2 measurements of relatively large distances. Theoretical justification of this method of measuring peak areas has been given by Bartlet.[134]

2.4542 Calibration Procedure

Although the area under any peak is proportional to the amount of compound present, the response factors $\left(f = \frac{\text{concentration}}{\text{peak area}}\right)$ of each fatty acid may differ slightly.

An internal standard method was employed to determine the relative response factor $\left(f_x = \frac{f \text{ sample fatty acid}}{f \text{ internal standard}}\right)$ of each of the main fatty acids present in chicken tissue. A standard mixture of C16:0, C18:0,

FIGURE X GLC TRACE OF CHICKEN NEUTRAL LIPID



C18:1, C18:2, and C18:3 methyl esters was purchased (Sigma Chemical Co. Ltd.), and 4 mg of C17:0 methyl ester (internal standard) added to give final concentration ratios of fatty acid to internal standard of 0.25, 0.5, 1.0, and 1.5. Each standard solution was chromatographed 6 times, using peak attenuation to ensure that all the components of each solution gave approximately equal peak heights.

Graphs of Area $\frac{\text{sample fatty acid}}{\text{internal standard}}$ vs. Concentration $\frac{\text{sample fatty acid}}{\text{internal standard}}$ were drawn (Figures XI, XII, XIII, XIV, XV) and the relative response factors (fx) determined from the reciprocal of the slopes:

$$fx = \frac{\text{C. fatty acid}}{\text{A. fatty acid}} \times \frac{\text{A. internal standard}}{\text{C. internal standard}}$$

$$= \frac{1}{\text{slope of standard curve}} \qquad (1)$$

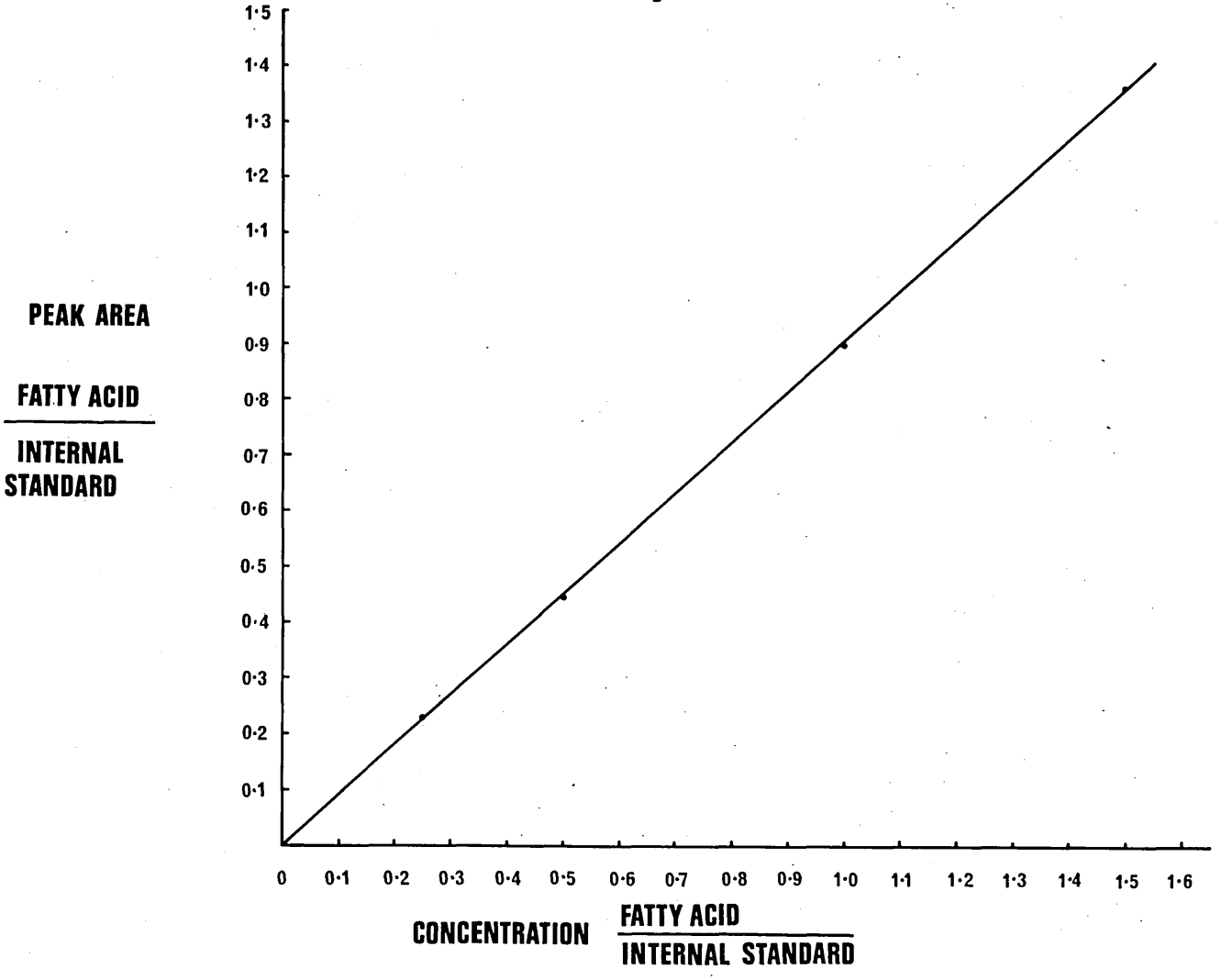
The graphs were linear over the entire test range, and the 6 replicates for each standard solution gave excellent reproducibility of results. The relative response factors calculated, were 1.10, 1.04, 1.03, 1.02 and 1.10 for C16:0, C18:0, C18:1, C18:2, and C18:3 respectively. Since these values were close to 1.00 and almost numerically identical, the mean value, 1.06 was assigned to those fatty acids which were not calibrated.

2.4543 Calculations

The amounts of individual fatty acids in test samples may be calculated directly from the calibration graphs, if the test solutions contain the same weight of internal standard as the calibration solutions. However, in this research, only the relative proportions of sample fatty acids were required, and not absolute amounts. Therefore, the addition of internal standard was not necessary. The concentration of each fatty acid was determined from the product of peak area, adjusted to account for attenuation, and relative response factor (see equation (1)). Each fatty acid was then expressed as a percentage of the total concentration:

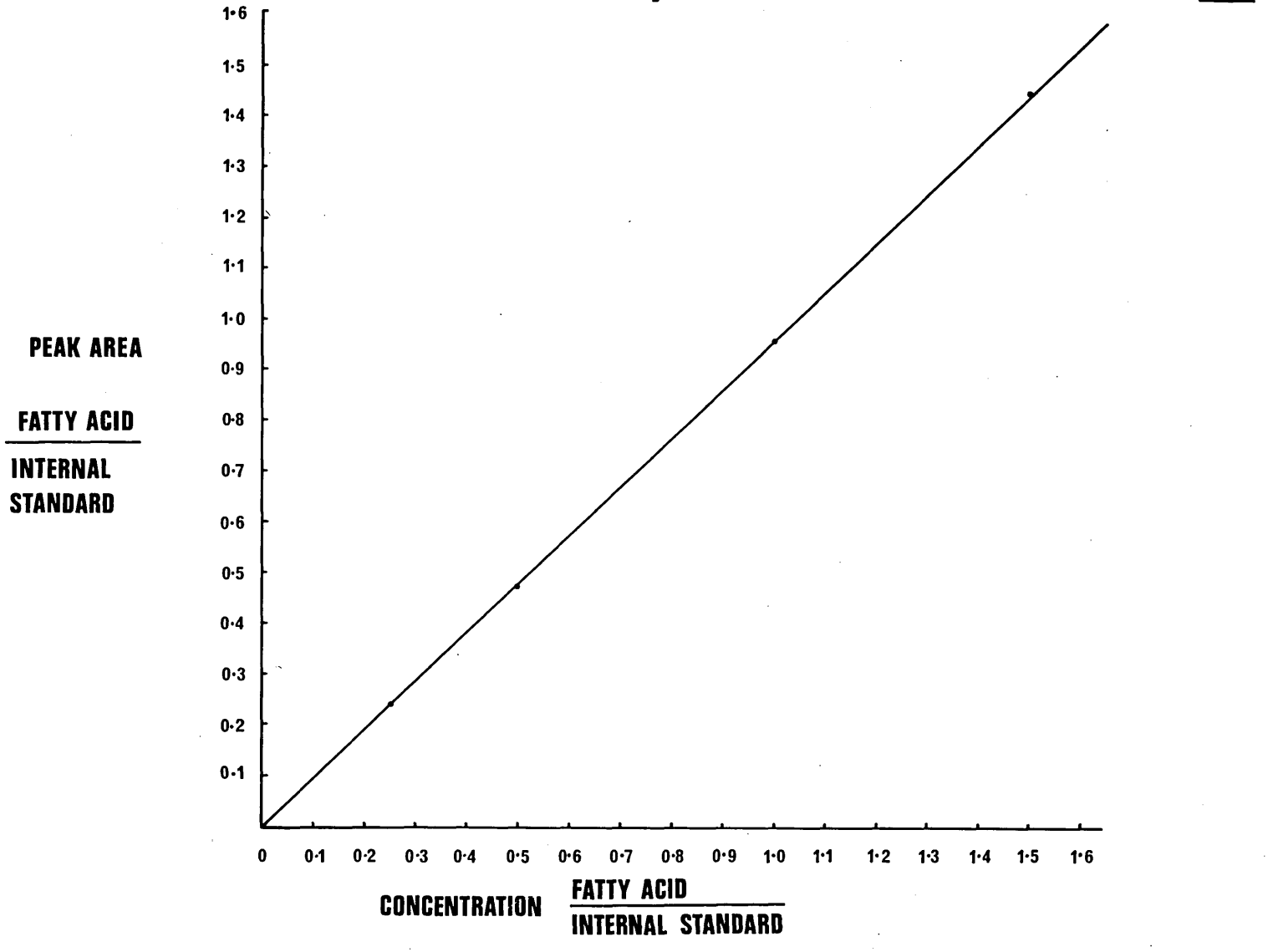
GLC Calibration Curve for Methyl Palmitate

FIGURE XI



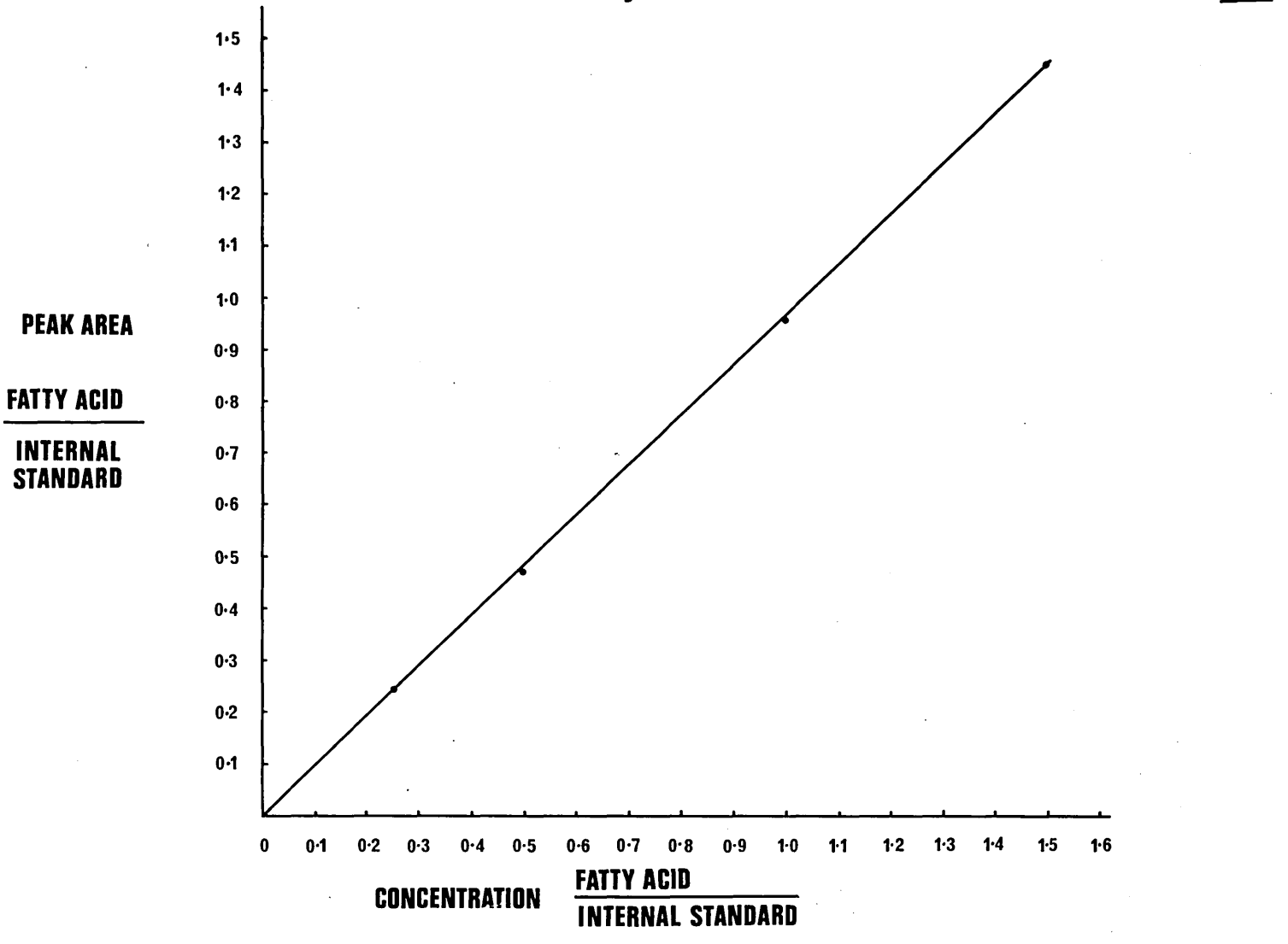
GLC Calibration Curve for Methyl Stearate

FIGURE XII



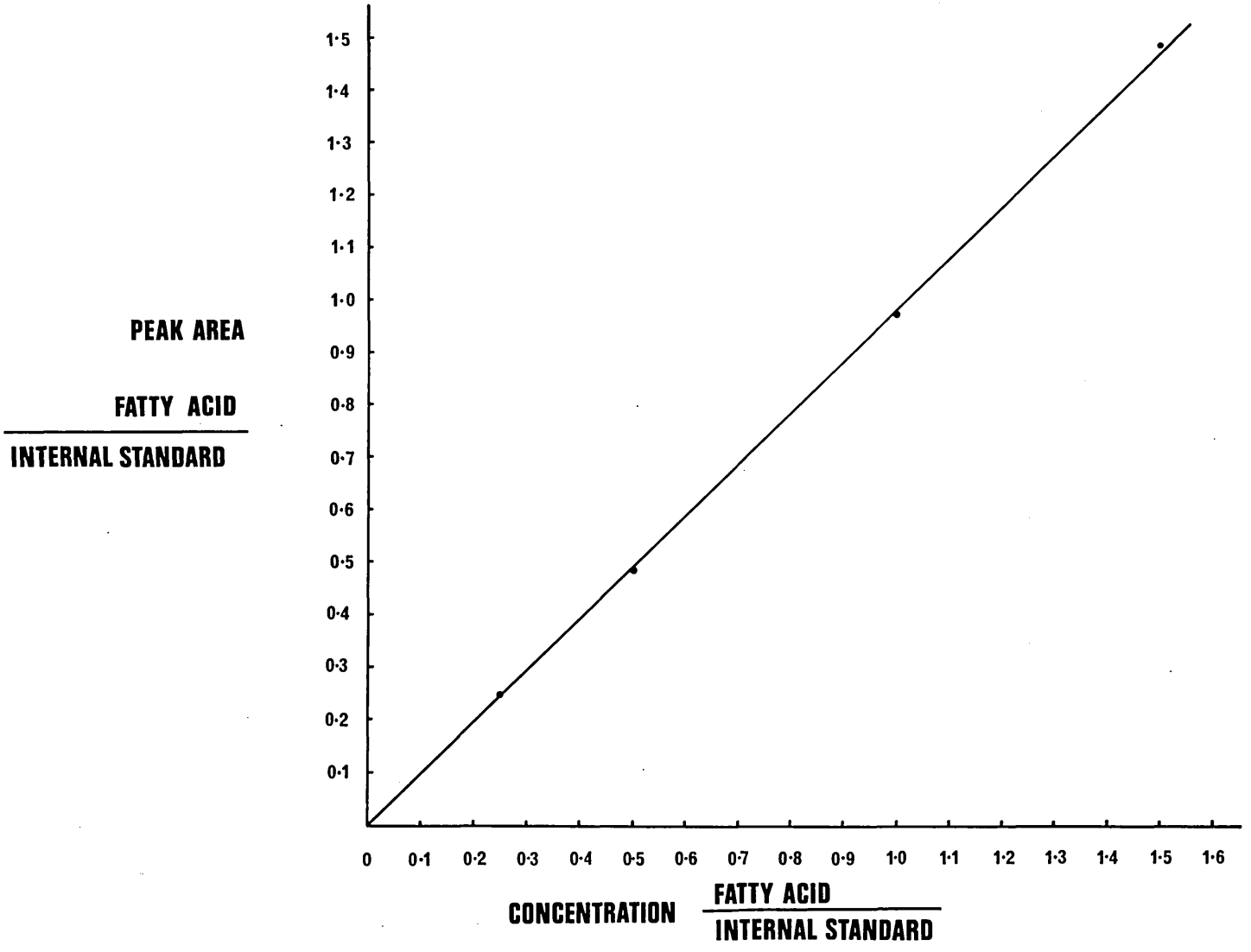
GLC Calibration Curve for Methyl Oleate

FIGURE XIII



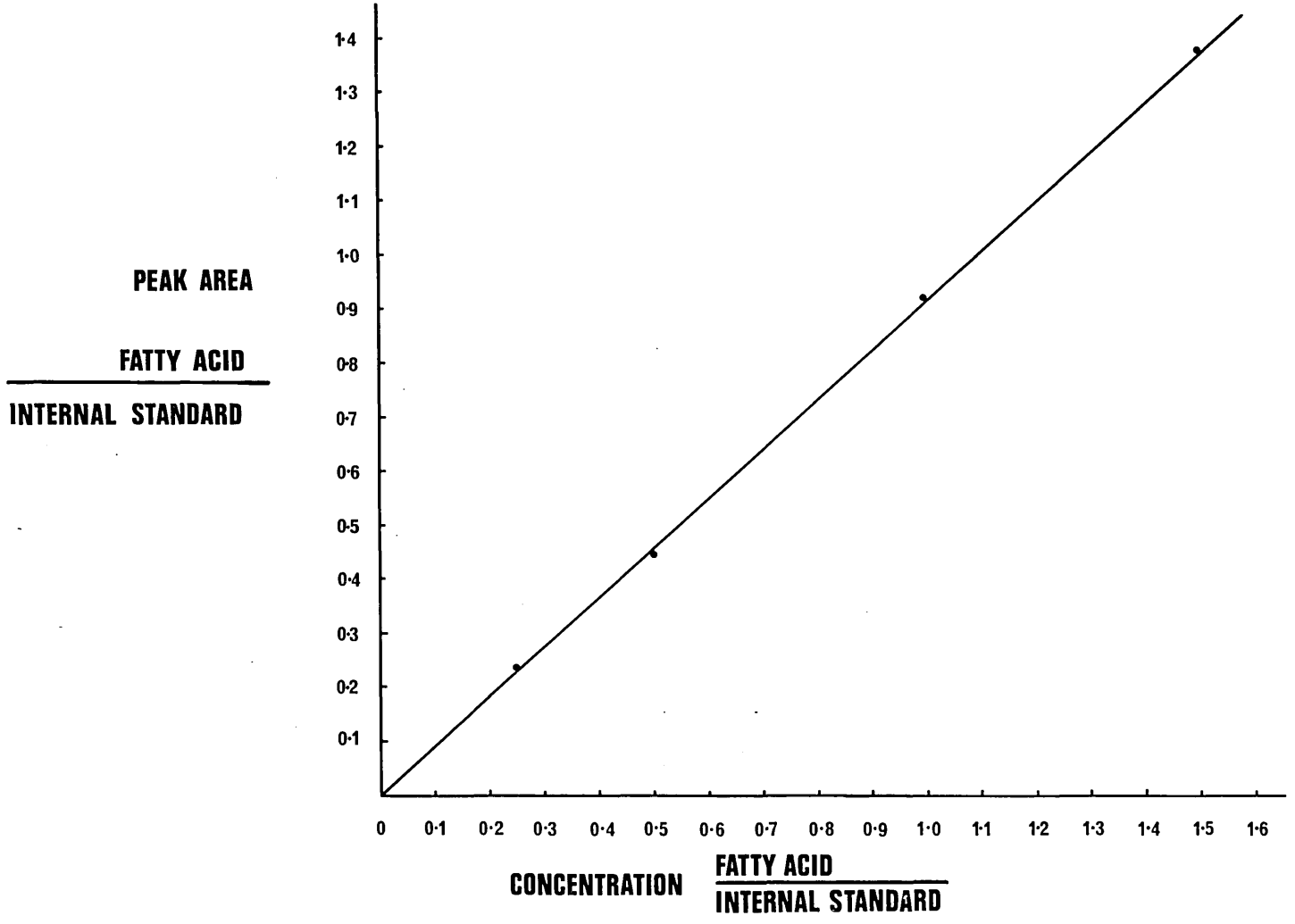
GLC Calibration Curve for Methyl Linoleate

FIGURE XIV



GLC Calibration Curve for Methyl Linolenate

FIGURE XV



$$\% \text{ fatty acid} = \frac{\text{concentration fatty acid}}{\sum \text{concentration fatty acid}} \times 100 \quad (2)$$

The internal standard method was used to determine the degree of methylation of chicken lipid samples (see section 2.443). Four milligrams of C17:0 methyl ester were added to a known weight of lipid prior to methylation, and the resulting esters were analysed by GLC. The percentage methylation was calculated from:

$$\% \text{ methylation} = \frac{\sum \text{concentration fatty acids} \times 4}{\text{sample weight (mg)}} \times 100 \quad (3)$$

Fatty acid analyses were carried out on various chicken samples during a 2 year period. Column performance during this time was monitered by periodically chromatographing calibration solutions, to see if changes in the column temperature (to maintain good separation on ageing columns - see section 2.452) altered the fatty acid distribution. These calibration solutions were stored under nitrogen at -18°C.

2.5 DETERMINATION OF PHOSPHORUS, SODIUM, CALCIUM, MAGNESIUM AND IRON

These ions were determined in chicken muscle, cook-out juices and thaw losses. The analyses were carried out by the Analytical Department of Albright and Wilson Ltd., on the solutions prepared in this laboratory as in section 2.51, using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES).

2.51 DIGESTION PROCEDURE

The following procedure, which was suggested by Albright & Wilson Ltd., was employed:

About 2g muscle or exactly 5 cm^3 thaw loss and cook-out juice, were accurately weighed and transferred to a 250 cm^3 tall form beaker. Ten cubic centimetres of concentrated sulphuric acid were added, and the mixture was heated until the sample charred. The beaker was then covered with a watch glass, and heating was continued until the sulphuric acid was nearly fuming, when concentrated nitric acid was added dropwise until the solution became clear. The solution was allowed to

cool and then 5 cm^3 nitric acid was added, and the solution boiled until the excess nitric acid had disappeared. After cooling, 5 cm^3 nitric acid and 1 cm^3 perchloric acid were added, and the solution was heated until the perchloric acid ceased to fume. The solution was then allowed to cool, when 150 cm^3 distilled water and a few anti-bumping stones were added, and the solution boiled for 20 minutes in order to hydrolyse any condensed phosphates. After the solution had cooled, it was made up to exactly 250 cm^3 and transferred to a plastic bottle and transported to Albright and Wilson Ltd.

Distilled water and polyphosphate injection solution blanks were determined as above.

2.52 CALCULATIONS

The concentrations of phosphorus, sodium, calcium, magnesium and iron in the digested solution were determined as micrograms per cubic centimetre of solution. The results were corrected for reagent blanks, and then these figures were used to calculate the concentration of each ion in the whole muscle and in the total volumes of thaw and cook losses.

The concentrations of phosphorus and sodium were reported as percentages (g per 100g or 100 cm^3 sample); and of calcium, magnesium and iron, as ppm (microg per g or cm^3 of sample).

2.6 DETERMINATION OF THE DEGREE OF HYDROLYSIS OF ADDED PHOSPHATES

2.61 THIN LAYER CHROMATOGRAPHIC PROCEDURE

Samples were prepared by macerating 10g muscle with 40 cm^3 of 10% trichloroacetic acid for 2 min. Trichloroacetic acid has been found to effectively halt the hydrolysis reaction, by Sutton.[113] The homogenate was then centrifuged at 0°C for 30 min. at 11000 G, and the supernatent taken for analysis.

The TLC procedure was adapted from that of Gibson and Murray.[135] Cellulose coated plates were used as support material, and the developing

solution was made up of isopropanol ($200cm^3$), n-propanol ($175cm^3$) trichloroacetic acid (25g), 0.88 ammonia ($1cm^3$), and water ($125cm^3$). Large plates (20 x 8.5 cm) were developed at room temperature for at least 12 hours, and smaller plates for 1 to 2 hours.

After development, the plates were dried in air and then sprayed with 1% ammonium molybdate, allowed to dry and sprayed with 1% stannous chloride in 10% hydrochloric acid. The colour was allowed to develop in the dark, when blue spots appeared on a white background. However, background colour was often very intense, and the developing reagent of Mikes[136] which gave better results, was used in the later stages of these experiments. This reagent was made up of 1% ammonium molybdate with $5cm^3$ 60% perchloric acid and $1cm^3$ hydrochloric acid per $100cm^3$ of solution. This solution was sprayed onto the dry developed plates, which were dried in an oven at 70^oC for a few minutes and then exposed to UV light, when blue spots appeared on a white background.

Sample spots were identified by comparison with orthophosphate, diphosphate and tripolyphosphate solutions. The Rf value of orthophosphate was 0.70, of diphosphate 0.40, and of tripolyphosphate 0.20. The spot present on the base line in samples of Puron 604 and treated chicken lipid solutions, was taken to be due to polyphosphates higher than tripolyphosphate.

The concentration of sample spots was calculated by eye from comparison of their colour intensity with standard polyphosphate solutions which were run alongside the samples on the TLC plates.

2.62 PHOSPHORUS-31 FOURIER TRANSFORM NUCLEAR MAGNETIC RESONANCE PROCEDURE

2.621 Principles of Nuclear Magnetic Resonance

Conventional nuclear magnetic resonance (NMR) involves either a continuous change of frequency or of magnetic field, over the resonance frequency range of the observed nucleus. Only a narrow frequency band contributes to the excitation of the resonance at any instant, which results in a low signal to noise ratio, leading to low sensitivity. Each

spectrum takes a matter of minutes to record.

Pulsed NMR consists of excitation of the sample by a short, intense pulse of radio-frequency energy; and measurement of the resulting free induction decay signal from the nuclear spins in the sample as a function of time. All of the frequencies of the molecule may be excited by the single high energy pulse in a period of micro-seconds, and the signal to noise ratio is improved compared to conventional NMR. Further improvements in sensitivity may be obtained by computer averaging of the decay signals from a large number of pulses. The computer averaged decay signal may be converted into a conventional NMR spectrum by Fourier transformation from the time domain to the frequency domain.

Since the ^{31}P nucleus is relatively insensitive to NMR, this Fourier transform NMR (FTNMR) technique using computer averaging is particularly useful for the analysis of phosphorus compounds, when well resolved spectra may be obtained in a few minutes.

2.622 Procedure

^{31}P-FTNMR has been used to detect added polyphosphates in chicken by O'Neill and Richards,[110] and the present method is a modification of this procedure.

Approximately 10 g samples of pectoralis major and leg muscle were excised from frozen chicken using a band-saw. The samples were packed in solid carbon dioxide and transported to the Laboratory of the Government Chemist, London, where they were stored in a freezer until required for analysis.

The samples were prepared for analysis by macerating the frozen muscle with 1 g of disodium ethylenediamine tetraacetic acid, which inhibits hydrolysis of the polyphosphates during subsequent thawing. Approximately 3 g of the macerate were tamped into a 10 mm o.d. NMR tube, and a capillary containing a D_2O solution of sodium methylenedi-

phosphonate as marker was inserted co-axially through the centre of the sample. The NMR spectra were recorded by Dr. Richards, using a JEOL PFT-100P spectrometer operating at room temperature and 40.3 MHz with deuteriumfield frequency lock. Each sample received 400 x 8μs pulses (flip angle 36°C) with a repetition time of 2.25 s. Data were collected in 4095 points with spectral observation and frequency filter settings of 5000 Hz. The resulting spectra were automatically integrated to obtain the peak areas.

Spectra of pure tripolyphosphate and injection solutions were also obtained.

2.623 Calculations

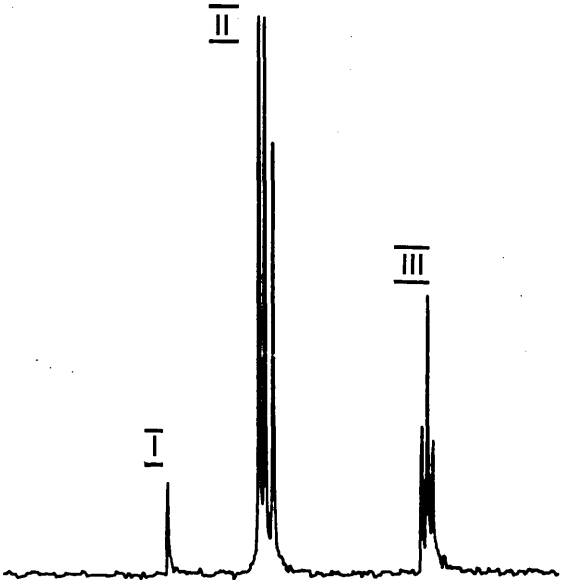
Species were identified by comparing the chemical shifts of sample peaks with those of tripolyphosphate (Figure XVIa). The splitting patterns of sample peaks (Figure XVIb) were more complicated than those of tripolyphosphate due to the presence of higher phosphates. Chemical shifts of polyphosphate species are pH sensitive[137], and therefore, slightly different values were obtained from the various samples relative to the standard of sodium methylenediphosphonate which is -16.8ppm from external 85% phosphoric acid.

The relative amounts of orthophosphate and higher phosphates were calculated from the integrated peak areas, which were normalised with respect to that of the standard which was assigned an integral of 1.0 arbitrary units.

The total added polyphosphate was calculated from the normalised areas of the higher phosphates and the excess orthophosphate arising from the hydrolysis of the added phosphates, relative to that of naturally occurring orthophosphate. In each case the level of naturally occurring orthophosphate was taken as that present in untreated chicken. The fraction of polyphosphate hydrolysed was then calculated from the excess orthophosphate and the total added phosphate:

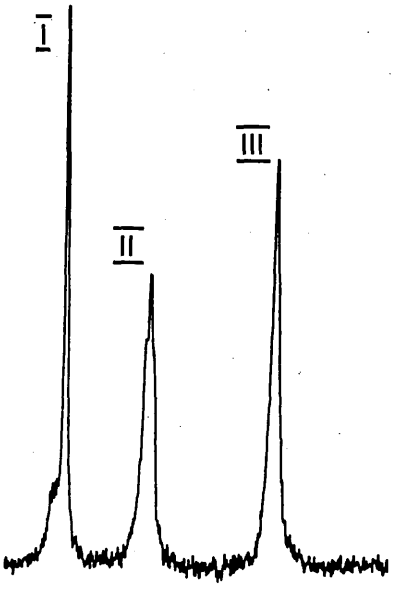
FIGURE XVI

^{31}P-FTNMR SPECTRA OF TRIPOLYPHOSPHATE AND CHICKEN MUSCLE



a) Tripolyphosphate containing diphosphate and orthophosphate.

The signals for orthophosphate, and the end and middle ^{31}P nuclei of higher phosphates are labelled I, II and III respectively.



b) Chicken muscle

$$\% \text{ polyphosphate hydrolysed} = \frac{\text{excess orthophosphate}}{\text{total amount polyphosphate added}} \times 100$$

2.7 DETERMINATION OF TOTAL HAEM

This simple method for estimating the blood content of exudate was a modification of that of Osner and Shrimpton,[26] Aliquots of thaw loss were diluted 10 times with sodium hydroxide ($0.1 mol.\ dm^{-3}$) and the solution filtered if not clear. The absorbancy at 415nm was determined, and the resulting value multiplied by the total volume of thaw loss. This value was taken as an estimate of the total haem content of the thaw loss. Values from all samples were compared, and no quantitative results were obtained.

2.8 DETERMINATION OF MICRO-ORGANISM CONTENT OF MINCED MUSCLE

The micro-organism content of minced muscle samples was determined by streaking samples onto nutrient Agar plates using a sterile wire. An attempt was made to obtain quantitative results by applying sterile gauze ($1cm^2$) to the surface of the muscle, and stamping this onto the plate. Plates were incubated at $37^{\circ}C$ for 3 days, and then the colony count was visually determined.

These determinations were not carried out under sterile conditions, and therefore, the results were thought to be of insufficient accuracy to quote in the results section of this work. However, since there was a distinct difference between polyphosphate-treated samples and untreated samples, it was thought that the results should be included, and they are reported in Appendix I.

3. RESULTS AND DISCUSSION

3.1 PRELIMINARY INVESTIGATION OF THE EFFECTS OF POLYPHOSPHATE TREATMENT ON RAW AND COOKED BATCH A CHICKENS AFTER 21 MONTHS STORAGE AT -18°C.

One polyphosphate treated chicken and one untreated chicken from batch A, which had been in frozen storage at -18°C for 21 months, were allowed to thaw in the bag at 4°C for 64 hours. Thaw loss and processing weight changes were calculated (Table Va). The pectoralis major muscles were excised and trimmed of fat and connective tissue. One muscle from each chicken was minced and held at 4°C. The other 2 muscles were cooked with no added fat or water in an oven at 350°C for 20 min. Cooking losses were calculated from the initial and drained muscle weights (Table Va). About 4g of each muscle was reserved at 4°C for organoleptic tests, the remainder being minced and held under the same conditions as the raw muscles. TBA and organoleptic tests were performed during holding. The organoleptic test used a 6 point hedonic scale rating for rancid odour and chicken flavour (a value of 6 represented no detectable rancid odour or very good chicken flavour). This test was performed by 1 person, and was therefore subjective, and no statistical weight could be given to these results. They did indicate, however, the general trends expected in tests of this type (Table Vb).

The TBA results clearly demonstrated the antioxidant effect of polyphosphates in cooked meat (Table Vc). However, the raw, treated muscle was also protected against autooxidation, which is contrary to other results (see section 1.43). No explanation can be offered for this result at this stage other than that the chickens had been stored for a considerable time before this experiment was carried out (see section 3.61).

Table Va shows that both the thaw and cook losses were reduced due to treatment, and also that the treated bird picked up less of the chill water than the untreated bird. However, the level of injection

(12.3%) was very high and would be considered illegal in some countries (see section 1.32). This high level of treatment could account for the poor texture and chicken flavour of the treated sample, although rancid odour was reduced compared to the control sample (Table Vb).

TABLE Va

UPTAKE OF POLYPHOSPHATE INJECTION AND CHILL WATER, AND THAW AND COOK LOSSES OF TREATED AND UNTREATED BATCH A CHICKENS AFTER 21 MONTHS STORAGE AT - 18°C

Chicken	Uptake of polyphosphate injection[a]	Uptake of chill water[b]	Thaw loss[c]	Cook loss of pectoralis major muscle[d]
Treated	12.3	2.2	1.1	24.1
Untreated	-	5.5	2.3	31.5

a. Percentage of eviscerated carcase
b. Percentage increase of eviscerated weight + injection weight after chill and drip.
c. Percentage of frozen carcase
d. Percentage of raw, wet muscle.

TABLE Vb

SUBJECTIVE ORGANOLEPTIC RATINGS OF COOKED PECTORALIS MAJOR MUSCLES DURING HOLDING AT 4°C

Days held at 4°C	Rancid odour[a]		Chicken Flavour[a]		Comments	
	treated	untreated	treated	untreated	treated	untreated
0	no odour	6	2	6	good light colour. Poor texture - too juicy	good texture
2	6	5	2	4	too juicy	dry
6	6	3	1	2	juicy	tough

a. 6 represents no detectable rancid odour or very good chicken flavour.

TABLE Vc

TBA NUMBERS OF RAW AND COOKED PECTORALIS MAJOR MUSCLES DURING HOLDING AT 4°C

Treatment		Days held at 4°C						
		0	2	4	6	7	10	11
Raw	treated	0.48	0.50	0.51	-	0.55	-	0.84
	untreated	0.94	5.36	8.06	-	10.94	-	-
Cooked	treated	1.25	0.50	-	0.71	-	1.09	-
	untreated	0.48	7.63	-	9.33	-	-	-

3.2 TBA ANALYSIS, AND LIPID AND FATTY ACID COMPOSITION OF RAW PECTORALIS MAJOR AND LEG MUSCLES OF TREATED AND UNTREATED BATCH A CHICKENS AFTER 36 MONTHS STORAGE AT -18°C

The preliminary experiment indicated that polyphosphate injection both increased water retention and decreased oxidative rancidity of raw and cooked chicken. It was decided to further investigate this antioxidant effect on raw chicken tissues and to compare TBA Numbers with lipid and fatty acid composition results of raw tissues.

The experiment was carried out as follows:

Three treated and 3 untreated chickens from batch A after 36 months storage at -18°C were taken and analysed as follows:

a) 1 treated and 1 untreated chicken thawed at room temperature for 5 hours.

b) Pectoralis major and leg muscles excised from 1 side of each chicken and minced. Leg muscles stored at -18°C until required ——→ c) 10g reserved at -18°C for TBA tests.

d) Total lipid extracted from each pectoralis major muscle, and then separated into neutral lipid and phospholipid

e) Each lipid class methylated and the fatty acid composition determined by GLC.

f) a) to e) repeated twice more

g) TBA tests performed on all 6 pectoralis major muscles

h) d) to g) repeated 3 times for leg muscles.

3.21 LIPID COMPOSITION

Table VIa shows the total lipid, neutral lipid and phospholipid contents of treated and untreated pectoralis major and leg muscles. The only statistically significant differences between treated and untreated samples occurred for the phospholipid content (when expressed as a percentage of wet tissue, but not total lipid) of pectoralis major muscles. The treated muscles contained significantly lower amounts than the untreated muscles, but the actual numerical differences were very small. No such differences were found for the leg muscles.

There was very little interchicken variation in the phospholipid contents (% wet tissue), whereas the neutral lipid contents (% wet tissue) of both pectoralis major and leg muscles showed relatively large interchicken variations. This suggests[9] that neutral lipid is affected to a far greater extent than phospholipid by the factors, such as diet and age, discussed in section 1.41. In addition there was a direct relationship between total lipid and neutral lipid levels, indicating that higher lipid levels arise primarily from the deposition of neutral lipid, as suggested by Marion and Miller,[47] Also, leg muscles contained more total lipid and a higher proportion of neutral lipid than pectoralis major muscles.

These general trends are in agreement with those discussed in section 1.41. However, it was not possible to directly compare the present results with those from other workers, because there is some evidence of lipase and phospholipase activity at temperatures down to -20°C (Fishwick, 1968, cited by El-Warraki et al,[50]).

TABLE VIa

TOTAL LIPID, NEUTRAL LIPID, AND PHOSPHOLIPID CONTENT OF RAW PECTORALIS MAJOR AND LEG MUSCLES OF TREATED AND UNTREATED BATCH A CHICKENS AFTER 36 MONTHS STORAGE AT -18°C

	MUSCLE															
	Pectoralis Major								Leg							
	treated				untreated				treated				untreated			
	1	2	3	M ±S.D.[a]	4	5	6	M±S.D.[a]	1	2	3	M ±S.D.	4	5	6	M ±S.I
total lipid (% of wet tissue)	0.84	0.84	0.70	$\pm^{0.79}_{0.08}$	0.98	0.87	0.90	$\pm^{0.92}_{0.06}$	2.94	4.15	2.11	$\pm^{3.07}_{1.03}$	2.07	2.12	2.00	$\pm^{2.06}_{0.06}$
neutral lipid (% of wet tissue)	0.45	0.46	0.31	$\pm^{0.41}_{0.08}$	0.57	0.45	0.47	$\pm^{0.50}_{0.06}$	2.42	3.64	1.66	$\pm^{2.57}_{1.00}$	1.54	1.69	1.55	$\pm^{1.59}_{0.08}$
neutral lipid (% of total lipid)	53.2	55.3	44.9	$\pm^{51.1}_{5.5}$	58.0	51.5	51.9	$\pm^{53.8}_{3.6}$	82.4	87.8	78.6	$\pm^{82.9}_{4.6}$	74.5	79.5	77.7	$\pm^{77.2}_{2.5}$
phospholipid (% of wet tissue)	0.39	0.38	0.39	$\pm^{0.39‡}_{0.01}$	0.41	0.42	0.43	$\pm^{0.42}_{0.01}$	0.52	0.51	0.45	$\pm^{0.49}_{0.03}$	0.53	0.43	0.45	$\pm^{0.47}_{0.05}$
phospholipid (% of total lipid)	46.8	44.7	55.1	$\pm^{48.9}_{5.5}$	42.0	48.5	48.1	$\pm^{46.2}_{3.6}$	17.6	12.2	21.4	$\pm^{17.1}_{4.6}$	25.5	20.5	22.3	$\pm^{22.8}_{2.5}$

a. Mean ± standard deviation

‡ Difference between treated and untreated significant at 99% level

3.22 FATTY ACID COMPOSITION

Table VIb shows the fatty acid composition of pectoralis major muscles, and Table VIc the fatty acid composition of leg muscles. The same general trends were observed for both pectoralis major and leg muscles.

It may be seen that the main fatty acids in neutral lipid and phospholipid are C16:0, C18:1 and C18:2; with phospholipids also containing large amounts of C18:0. Phospholipids also contained larger amounts of C20:4 than the neutral lipids. These general trends are in agreement with the literature (see section 1.41). However, higher levels of C20:4 were expected to be present in the phospholipids of both tissues. The low levels found may be due to autooxidation of this polyunsaturated fatty acid during the extended frozen storage period of these chickens prior to analysis.

No difference in fatty acid composition between treated and untreated leg or pectoralis major muscles were apparent from these figures. However, interchicken differences may have obscured any differences due to treatment. Therefore, to help account for the inter-chicken variations, the level of C16:0 was assumed to be constant (i.e. unaffected by autooxidation), and the amounts of total unsaturated acids were calculated with respect to C16:0 for each set of figures. Again, there were no significant differences between treated and untreated muscles.

3.23 CORRELATION COEFFICIENTS BETWEEN LIPID AND FATTY ACID COMPOSITIONS

Table VId shows that there were statistically significant negative correlation coefficients between the neutral lipid content (expressed as a percentage of the total lipid) and the amounts of C18:2 and of the total amount of unsaturated fatty acids in the neutral lipid of pectoralis major treated and untreated muscles. A negative correlation for C18:2, but a positive correlation for unsaturated acids,

TABLE VIb FATTY ACID COMPOSITION[a] of NEUTRAL LIPID AND PHOSPHOLIPID OF RAW PECTORALIS MAJOR MUSCLES

FATTY ACID[b]	LIPID															
	Neutral Lipid								Phospholipid							
	treated				untreated				treated				untreated			
	1	2	3	M±S.D.[c]	4	5	6	M±S.D.[c]	1	2	3	M±S.D.[c]	4	5	6	M±S.D.
C14:0	1.2	0.9	0.9	-	1.0	1.1	1.1	-	0.7	0.9	0.9	-	0.5	0.5	0.5	-
C14:2	-	-	-	-	-	-	-	-	3.6	4.4	4.6	4.2 ±0.5	4.3	5.0	5.9	5.1 ±0.8
C16:0	24.9	27.9	24.4	25.7 ±1.9	28.4	25.4	28.2	27.3 ±1.7	31.2	33.1	30.7	32.1 ±1.0	30.7	34.4	32.8	32.6 ±1.9
C16:1	6.2	8.9	6.0	7.0 ±1.6	8.3	7.4	8.2	8.0 ±0.5	-	-	-	-	-	-	-	-
C18:0	7.6	6.1	6.2	6.6 ±0.8	6.8	7.0	6.8	6.9 ±0.1	13.1	12.0	11.6	12.2 ±0.8	11.0	12.0	10.9	11.3 ±0.6
C18:1	37.7	37.8	35.1	36.9 ±1.5	38.3	30.9	33.4	34.2 ±3.8	26.8	31.6	28.8	29.1 ±2.4	30.2	27.4	28.4	28.7 ±1.4
C18:2	16.3	13.5	20.3	16.7 ±3.4	12.6	20.2	16.9	16.6 ±3.8	16.8	13.1	16.0	15.3 ±1.9	15.8	16.3	15.9	16.0 ±0.3
C18:3	2.1	2.0	2.1	2.1 ±0.1	2.3	2.4	2.0	2.3 ±0.2	0.4	0.5	0.5	-	0.6	0.3	0.7	-
C20:4	1.9	1.2	2.6	1.9 ±0.7	1.0	2.9	1.4	1.8 ±1.0	3.8	2.1	3.2	3.0 ±0.9	3.0	2.1	2.6	2.6 ±0.5
C_B	1.7	1.3	1.8	1.6 ±0.3	1.0	1.9	1.4	1.4 ±0.5	2.5	1.7	1.2	1.8 ±0.7	3.1	0.8	1.4	1.8 ±1.2
% total unsaturated	66.4	65.0	68.6	66.7 ±1.8	63.7	66.4	63.8	64.6 ±1.5	55.0	54.0	55.3	54.8 ±0.7	57.7	53.1	56.0	55.6 ±2.3
% total unsaturated / C16:0	2.7	2.3	2.8	2.6 ±0.3	2.3	2.6	2.3	2.4 ±0.2	1.8	1.6	1.8	1.7 ±0.1	1.9	1.5	1.7	1.7 ±0.2

a. Values expressed as a % of total fatty acids

b. Also trace (< 1.0%) amounts of C20:0 and C_A present

c. Mean ± standard deviation

TABLE VIc FATTY ACID COMPOSITION[a] OF NEUTRAL LIPID AND PHOSPHOLIPID OF RAW LEG MUSCLES

FATTY ACID[b]	LIPID															
	Neutral Lipid								Phospholipid							
	treated				untreated				treated				untreated			
	1	2	3	M±S.D.[c]	4	5	6	M±S.D.[c]	1	2	3	M±S.D.[c]	4	5	6	M±S.D.
C14:0	1.0	0.9	2.4	1.4 ±0.8	0.9	1.0	2.3	1.4 ±0.8	0.6	-	0.4	-	0.4	0.6	0.6	-
C14:2	-	-	-	-	-	-	-	-	4.4	-	4.1	4.3 ±0.2	4.7	3.9	3.3	4.0 ±0.7
C16:0	26.9	27.9	26.3	27.0 ±0.8	27.2	25.6	27.0	26.6 ±0.9	26.1	-	28.4	27.3 ±1.6	26.3	31.1	28.9	28.8 ±2.4
C16:1	8.4	11.3	7.7	9.1 ±1.9	9.2	10.0	9.6	9.6 ±0.4	-	-	-	-	-	-	-	-
C18:0	6.4	4.8	8.1	6.4 ±1.7	6.6	6.5	7.4	6.8 ±0.5	24.5	-	26.3	25.4 ±1.3	24.6	24.7	25.2	24.8 ±0.3
C18:1	39.6	39.5	34.3	37.8 ±3.0	36.7	31.2	32.7	33.5 ±2.8	24.3	-	24.0	24.2 ±0.2	25.9	22.9	24.3	24.4 ±1.5
C18:2	13.4	12.4	15.7	13.8 ±1.7	14.5	19.2	15.8	16.5 ±2.4	13.4	-	12.6	13.0 ±0.6	12.6	13.3	13.3	13.1 ±0.4
C18:3	2.0	2.0	2.2	2.1 ±0.1	1.8	2.4	2.4	2.2 ±0.3	0.7	-	0.8	-	0.8	1.1	0.8	-
C20:4	1.2	0.8	2.0	1.3 ±0.6	1.7	2.6	1.8	2.0 ±0.5	4.4	-	2.6	3.5 ±1.3	2.8	1.7	2.6	2.4 ±0.6
C_B	0.8	0.5	0.9	-	1.0	0.9	0.9	-	0.8	-	0.4	-	1.1	0.3	0.5	-
% total unsaturated	65.7	66.7	63.2	65.2 ±1.8	65.4	66.7	63.5	65.2 ±1.6	48.7	-	44.9	46.8 ±2.7	48.7	43.8	45.3	45.9 ±2.5
% total unsaturated / C16:0	2.4	2.4	2.4	2.4 ±0.0	2.4	2.6	2.4	2.5 ±0.1	1.9	-	1.6	1.8 ±0.2	1.9	1.4	1.6	1.6 ±0.3

a. Values expressed as a % of total fatty acids.

b. Also trace (< 1.0%) amounts of C20:0 and C_A present

c. Mean ± standard deviation.

TABLE VId

CORRELATION COEFFICIENTS[a] BETWEEN NEUTRAL LIPID CONTENT (% of total lipid) AND FATTY ACIDS

MUSCLE	FATTY ACID		
	C16:0	C18:2	% total unsaturated
Pectoralis Major	+0.73	-0.88°	-0.82°
Leg	+0.41	-0.53	+0.51

a. Based on data in Tables VIa, VIb and VIc

o Significant at 95% level

TABLE VIe

TBA NUMBERS

MUSCLE	TREATMENT							
	Treated				Untreated			
	1	2	3	M±SD[a]	4	5	6	M±SD
Pectoralis Major	0.46	0.43	0.17	0.35 ± 0.16	1.07	0.96	0.42	0.82 ± 0.35
Leg	1.99	0.92	0.75	1.22 ± 0.67	0.47	0.84	0.65	0.65 ± 0.19

a. Mean ± standard deviation

was found for leg muscles, but these were not statistically significant. There were high, but not statistically significant, positive correlation coefficients between the neutral lipid content and the amount of C16:0 in the neutral lipid of pectoralis major and leg muscles. No such high correlation coefficients were found for the phospholipid fractions of either pectoralis major or leg muscles.

It should be remembered that the amounts of C18:2 in the neutral lipid of both pectoralis major and leg muscles, showed greater interchicken variation than any of the other main fatty acids (see Tables V1b and V1c).

These observations may be partly explained by the points discussed in section 1.41 i.e. dietary C18:2 is deposited in chicken tissues to a larger extent than other dietary fatty acids, and also, that neutral lipid fractions exhibit most pronounced changes in the 16 and 18- carbon fatty acids, whilst phospholipids show most marked changes in the longer chain fatty acids as a result of diet. Also, higher lipid levels arise primarily from the deposition of neutral lipid. Lack of correlation between long chain polyunsaturated fatty acids and the amount of phospholipid could be because only small amounts of these acids are present, and calculation of their amounts is thus subject to high experimental error.

In terms of diet, a positive correlation between the amounts of neutral lipid and C18:2 would have been expected, because the greater the amount of total lipid (presumably derived from dietary fat,) the greater the amount of neutral lipid and hence C18:2 is expected. However, statistically significant negative correlations were obtained between neutral lipid and C18:2. This may only be accounted for by the fact that C18:2 is susceptible to autooxidation, and its level will therefore be controlled by factors other than diet. The positive correlation between neutral lipid and C16:0 is as would be expected from diet, and the fact that it is relatively insusceptible to oxidation.

3.24 TBA NUMBERS

Table VIe shows the TBA numbers of the freshly excised pectoralis major and leg muscles after they had been stored at -18°C for approximately 2 weeks. There were no large differences in TBA Numbers between raw treated and untreated pectoralis major muscles, as found in the preliminary experiment. However, the mean TBA Number of treated muscles was lower than that of untreated muscles, and this difference approached significance. In leg muscles, however, the mean TBA Number of treated muscles was higher than for untreated muscles, but this difference was not significant. All of the treated pectoralis major muscles, and most of the other muscles, had TBA Numbers of less than 1.0, the value at which it has been suggested that rancidity first becomes organoleptically detected.[88]

3.25 CORRELATION COEFFICIENTS BETWEEN TBA NUMBERS AND FATTY ACID LEVELS

Since the TBA Test measures a by-product of the autooxidation of fatty acids, a correlation between these 2 values may be expected to exist. In fact, Marion et al,[58] reported such correlations between the amounts of fatty acids in the neutral lipid and in 2 phospholipid fractions (cephalin and lecithin) of raw pectoralis major muscles, and their TBA Numbers (see Table VIf for values). Also Bartov and Bornstein,[60] found that increases in the level of C18:2 derived from soybean and milo oils, increased the susceptibility of chicken tissues to oxidation. However, both these reports dealt with chicken lipids having very different fatty acid compositions due to varying dietary fats, and any correlations between TBA Numbers and fatty acids would be expected to be magnified.

Table VIf shows the correlation coefficients between TBA Numbers and the fatty acid levels of the neutral lipid and phospholipid fractions of both pectoralis major and leg muscles. These coefficients were calculated from the combined values for treated and untreated muscles,

TABLE VIf

CORRELATION COEFFICIENTS[a] BETWEEN TBA NUMBERS AND FATTY ACIDS

MUSCLE	LIPID	FATTY ACID							
		total unsaturated	C16:0	C16:1	C18:0	C18:1	C18:2	C20:4	C_B
Pectoralis Major	neutral[b]	-0.48	+0.34 (+0.20)[d]	+0.39 (-0.42)	+0.35 (-0.03)	-0.09 (-0.60)	-0.29 (+0.80)	-0.12 (+0.16)	-0.32
	phospholipid[b]	+0.16	+0.01 (+0.29)[e1] (+0.17)[e2]	-	-0.19 (-0.19) (-0.05)	+0.01 (-0.45) (-0.05)	+0.17 (+0.14) (+0.27)	-0.27 (-0.48) (-0.51)	+0.33
Leg	neutral[b]	+0.27	+0.02	-0.23	-0.23	+0.54	-0.34	-0.40	-0.31
	phospholipid[c]	+0.40	-0.37	-	-0.34	-0.21	+0.58	+0.80	+0.09

a. Based on data in Tables VIb, VIc and VIe

b. 0.81 required for significance at 95% level.

c. 0.88 required for significance at 95% level.

d. Figures in brackets are results of Marion et al[58] where 0.21 required for significance at 95% level

e_1 and e_2 Marion et al,[58] results for cephalin and lecithin lipid fractions respectively.

since there were no significant differences in fatty acid composition or TBA Numbers between the 2 treatments.

It may be seen that although the correlation coefficients were not statistically significant, they did show the general relationships expected between the degree of autooxidation and fatty acid levels. The 16- and 18- carbon fatty acids of neutral lipid, and the polyunsaturated 20 plus- carbon fatty acids of phospholipid, appeared to be the most important indicators of the degree of autooxidation in both pectoralis major and leg muscles.

One would expect negative correlation coefficients between unsaturated fatty acids and TBA Numbers, and positive correlation coefficients between saturated fatty acids and TBA Numbers. The most notable exceptions to this were the positive correlation between the level of C_B in the phospholipid fraction of pectoralis major muscles and their TBA Numbers; and the high positive correlation between C20:4 in the phospholipid fraction of leg muscles and their TBA Numbers. However, since each fatty acid is expressed as a percentage of the total amount of fatty acids, losses of one acid automatically causes an increase in the others. Also, the amounts of the long-chain fatty acids were small (less than 4%), and their determination is thus subject to high experimental error.

3.26 CORRELATION COEFFICIENTS BETWEEN TBA NUMBERS AND LIPID LEVELS

Table VIg shows the correlation coefficients between TBA Numbers and lipid levels for treated, untreated and treated plus untreated muscles. The correlation coefficients between TBA Numbers and phospholipid levels were calculated for treated and untreated separately, as well as in combination because there was a significant difference in the phospholipid content (% of wet tissue) between treated and untreated muscles (see Table VIa). There were no

TABLE VIg CORRELATION COEFFICIENTS BETWEEN TBA NUMBERS AND LIPID LEVELS

MUSCLE	TREATMENT	LIPID				
		Total lipid as % tissue	Neutral lipid as % tissue	Neutral lipid as % total lipid	Phospholipid as % tissue	Phospholipid as % total lipid
Pectoralis Major	treated[b]	+0.99	+0.99	+0.96	-0.42	-0.96
	untreated[b]	+0.40	+0.50	+0.59	-0.93	-0.59
	treated + untreated[c]	+0.79 (-0.64)[d]	+0.77	+0.65	+0.46 (-0.78)	-0.65 (+0.40)
Leg	treated[b]	+0.02	-0.01	+0.03	+0.71	-0.03
	untreated[b]	+0.43	+0.90	+0.98	-0.94	-0.98
	treated + untreated[c]	+0.39 (-0.39)	+0.38	+0.48	+0.32 (+0.74)	-0.48 (+0.59)

a. Based on data in Tables VIa and VIe

b. 0.997 required for significance at 95% level

c. 0.81 required for significance at 95% level

d. Figuresin brackets are results of Wilson et al,[138] for untreated cooked muscles, where 0.997 required for 95% significance.

significant differences between treated and untreated muscles for the other lipid levels, but the separated values were also calculated. However, any numerical differences between these values for treated and untreated muscles, are obviously due to inherent inter-chicken variations, and not to polyphosphate treatment.

Positive correlation coefficients were obtained between TBA Numbers and total lipid and neutral lipid (% of total lipid and wet tissue) levels, and these approached significance in the case of pectoralis major muscles. These observations would seem to suggest that neutral lipid plays a major role in raw tissue oxidation. This bears out the findings of Shorland,[39] who found that the fatty acids of neutral lipid of raw beef and lamb after prolonged frozen storage, had undergone considerably greater oxidation than those of phospholipids. It seems, therefore, that phospholipids are protected against oxidation during frozen storage, presumably by their close association with muscles proteins. However, in the present work there were positive correlation coefficients between TBA Numbers and phospholipid (% of wet tissue) levels for treated plus untreated muscles, but these values were small and statistically insignificant. Also, these coefficients for treated and untreated pectoralis major muscles separately, were negative, and in the case of untreated muscles the value approached significance. This finding, along with the fact that the phospholipid contents of treated and untreated muscles were significantly different, seems to indicate that polyphosphates may have some direct effect on the phospholipids of pectoralis major muscles. However, this evidence is too tenuous to pursue this discussion.

All of these correlation coefficients were opposite in sign to those obtained in untreated cooked chicken muscles by Wilson et al,[138] (see Table VIg for values). Their correlations were not

statistically significant, and, in fact, were calculated from the results obtained from only 3-chicken samples. However, it was thought that it was valid to compare these results with the present ones, since the numerical values of the coefficients from both experiments were relatively high. Wilson et al, interpreted their results as evidence of phospholipids playing the major role in cooked muscle autooxidation; and that this was because of their high polyunsaturated fatty acid content. Since the present results show that it is the neutral lipids which play the major role in raw muscle autooxidation, it is suggested that heat may loosen any phospholipid-protein associations, and thus allow phospholipid oxidation to proceed (see section 3.78).

3.3 INVESTIGATIONS OF THE HYDROLYSIS OF ADDED POLYPHOSPHATES IN CHICKEN TISSUES

In the previous experiment, polyphosphate was found to have very little detectable effect on the lipid and fatty acid composition of raw chicken muscles. This approach, therefore, was temporarily abandoned in order to investigate the rate of hydrolysis of added polyphosphates in chicken tissues. Few previous reports of this have been found in the literature, although the rate of hydrolysis of polyphosphates in other meats at refrigerator and room temperature has been investigated (see section 1.64). As explained in section 1.64, it is very important to study the hydrolysis of added polyphosphate, since orthophosphate, the ultimate hydrolysis product of polyphosphates, is not effective as an antioxidant and has less effect on moisture losses than diphosphate and tripolyphosphate.

3.31 DURING STORAGE AT 4°C

Fifty grams of breast muscle from an untreated chicken from batch B were homogenised with 0.25 g of sodium tripolyphosphate. Ten grams of this mixture were taken immediately after mixing, and homogenised with 30 cm^3 of 10% trichloroacetic acid, and analysed for polyphosphates by the TLC method in section 2.61. The remaining muscle was stored at 4°C, and analysed for polyphosphates as before after 18 and 23 hours storage.

Immediately after mixing, the tripolyphosphate content had decreased by about 70%, and none was detected after 18 and 23 hours storage. Only a trace amount of diphosphate was detected after 18 hours, and none after 23 hours. However, this method lacked sensitivity, since it was calculated that tripolyphosphate could only be detected if more than 15% of the original amount remained. Nevertheless, this rapid hydrolysis on mixing and during short-term storage has been reported in other meats by various workers (see section 1.64).

Later experiments using ^{31}P-FTNMR gave much more precise measurements than the TLC technique. It was decided to determine if any hydrolysis occurred during frozen storage of treated chickens, since preliminary experiments using the TLC technique (not reported here) showed that polyphosphate could not always be detected in chickens which had been in frozen storage for periods of greater than 5 months.

The pectoralis major muscles of 3 treated chickens from batch A and batch B and 4 chickens from batch C, which had been in frozen storage for periods of 43, 15 and 5 months respectively, along with 1 untreated chicken from batch C, were tested according to section 2.622.

Table VIIa shows the levels of orthophosphate and higher phosphates in the muscles, and also the relative amounts of total added phosphate and the fraction of added polyphosphate hydrolysed. The results showed that hydrolysis had proceeded to a great degree during 15 months, and especially during 43 months; but only to a very small degree during 5 months storage, with 1 exception. This anomaly in the 5 month sample, when 40% hydrolysis had occurred, may be explained by a relatively small amount of added phosphate being in contact with normal levels of phosphatases. However, this chicken had been injected with the same amount of phosphate as the other chickens and the very small amount detected was presumably due to sampling error, which is discussed below.

The only other results of this nature which have been in the literature are those of Jozefowicz et al,[111] and O'Neill and Richards,[110] who state that polyphosphates undergo very little hydrolysis during 6 months frozen storage of the treated chicken. However, these workers did not present quantitative results, as in the present experiment.

TABLE VIIa

POLYPHOSPHATE SPECIES PRESENT IN THE PECTORALIS MAJOR MUSCLES OF BATCH A, B AND C CHICKENS AFTER 43, 15 AND 5 MONTHS' STORAGE AT -18°C RESPECTIVELY

Storage time (months)	Integrals[a]		Relative total added phosphate[a,b]	Fraction of polyphosphate hydrolysed (%)[c]
	Ortho-phosphate	Higher phosphates		
5	7.2	6.7	7.0	4
	6.7	13.5	13.5	0
	7.7	1.2	2.0	40
	7.1	24.0	24.2	1
15	7.7	7.8	8.6	9
	8.3	3.8	5.2	27
	10.2	7.1	10.4	32
43	11.0	0	4.1	100
	16.4	4.9	14.4	66
	12.5	1.0	6.6	85
Untreated control	6.9	-	-	-

a. Normalised relative to the capillary solution of sodium methylene diphosphonate with an integral of 1.0 arbitrary units.

b. Excess abundance of phosphorus nuclei over that in untreated sample.

c. Fraction of polyphosphate phosphorus nuclei appearing as orthophosphate.

Although these results show a consistent trend of hydrolysis with frozen storage time, two approximations were made in the procedure. First, the percentage hydrolysis was calculated taking the level of naturally occurring orthophosphate in chicken muscle as that present in only 1 untreated chicken. This was thought to be justifiable because the naturally occurring orthophosphate is present in relatively large amounts compared to the injected amount, and also the natural amount is reasonably constant between chickens (see sections 3.42 and 3.791). Second, differences in the location of the actual sample site relative to the injection site, which was not exactly known, were likely to produce errors because of the unknown efficiency of distribution of injected phosphates.

Since orthophosphate, the ultimate hydrolysis product of polyphosphates, is not effective as an antioxidant (and relatively ineffective in controlling WHC), the fact that hydrolysis of polyphosphates has been found to occur during frozen storage is of considerable importance when considering the mode of action of polyphosphates. This will be further discussed in section 4.2.

This ^{31}P-FTNMR technique only became available at the end of this research, and more detailed studies of the distribution and hydrolysis of polyphosphates added to chicken were not possible. However, some of the leg muscles from the batch B and batch C chickens used here were analysed for polyphosphates using this technique, and the results are mentioned in sections 3.42 and 3.791.

3.4 TBA ANALYSIS, MOISTURE CONTENT, AND POLYPHOSPHATE SPECIES AND TOTAL PHOSPHORUS CONTENTS OF RAW AND COOKED PECTORALIS MAJOR, PECTORALIS MINOR AND LEG MUSCLES OF TREATED AND UNTREATED BATCH B CHICKENS AFTER 18 WEEKS STORAGE AT -18°C.

This experiment was designed to investigate the distribution of injected polyphosphates in chicken, since very little published information is available on this subject (see section 1.2). The TBA Numbers of various samples were determined to see if any correlation existed between phosphorus content and degree of oxidation.

The experimental design was as follows:

a) 3 treated and 3 untreated chickens from batch B after 18 weeks storage at -18°C were thawed at 4°C for 18 hours.

b) 1 pectoralis major, pectoralis minor and leg muscle excised from each chicken and stored at -18°C until required.

c) 5g each treated pectoralis major muscle immediately analysed for polyphosphate species by the TLC technique. TBA tests performed on 5g each muscle. Remainder stored at -18°C.

d) 2g each muscle reserved for total phosphorus and water determinations. Remainder cooked by heating in glass jars at 85-90°C for 45 min. Cooled and treated as in c), except remainder stored at 4°C for 2 or 3 days, then TBA tests performed on 5g each muscle.

e) Water determinations and digestion procedure for total phosphorus carried out on 1g amounts of each raw and cooked muscle.

f) c) to e) performed on pectoralis minor and then leg muscles.

g) All digested solutions sent to Albright and Wilson for total phosphorus analyses.

3.41 TBA NUMBERS

Table VIIIa shows that there were no statistically significant differences in TBA Numbers between treated and untreated muscles when raw or freshly cooked. However, all mean values for treated muscles were lower than those for untreated muscles. There were relatively large interchicken variations in TBA Numbers for all 3 types of muscle, and no clear inter-muscle differences were apparent. All values were below 1.00, as would be expected for fresh raw muscles and freshly cooked muscles after a relatively short period of frozen storage.

As expected, the TBA Numbers of cooked treated pectoralis major muscles were significantly lower than those of untreated muscles after 2 days holding. Unfortunately, the TBA Numbers of cooked treated pectoralis minor and leg muscles were not determined, and therefore, the effect of polyphosphate treatment on these 2 muscles could not be determined. The TBA Numbers of these untreated muscles were very high after 3 days holding.

All untreated cooked muscles after 2 or 3 days of holding had very much higher TBA Numbers than those found by Jacobson and Koehlar,[40] (see Table 11, section 1.4). However, these workers used the TBA test of Tarladgis et al,[122] which does not use acid-heat treatment (see section 2.31 for discussion of this point), and lower values may have been expected. However, the freshly cooked values for the present experiment and those of Jacobson and Koehlar were similar. Also, Wilson et al,[138] who used the same TBA test as in the present experiment, found much higher TBA Numbers than the present ones, for both raw and cooked chicken after 0 and 2 days holding at 4°C (see section 3.521 for their cooked values). Thomson,[86] who used the TBA test of Turner et al,[87] reported similar TBA Numbers to the present ones for both treated and untreated cooked muscles during refrigerated storage. It should be noted that each of these 3 sets of workers tested chicken muscles which were

TABLE VIIIa

TBA NUMBERS OF RAW AND COOKED PECTORALIS MAJOR, PECTORALIS MINOR AND LEG MUSCLES OF TREATED AND UNTREATED BATCH B CHICKENS AFTER 18 WEEKS STORAGE AT - 18°C AND DURING HOLDING OF THE COOKED MUSCLES AT 4°C

MUSCLE	TREATMENT															
	Raw[a]								Cooked							
	treated				untreated				treated				untreated			
	1	2	3	M±S.D.[b]	4	5	6	M±S.D.[b]	1	2	3	M±S.D.[b]	4	5	6	M±S.D.
Pectoralis Major	0.86	0.46	0.45	0.58 ± 0.25	0.93	0.53	NT[c]	0.73 ± 0.28	[d]0.71	0.86	0.58	0.72 ± 0.14	0.73	0.58	NT	0.66 ± 0.11
									[e]0.80	0.80	2.60	1.40 ± 1.04	6.64	5.81	8.30	6.92 ± 1.27
Pectoralis Minor	0.28	0.58	0.54	0.47 ± 0.16	0.48	0.61	NT	0.55 ± 0.09	NT	NT	NT		[f]4.83	4.59	5.06	4.83 ± 0.24
Leg	0.40	0.42	0.52	0.45 ± 0.06	0.26	0.71	0.67	0.55 ± 0.25	NT	NT	NT		[f]8.10	7.56	7.38	7.68 ± 0.37

a. Raw values obtained immediately after excision of muscles

b. Mean ± standard deviation

c. Not tested

d. Freshly cooked

e. After holding for 2 days at +4°C

f. After holding for 3 days at +4°C

‡ Difference between treated and untreated significant at 99% level

different in all aspects including source, treatment, cooking method, and storage conditions, from each other and from the present experiment. Therefore, these 3 comparisons highlight the difficulty of comparing TBA Numbers from different sources, owing, not only to the use of different TBA tests, but to the actual samples tested and their treatment.

3.42 TOTAL PHOSPHORUS

Table VIIIb shows the total phosphorus contents of raw and cooked pectoralis major, pectoralis minor and leg muscles. It may be seen that the total phosphorus content of untreated muscles showed very little interchicken variation for each of the 3 types of muscle. The phosphorus levels in all 3 types of cooked untreated muscle were similar to each other, whilst the raw leg muscles contained lower amounts of phosphorus than the other 2 types of raw muscle.

The values for the raw pectoralis major muscles were similar to those obtained by Grey et al,[139] on the entire breast muscle of untreated and commercially treated broilers. The ranges of their values were, 0.37 - 0.44%, and 0.20 - 0.23% for treated and untreated muscles respectively. Their mean values are given in Table VIIIb.

Both raw and cooked pectoralis major and pectoralis minor muscles from treated chickens contained significantly more phosphorus than muscles from untreated chickens. This shows that added phosphorus is present in both these breast muscles, although the major muscles contained more added phosphorus than the minor muscles. The minor muscle is situated behind the major muscle into which the injection is given, and therefore it appears that the injected polyphosphate is not evenly distributed throughout the carcase.

There were no statistically significant differences in phosphorus content between the leg muscles from treated and untreated chickens when they were either raw or cooked. This is in agreement

TABLE V111b

TOTAL PHOSPHORUS CONTENT[a] OF RAW AND COOKED MUSCLES AFTER 18 WEEKS STORAGE AT -18°C

MUSCLE	TREATMENT															
	Raw								Cooked							
	treated				untreated				treated				untreated			
	1	2	3	M±S.D.[b]	4	5	6	M±S.D.	1	2	3	M±S.D.	4	5	6	M±S.D.
Pectoralis Major	0.364	0.364	0.323	0.350 ± 0.024 (0.40)[c]	0.220	0.220	0.226	0.222 ± 0.003‡ (0.23)[c]	0.397	0.382	0.389	0.389 ± 0.008	0.234	0.237	0.246	0.239 ± 0.006‡
Pectoralis Minor	0.255	0.282	0.270	0.269 ± 0.014	0.229	0.224	0.223	0.225 ± 0.003‡	0.308	0.387	0.315	0.337 ± 0.044	0.234	0.228	0.237	0.233 ± 0.005°
Leg	0.184	0.185	0.205	0.191 ± 0.012	0.185	0.191	0.187	0.188 ± 0.003	0.268	0.225	0.252	0.248 ± 0.022	0.240	0.231	0.231	0.234 ± 0.005

a. Values expressed as a percentage of wet muscle

b. Mean ± standard deviation

c. Figures in brackets are results of Grey et al,[139]

° Difference between treated and untreated significant at 95% level

‡ " " " " " " " 99% level

‡‡ " " " " " " " 99.9% level.

with results obtained in the experiment reported in section 3.32. These were, that no added phosphates were found in the leg muscles from treated chickens which had been stored at -18°C for 5 or 15 months.

These findings that added phosphate is not reaching the leg muscles of chickens injected with polyphosphates under normal commercial conditions has not been reported in such detail before, although the inconclusive evidence of Truman and Dickes,[21] did point to this fact (see section 1.2); and, also, Grey et al[18] stated that leg muscles did not contain added phosphates, but they gave no experimental values (see section 1.33). However, it is recognised that turkeys so treated require injection into the leg, as well as the breast muscles, to ensure adequate distribution (see section 1.2).

Each cooked muscle contained a higher concentration of phosphorus than the corresponding raw muscles. Since the results were expressed as a percentage of wet tissue weight, this higher concentration in cooked muscle is due to the fact that the ratio of phosphorus to muscle is increased in the cooked muscle because of moisture loss during cooking. It was not possible to accurately determine if phosphorus was lost during cooking by comparing dry muscle values, because the cook losses were only calculated approximately from cook-out juice volumes. However, from the approximate figure obtained, it appeared that sample number 1 lost about 3.5% phosphorus during cooking, number 2 about 6.5%, and number 3 did not appear to have lost any phosphorus. All untreated pectoralis major muscles lost phosphorus during cooking, but the values are not given because they were approximate. For this reason, phosphorus losses of the other 2 types of muscle were not calculated. The reason for quoting treated pectoralis major results is that these may be compared with the TBA Numbers of the cooked muscles (see section 3.43), whereas the TBA Numbers of the other 2 types of cooked muscle were not determined.

3.43 CORRELATION COEFFICIENTS BETWEEN TBA NUMBERS AND TOTAL PHOSPHORUS

Since polyphosphates reduce the TBA Numbers of cooked muscles, a correlation coefficient between phosphorus content and TBA Numbers may be expected to exist. In fact, Thomson,[86] reported a significant negative correlation between percentage phosphorus and TBA Numbers of polyphosphate treated commercially cooked fryer chickens, but the phosphorus results to support this claim were not given.

In the present work, a correlation coefficient of -0.49 was found between TBA Numbers for freshly cooked treated pectoralis major muscles and their phosphorus content (% wet tissue). This coefficient was not statistically significant. Since the correlation was negative, it would appear that high phosphorus levels in cooked muscles, which presumably arise from high levels of treatment, result in reduced TBA Numbers. However, because of the effect of cook losses, as discussed in the previous section, a more valid comparison would be obtained by taking the phosphorus content as a percentage of dry muscle weight. When the approximate (see section 3.42) dry weight figures were used, a negative correlation coefficient of similar magnitude to the above was found.

However, these correlations assume that there is no inter-chicken variation in natural phosphorus content. When this is taken into account by comparing the difference in phosphorus content between the raw and cooked treated muscles and their TBA Numbers, negative correlations were again obtained. In this case the correlation coefficient was -0.96 when phosphorus was calculated as both a percentage of wet and dry muscle. Despite this high numerical value, the correlation was not statistically significant, ~~presumably~~ because the sample size was small. This correlation means that as the difference in phosphorus content between raw and cooked muscles increases, the TBA Number of the freshly cooked muscles decreases. Since muscles lose

phosphorus during cooking (see section 3.42), it follows that, the greater the amount of phosphorus lost during cooking, the lower the TBA Number of the cooked muscle is. However, sample number 3, which did not appear to lose any phosphorus during cooking, and has a lower TBA Number than the other 2 muscles when freshly cooked had a much higher TBA Number than the other 2 muscles after holding for 2 days.

It is obvious that phosphorus levels alone cannot adequately explain these findings, since, for instance, loss of phosphorus during cooking may be related to loss of prooxidant metal ions. These points are discussed further in sections 3.710 and 3.711, where more detailed ionic analyses were performed.

3.44 MOISTURE CONTENT

Table VIIIc shows that treated pectoralis major muscles contained significantly more water than the untreated muscles, presumably as a direct result of polyphosphate injection. There was no difference, however, between treated and untreated pectoralis minor muscles, despite the fact that treated muscles contained significant amounts of added phosphorus. There were no differences between treated and untreated leg muscles, although the values were higher than previously reported by Truman and Dickes.[21]

3.45 POLYPHOSPHATE SPECIES

No tripolyphosphate was found in any of the muscles immediately after thawing, and diphosphate was detected in only 1 of the treated pectoralis major muscles. However, polyphosphates higher than tripolyphosphate were found in each of the treated raw and cooked pectoralis major and pectoralis minor muscles; but none were detected in any of the treated leg muscles, which corresponds to the findings that leg muscles contain no added total phosphorus.

Even allowing for the relative insensitivity of the TLC method used here, compared to the ^{31}P-FTNMR method used in the other experiment

TABLE VIIIC

MOISTURE CONTENT[a] OF RAW MUSCLES AFTER 18 WEEKS STORAGE AT -18°C

MUSCLE	TREATMENT							
	Treated				Untreated			
	1	2	3	Mean ± standard deviation	4	5	6	Mean ± standard deviation
Pectoralis Major	75.9	74.7	75.5	75.4 ± 0.6	71.7	71.7	72.1	71.8 ± 0.2‡‡
Pectoralis Minor	71.4	70.0	68.5	70.0 ± 1.1	69.7	71.4	70.9	70.7 ± 0.9
Leg	76.7	75.6	75.4	75.9 ± 0.7	74.9	73.0	74.9	74.3 ± 1.1

a. Values expressed as a percentage of wet muscle

‡‡ Difference between treated and untreated significant at 99.9% level.

(see section 3.32), these findings for pectoralis major muscles were not expected. These present chickens had been stored at -18°C for only 18 weeks, and previously (see section 3.32), very little hydrolysis had taken place in pectoralis major muscles during 5 months storage at -18°C. Since the present breast muscles contained significant amounts of added total phosphorus, it would seem that rapid hydrolysis of polyphosphates does occur during thawing; as suggested by O'Neill and Richards,[110] (see section 1.64). This present experiment, therefore, was probably not valid; but it was performed before the ^{31}P-FTNMR technique and other workers findings, were discovered. It should be mentioned here that no added phosphates were detected by ^{31}P-FTNMR in leg muscles from treated chickens which had been in frozen storage for 5 and 15 months (see section 3,32 for experimental details).

3.5 TBA ANALYSIS OF RAW AND COOKED PECTORALIS MAJOR AND LEG MUSCLES FROM TREATED AND UNTREATED BATCH B CHICKENS AFTER 6 MONTHS STORAGE AT -18°C DURING HOLDING AT 4°C AND -18°C; AND CALCULATION OF COOK LOSSES

This experiment was carried out to obtain more TBA results of raw chicken muscles because of the so far conflicting findings of the effect of polyphosphates on these TBA Numbers. Also, it was necessary to obtain TBA results of cooked leg muscles to support the findings that leg muscles from treated chickens contain no added polyphosphates.

In addition the cook losses of the muscles were calculated.

The experimental design was as follows:

a) 3 treated and 3 untreated chickens from batch B after 6 months storage at -18°C were thawed at room temperature for 22 hours.

b) Both pectoralis major muscles excised from each chicken and stored at -18°C until required.

c) 1 pectoralis major muscle from each chicken minced.

d) TBA tests performed on 5 g each muscle. Half of remainder stored at 4°C and half at -18°C.

e) TBA tests performed on 5 g each during 11 days storage at 4°C and 3 months storage at -18°C.

f) Other pectoralis major muscle from each chicken cooked by heating in glass jars at 85-90°C for 45 min. Cooled and minced, and then treated as in d) and e).

g) Micro-organism content of some muscles determined (see Appendix 1)

h) Performed b) to g) on leg muscles.

3.51 COOK LOSSES

Table 1Xa shows that there were no significant differences in cook losses between treated and untreated pectoralis major or leg muscles, although polyphosphates are known to decrease cook losses (see

TABLE 1Xa

COOK LOSSES[a] OF PECTORALIS MAJOR AND LEG MUSCLES OF TREATED AND UNTREATED BATCH B CHICKENS AFTER 6 MONTHS STORAGE AT - 18°C

MUSCLE	TREATMENT							
	treated				untreated			
	1	2	3	M±S.D.[b]	4	5	6	M±S.D.[b]
Pectoralis Major	32.5	27.2	27.1	28.9 ± 3.1	27.0	25.9	24.8	25.9 ± 1.1
Leg	26.8	26.7	27.7	27.1 ± 0.6	24.8	29.5	29.3	27.9 ± 2.7

a. Values expressed as a percentage of raw muscle

b. Mean ± standard deviation.

section 1.33). However, the steam method of cooking used here, may have been expected to mask any differences due to treatment because of the saturated water vapour atmosphere. In fact, Table 1a shows that the cook loss of 1 treated pectoralis major muscle after roasting with no added fat, was much lower than that of 1 untreated muscle. Also, it seems likely that increased cook yields of whole and portioned chickens (as discussed in section 1.33), are not reflected in the individual muscles because skin, tendons, and connective tissue have been removed.

3.52 TBA NUMBERS

3.521 During Holding at 4°C

Table 1Xb shows the TBA Numbers of cooked muscles during holding at 4°C, and Table 1Xc, the TBA Numbers of raw muscles during holding at 4°C.

For cooked muscles, the difference between treated and untreated pectoralis major muscles was always highly significant throughout 9 days of holding. This again indicates that polyphosphates inhibit auto-oxidation in cooked chicken tissues (cf. Tables Vc and VIIIa). However, there were no significant differences between treated and untreated leg muscles throughout 5 days of holding. This was the first time in this work that the TBA Numbers of cooked leg muscles had been determined. This lack of difference between leg muscles from treated and untreated chickens clearly corresponds to the findings in sections 3.42 and 3.791, that injected polyphosphate does not reach the leg muscles.

By comparing these results from pectoralis major and leg muscles, it may be seen that the TBA Numbers of leg muscles after 2 days of holding were very much lower than those of untreated pectoralis major muscles after 1 day. However, the values for leg muscles after 5 days were higher than those of untreated pectoralis major muscles after 7 and 9 days. Thus, cooked leg muscles were found to undergo auto-oxidation at a slower rate than pectoralis major muscles. This delayed onset of rancidity in leg muscles compared to pectoralis major muscles was not found by Wilson et al,[138] whose mean TBA Numbers for 3 cooked minced, muscles were, 3.96 ± 1.03 for dark muscles and 3.13 ± 1.76 for white muscle immediately after cooking. These values rose to 9.20 ± 0.69 and 8.60 ± 0.72, respectively, after 2 days holding at 4°C (however, see discussion in section 3.41, about the difficulty of comparing TBA Numbers from different sources).

TABLE 1Xb

TBA NUMBERS OF COOKED MUSCLES DURING HOLDING AT 4°C

DAYS HELD AT 4°C	MUSCLE															
	Pectoralis Major								leg							
	treated				untreated				treated				untreated			
	1	2	3	M±S.D.[a]	4	5	6	M±S.D.	1	2	3	M±S.D.	4	5	6	M±S.D.
1	0.90	1.25	1.33	1.16 ± 0.23	5.27	8.64	8.57	7.49‡‡ ± 1.93	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-		2.43	3.01	3.50	2.98 ± 0.54	1.79	2.82	3.45	2.67 ± 0.84
5	-	-	-	-	-	-	-	-	12.53	20.96	18.72	17.40 ± 4.37	11.59	13.64	19.16	14.80 ± 3.92
7	0.79	1.11	1.99	1.30 ± 0.62	8.89	11.52	12.38	10.93‡‡ ± 1.82	-	-	-	-	-	-	-	-
9	0.54	1.62	1.95	1.37 ± 0.74	9.11	12.06	12.60	11.26‡‡ ± 1.88	-	-	-	-	-	-	-	-

a. Mean ± standard deviation

‡ Difference between treated and untreated significant at 99% level

‡‡ " " " " " " " 99.9% level

TABLE 1Xc

TBA NUMBERS OF RAW MUSCLES DURING HOLDING AT 4°C

DAYS HELD AT 4°C	MUSCLE															
	Pectoralis Major								Leg							
	treated				untreated				treated				untreated			
	1	2	3	M±S.D.[a]	4	5	6	M±S.D.[a]	1	2	3	M±S.D.	4	5	6	M±S.D.[a]
2	1.05	1.04	1.30	1.13 ± 0.15	1.33	1.56	1.12	1.34 ± 0.22	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-		0.97	1.07	0.96	1.00 ± 0.006	0.55	0.96	0.54	0.68 ± 0.24
5	2.55	3.75	2.82	3.04 ± 0.63	2.68	1.40	1.93	2.00 ± 0.64	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-		1.47	2.24	1.10	1.60 ± 0.58	1.21	1.13	0.54	0.96 ± 0.37
8	2.66	4.57	3.68	3.64 ± 0.96	2.25	2.82	1.25	2.11 ± 0.79	-	-	-	-	-	-	-	-
9	3.03	4.04	4.03	3.70 ± 0.58	1.90	3.93	1.40	2.41 ± 1.34	-	-	-	-	-	-	-	-
11									0.82	1.84	0.94	1.20 ± 0.56	0.75	1.71	0.86	1.11 ± 0.53

a. Mean ± standard deviation

For raw muscles, the mean TBA Numbers of treated muscles were generally higher than those of untreated muscles, but these differences were not significant. Therefore, polyphosphate treatment was not found to inhibit autooxidation in raw muscles, as it was in section 3.1 (also, cf. Table Xa).

By comparing these results from pectoralis major and leg muscles, it may be seen that the mean TBA Numbers of leg muscles were lower than those of pectoralis major muscles throughout the 11 day holding period. This would seem to correspond to the discussion in section 3.26, that phospholipids are protected against autooxidation in raw muscles by their close association with muscle proteins. This is because leg muscles contain a greater amount of phospholipids than pectoralis major muscles (Table VIa), and therefore autooxidation would proceed less rapidly in leg muscles than pectoralis major muscles. However, from the discussion in section 3.26, it may be seen that neutral lipids are presumed to play a major role in raw muscle oxidation. Since leg muscles contain much larger amounts of neutral lipids than pectoralis major muscles, (Table VIa) it may be expected that raw leg muscles would be more susceptible to oxidation than raw pectoralis major muscles. Because they have been found not to be, must be a reflection of the small polyunsaturated fatty acid content of neutral lipids compared to phospholipids.

It is surprising to note that the TBA Numbers of raw treated pectoralis major muscles were higher than those of cooked treated pectoralis major muscles, which were very low throughout the holding period. Thomson,[86] also found that cooked treated muscles had very low TBA Numbers throughout 14 days of refrigerated storage (see section 1.43 for the values). Heat would be expected to accelerate the auto-oxidative process, resulting in a higher degree of rancidity in cooked muscles compared to raw muscles. That this was not found to be the

case, could be because the cooked muscles were in the latter stages of rancidity when the amount of malonaldehyde had been depleted (see section 2.31), leading to low TBA Numbers. However, this does not explain why the untreated cooked muscles had very high TBA Numbers throughout the holding period.

It seems that polyphosphate treatment could result in cooked muscles having very low TBA Numbers by some mechanism other than inhibition of autooxidative rancidity. For instance, polyphosphates may act by destroying carbonyl by-products of autooxidation and not by inhibiting oxygen attack on fatty acids. From the present experiment it is not possible to determine absolutely which, if either, of these 2 mechanisms is correct. However, since raw treated muscles have very similar TBA Numbers to untreated muscles, it seems that polyphosphates do not directly interfere with the initial stages of autooxidation. Rao et al,[90,91] also encountered difficulties in interpreting their carbonyl results which are reported in section 1.52.

3.522 <u>During Holding at -18°C</u>

Table 1Xd shows the TBA Numbers of raw muscles during 4 months holding at -18°C, and Table 1Xe the TBA Numbers of cooked muscles during 3 months holding at -18°C.

For raw muscles, it may be seen that there were no significant differences between treated and untreated muscles. This again suggests that polyphosphates do not inhibit autooxidation in raw muscles. For pectoralis major muscles, the TBA Numbers after 4 months at -18°C were similar to those obtained after 2 days holding at 4°C. This suggests that little or no oxidation had occurred during 4 months frozen storage. For leg muscles, however, the mean values obtained after 4 months at -18°C, were much higher than those obtained after 11 days holding at 4°C. These leg muscle values during frozen storage were very variable, ranging from 0.86 to 9.11. The only explanation which may be offered for this variability is that, blood vessels could not always be easily

TABLE 1Xd

TBA NUMBERS OF RAW MUSCLES DURING HOLDING AT -18°C

MONTHS HELD AT -18°C	1 MUSCLE	TREATMENT							
		treated				untreated			
		1	2	3	M±S.D.[a]	1	2	3	M±S.D.[a]
4	Pectoralis Major	1.20	1.70	1.49	1.46 ± 0.25	1.27	1.21	1.21	1.23 ± 0.04
	Leg	4.11	5.49	1.58	3.73 ± 1.98	9.11	0.86	1.28	3.75 ± 4.65

a. Mean ± standard deviation

TABLE 1Xe

TBA NUMBERS OF COOKED MUSCLES DURING HOLDING AT -18°C

MONTHS HELD AT -18°C	MUSCLE	TREATMENT							
		treated				untreated			
		1	2	3	M±S.D.[a]	4	5	6	M±S.D.[a]
3	Pectoralis Major	0.99	1.16	1.74	1.30 ± 0.39	7.35	10.73	11.17	9.75 ± 2.09
	Leg	20.59	29.00	26.46	-	18.85	24.87	25.88	-

a. Mean ± standard deviation

separated from the leg muscles during excision and mincing. Thus, samples of leg muscles probably contained varying amounts of pro-oxidant haem compounds, which contributed to increased development of rancidity during 4 months at -18°C, but not to such a marked extent during 11 days at 4°C (cf. Table 1Xc).

For cooked muscles, the difference between treated and untreated pectoralis major muscles was highly significant, but this was not true of leg muscles. These findings are as expected from the results of cooked muscles during holding at 4°C. The values for leg muscles were very much higher than those of pectoralis major muscles, which corresponds to the results obtained on the 5th day of holding at 4°C (see section 3.521). By comparing the results at 4°C and -18°C, it also appeared that autooxidation had proceeded in the leg muscles during frozen storage, and, perhaps, in the untreated pectoralis major muscles.

Thus, autooxidation was found to have occurred in untreated cooked muscles during 3 months frozen storage, but not in raw muscles (except for some leg muscles), see above) during 4 months frozen storage. This obviously reflects the greater susceptibility of cooked muscles to autooxidation compared to raw muscles.

3.6 TBA ANALYSIS OF RAW PECTORALIS MAJOR AND LEG MUSCLES OF TREATED AND UNTREATED BATCH A CHICKENS AFTER 47 MONTHS STORAGE AT -18°C; AND PRELIMINARY THAW LOSS STUDIES.

This experiment was carried out to see if during further prolonged frozen storage, raw treated batch A chickens were protected against autooxidation as onewas found to be after 21 months frozen storage (section 3.1). Subsequent TBA analyses of raw muscles did not conclusively show this protection of raw treated muscles (see sections 3.24, 3.41 and 3.52).

The experiment was carried out as follows:

Two treated and 2 untreated chickens from batch A after 47 months storage at -18°C were placed on 2 trays and thawed at room temperature for 16 hours. The pectoralis major and leg muscles from 1 side of each chicken were excised and minced. All muscles gave off rancid odours. TBA tests were carried out during holding at 4°C.

3.61 TBA NUMBERS

Table Xa shows the TBA Numbers of treated and untreated pectoralis major and leg muscles during holding at 4°C for 8 days. The mean TBA Numbers of treated muscles were lower than those of untreated muscles for both pectoralis major and leg muscles throughout the 8 day holding period. These differences were not statistically significant, due in part no doubt to the small sample size and relatively large standard deviations. However, it should be noted that except for the 8th day of storage, both treated pectoralis major muscles had lower TBA Numbers than the 2 untreated muscles. This was not true of the leg muscles.

For both pectoralis major and leg muscles, all values were lower than those of batch B chickens after 6 months storage at -18°C, during holding at 4°C (section 3.521), Table IXc). A possible explanation of this is that the present chickens had undergone considerable auto-oxidation during their 47 months of frozen storage (all muscles gave off rancid odours after excision); and malonaldehyde, the autooxidation

TABLE Xa

TBA NUMBERS OF RAW PECTORALIS MAJOR AND LEG MUSCLES OF TREATED AND UNTREATED BATCH A CHICKENS AFTER 47 MONTHS' STORAGE AT - 18°C, DURING HOLDING AT 4°C.

DAYS HELD AT 4°C	MUSCLE											
	Pectoralis Major						Leg					
	Treated			Untreated			Treated			Untreated		
	1	2	M±S.D.[a]	3	4	M±S.D.[a]	1	2	M±S.D.	3	4	M±S.D.[a]
0	0.47	0.48	0.48 ± 0.01	0.78	0.60	0.69 ± 0.13	-	-		-	-	
1	-	-		-	-		0.37	0.40	0.39 ± 0.02	0.29	0.67	0.48 ± 0.27
4	0.59	0.58	0.59 ± 0.01	1.62	0.81	1.22 ± 0.57	-	-		-	-	
5	-	-		-	-		1.01	0.70	0.86 ± 0.22	0.65	2.38	1.52 ± 1.22
8	0.80	0.29	0.55 ± 0.36	1.33	0.60	0.97 ± 0.52	0.98	0.77	0.88 ± 0.15	0.60	3.02	1.81 ± 1.71

a. Mean ± Standard deviation

product which is measured in the TBA test, is not a stable end-product of autooxidation (see section 2.31). Therefore, lower concentrations of this aldehyde would be expected to be present in rancid muscles compared with those which had only been stored for 6 months at -18°C. However, if this were the case, then it may seem inconsistent that the TBA Numbers of the present muscles increased during holding. It should be remembered, however, that TBA Numbers have been shown to vary inconsistently during holding (see Table 1Xc); and, also, that the level of malonaldehyde present at any one time is thought to depend on the amount of available oxygen.[120] It seems reasonable to assume that auto-oxidation during 47 months frozen storage leads to the production (and destruction) of near the maximum amount of malonaldehyde from the most oxidation-susceptible fatty acids, whilst the more inert acids undergo oxidation during holding at 4°C.

These TBA results agree with those of sections 3.24 (Table Vle), 3.41 (Table Vlla), and 3.521 (Table 1Xc), in that no definite inhibition of autooxidation in raw treated muscles has been found. In particular, this experiment failed to confirm the results of experiment 3.1, where it was found that an untreated pectoralis major muscle from this batch of chickens after 21 months of frozen storage, had very much higher TBA Numbers throughout 7 days holding at 4°C than a treated muscle.

3.62 TOTAL HAEM CONTENT OF THAW LOSS

The combined thaw losses from the treated chickens seemed to contain more blood than the combined thaw losses of the untreated chickens. This observation was confirmed by measurement of the total haem content of aliquots of both thaw losses by the method of section 2.6. The values (absorbance x total volume) for treated and untreated thaw losses were 5.7 and 2.6 respectively.

This difference in thaw loss composition between treated and untreated chickens had not been noticed in earlier experiments, and no accounts

have been found in the literature. It seems likely that disruption of muscle protein structure caused by polyphosphates (see section 1.6), would lead to loss of haemoproteins. Also, removal of prooxidant haem compounds would be expected to inhibit autooxidation, and, therefore, these observations have very important implications. Further studies on the total haem content of thaw losses are reported in section 3.72.

3.7 TBA ANALYSIS AND LIPID, FATTY ACID AND IONIC (PHOSPHORUS, IRON, SODIUM, CALCIUM, AND MAGNESIUM) COMPOSITION OF COOKED TREATED AND UNTREATED BATCH C CHICKEN MUSCLES AND JUICES AFTER 2 WEEKS STORAGE AT -20°C; AND CALCULATION OF THEIR COOK-LOSSES, AND OF THE UPTAKE OF CHILL WATER FOR BATCH A AND BATCH C CHICKENS.

Most of the previous experiments were carried out on chickens which had been in frozen storage for longer periods than would be used in the retail trade. It was therefore thought necessary to check some of the previous findings against those obtained from chickens after short term frozen storage. Also, previous fatty acid composition results of raw muscles failed to distinguish between treated and untreated samples, and it was thought that results of cooked muscles would be far more likely to do this. In addition to repeating previous analyses, the ionic composition of muscles and juices were determined in order to check conflicting literature reports of the metal chelation properties of polyphosphates in meat. Sodium analyses were carried out to see if this ion was distributed throughout the carcase in a similar way to polyphosphate.

The experiment was carried out as follows:

Six treated (T1 to 6) and 6 untreated (U1 to 6) chickens from batch C after 2 weeks storage at -20°C, were inverted in large funnels and thawed at room temperature for 16 hours, and the thaw-drips collected in measuring cylinders. The amount of chill water these chickens had absorbed was known from weights taken in the factory. The pectoralis major and leg muscles from 1 side of each chicken were excised and stored at -20°C until required for cooking, when they were thawed and cooked by heating in glass jars at 85 - 90°C for 45 minutes. The muscles were cooled, minced, and divided into portions for the various analyses. At all times the muscles were returned to the freezer as quickly as possible when not immediately required for analysis, except for the muscles which were allowed to become rancid by leaving at room temperature for 5 days. Phosphorus, iron, sodium, calcium and magnesium (P,Fe,Na,Ca, and Mg,

respectively) determinations were carried out by Albright & Wilson Ltd. on solutions prepared as in section 2.51.

The analyses were performed on various muscles and juices as follows:

SAMPLE	ANALYSES
Thaw loss	Total haem and P,Fe,Na,Ca, and Mg content of T1 to 6 and U1 to 6 .
Freshly cooked muscle	Lipid composition and fatty acid composition of the neutral lipid and phospholipid of pectoralis major muscles of T1, 2 and 3, and U1, 2 and 3.
	P,Fe,Na,Ca, and Mg content of pectoralis major muscles of T1 to 6 and U1 to 6, and of leg muscles of T1 to 6 and U1 to 6.
	TBA Numbers of pectoralis major of T1, 2 and 3, and U1, 2 and 3, and of leg muscles of T1 to 6 and U1 to 6, after 2 and 6 weeks storage at -20°C, respectively.
Cook-out juices	Fatty acid composition of total lipid, and P,Fe, Na,Ca, and Mg content of pectoralis major muscles T1 to 6 and U1 to 6, and of leg muscles T1 to 6 and U1 to 6.
Rancid cooked muscle	Lipid composition of pectoralis major muscles T1 to 6 and U1 to 6.
	Fatty acid composition of neutral lipid and phospholipid of pectoralis major muscles T1, 2 and 3, and U1, 2 and 3; and of total lipid of pectoralis major muscles T4, 5 and 6, and U4, 5 and 6.

3.71 UPTAKE OF CHILL WATER

Table XIa shows that there was no significant difference in the amount of chill water absorbed between treated and untreated chickens; although the mean value for treated chickens was lower than that for untreated chickens. However, the mean values for the total number of 11 treated and 11 untreated batch C chickens were, 4.8 ± 1.0 and 6.2 ± 1.2, respectively, and this difference was statistically significant. Also, the mean values for the total number of 12 treated and 12 untreated batch A chickens were, 3.0 ± 1.0 and 5.2 ± 2.5, respectively, and this difference was statistically significant. The chill water uptakes of batch B chickens were not known.

Uptake of chill water is governed by the conditions in the chill tank, and by the physical characteristics of the carcase (see section 1.32).

TABLE X1a

UPTAKE OF CHILL WATER, AND AMOUNT AND TOTAL HAEM CONTENT OF THAW LOSSES FROM TREATED AND UNTREATED BATCH C CHICKENS AFTER 2 WEEKS STORAGE AT - 20°C

Treatment	Uptake of chill water[a,d]	Thaw loss[b,d,]	Total Haem in thaw liquor[c,d]
treated	5.1 ± 1.2	4.9 ± 0.7	2.6 ± 0.3
untreated	5.6 ± 0.9	4.4 ± 0.7	2.3 ± 0.4

a. Percentage increase (of eviscerated weight + injection weight) after chill and drip

b. Percentage of frozen carcase

c. Absorbance at 415mm (concentration) x total volume

d. Mean ± standard deviation of 6 chickens.

TABLE X1b

COOK LOSSES[a] OF PECTORALIS MAJOR AND LEG MUSCLES

TREATMENT	MUSCLE	
	Pectoralis Major	Leg
treated[b]	23.6 ± 1.1	27.8 ± 1.2
untreated[b]	23.1 ± 1.5	25.1 ± 2.5

a. Values expressed as a percentage of raw muscle.

b. Mean ± standard deviation of 6 muscles.

Therefore, the differences in the levels of uptake between batch C and and batch A chickens were to be expected. However, it could be significant that treated batch A chickens, which had absorbed less chill water than treated batch C chickens, had received a larger percentage injection than batch C chickens (the mean values were, 9.6% and 5.1% respectively). There was no relationship, however, between the levels of injection and amount of chill water uptake for the batch A and batch C chickens separately. This may have been because there was little interchicken variation in injection levels for each of the separate batches of chickens.

These results are in agreement with those of Grey et al,[18] in that commercial polyphosphate injection has no consistent effect on the level of chill water uptake (see section 1,33). However, their mean value for percentage chill water uptake of 1019 untreated chickens was 7.8%, and of 957 treated chickens 7.6%. These 2 values are very similar to each other, but they seemed to have calculated them as:- weight after chilling -(eviscerated weight + injection weight) ÷ eviscerated weight only x 100; and not as in the present experiment:- ÷ eviscerated weight + injection weight. Thus, their method of calculation gives higher treated values than would be obtained by the present method. The present results may be explained by considering the following:

Water taken up during chilling is thought to accumulate between the skin and tissue, and to only slowly diffuse into the muscle.[27] Presumably, therefore, only a certain amount of water may be readily absorbed during the 30 to 45 minutes that these chickens spent in the chill tank. The injected chickens already contain added water, some of which has, almost certainly, been absorbed by the subcutaneous tissue. Therefore, it is suggested that, polyphosphate injected chickens absorb less chill water than untreated chickens because the subcutaneous tissue, the major site of water accumulation, is partly hydrated. This theory

is supported by the fact that chickens from batch C which had received 5% injections of water only, absorbed less chill water than uninjected chickens. In fact, their mean value (of 6 chickens) was 5.1% which was very similar to the mean value of 4.8% for polyphosphate injected chickens.

3.72 AMOUNT AND TOTAL HAEM CONTENT OF THAW LOSSES

Table XIa shows that there was no significant difference in the amount of thaw loss between treated and untreated chickens. This contradicts the results shown in Table Va and the results of Van Hoof and Daelman,[17] (see section 1.33), where treated chickens were found to have reduced thaw losses compared to untreated chickens. However, Grey et al,[18] and Truman and Dickes,[21] found no significant difference in thaw loss values between treated and untreated chickens, (see section 1.33).

It is impossible to account for the differences between the present results and those from other sources, because the WHC of meat is subject to several factors (see section 1.31 and 1.32).

Table XIa also shows that there was no significant difference in the total haem content of the thaw losses between treated and untreated chickens, although the mean value for treated chickens was greater than that for untreated chickens. In addition, both mean values were similar to that of untreated thaw losses from chickens after 47 months frozen storage (see section 3.62). However, these latter treated chickens lost more than twice as much total haem than the present treated chickens, which had been frozen for only 2 weeks. It would seem, therefore, that polyphosphate-induced denaturation of muscle proteins which leads to loss of haem compounds (see section 3.62) occurs during extended frozen storage but not during short-term storage.

Therefore, polyphosphate treatment was found to have no effect on either the amount or total haem content of thaw losses after short-term frozen storage.

3.73 COOK LOSSES

Table XIb shows that there was no significant difference in cook loss between treated and untreated pectoralis major or leg muscles. These results correspond to those of section 3.51 (Table IXa), where they are discussed with respect to the method of cooking.

3.74 LIPID COMPOSITION OF PECTORALIS MAJOR MUSCLES

Table XIc shows the total lipid, neutral lipid and phospholipid contents of treated and untreated pectoralis major muscles, when freshly cooked and rancid. It may be seen that there were no significant differences between treated and untreated muscles for any of the lipid fractions. Also, there were no consistent differences in individual muscles between the values for freshly cooked and rancid samples (for example, not all rancid muscles had decreased lipid levels compared to freshly cooked muscles).

It may be seen that the lipid composition of these cooked pectoralis major muscles shows greater interchicken variation than that of raw muscles, as determined in section 3.21, Table VIa, This was to be expected, since cook-out juices contain some lipid material (see section 3.754); and, also, loss of fluid on cooking will lead to variations in the ratio of lipid to wet muscle. Because of the effects of factors such as age, diet and storage time on the lipid composition of chicken muscles (see section 1.41) it was not possible to directly compare these results with those of raw muscles in section 3.2.

3.75 FATTY ACID COMPOSITION

3.751 Freshly Cooked Pectoralis Major Muscles

Table XId shows that the neutral lipid of freshly cooked T1 and T3 contained no C18:2 and very high levels of C16:0. However, C18:2 was present in the neutral lipid of these rancid same muscles (Table XIf), and also in the phospholipid fractions of both freshly cooked and rancid samples. This suggests that these anomalous results

TABLE XIc

TOTAL LIPID, NEUTRAL LIPID AND PHOSPHOLIPID CONTENT OF FRESHLY COOKED AND RANCID PECTORALIS MAJOR MUSCLES

LIPID	STATE OF MUSCLE	TREATMENT													
		treated							untreated						
		T1	T2	T3	T4	T5	T6	M ± S.D.[a]	U1	U2	U3	U4	U5	U6	M ± S.D.[a]
total lipid[b]	freshly cooked	0.86	1.00	1.15	-	-	-	1.00 ± 0.15	1.33	0.82	1.20	-	-	-	1.12 ± 0.27
	rancid cooked	1.01	0.75	1.07	-[c]	0.73	-[c]	0.89 ± 0.18	1.08	0.94	1.02	1.04	0.95	0.81	0.97 ± 0.10
Neutral lipid[b]	freshly cooked	0.40	0.37	0.58	-	-	-	0.45 ± 0.11	0.64	0.38	0.61	-	-	-	0.54 ± 0.14
	rancid cooked	0.49	0.27	0.56	-	-	-	0.44 ± 0.15	-[c]	0.44	0.53	-	-	-	0.49 ± 0.06
phospho-lipid[b]	freshly cooked	0.46	0.63	0.57	-	-	-	0.55 ± 0.09	0.69	0.44	0.59	-	-	-	0.57 ± 0.13
	rancid cooked	0.52	0.48	0.51	-	-	-	0.50 ± 0.02	-[c]	0.50	0.50	-	-	-	0.50 ± 0.00

a. Mean ± standard deviation

b. Percentage of cooked tissue

c. Spoiled

TABLE XId

FATTY ACID COMPOSITION[a] OF NEUTRAL LIPID AND PHOSPHOLIPID OF FRESHLY COOKED PECTORALIS MAJOR MUSCLES

FATTY ACID	LIPID													
	NEUTRAL						PHOSPHOLIPID							
	treated			untreated			treated				untreated			
	T1	T2	T3	U1	U2	U3	T1	T2	T3	M±S.D.[b]	U1	U2	U3	M±S.D.[b]
C14:0	1.7	1.3	2.0	1.3	1.3	1.4	0.9	0.9	0.9	0.9 ± 0.0	0.8	0.7	0.6	0.7 ± 0.1
C14:2	-	-	-	-	-	-	5.3	6.5	2.6	4.8 ± 2.0	5.6	4.1	4.1	4.6 ± 0.9
C16:0	41.4	29.7	47.3	27.1	27.7	28.3	32.4	30.6	33.7	32.2 ± 1.6	27.8	31.5	33.5	30.9 ± 2.9
C16:1	2.6	4.7	1.7	5.3	6.1	5.8	-	-	-	-	-	-	-	-
C18:0	10.1	7.1	11.1	6.5	6.2	6.5	9.6	10.0	10.8	10.1 ± 0.6	11.6	10.6	10.1	10.8 ± 0.8
C18:1	38.5	38.2	36.0	41.0	39.8	40.0	27.4	25.4	30.1	27.6 ± 2.4	27.9	26.6	28.4	27.6 ± 0.9
C18:2	-	14.7	-	14.7	14.6	14.7	14.1	14.0	13.3	13.8 ± 0.4	15.7	14.8	14.6	15.0 ± 0.6
C.20:0	3.3	0.3	0.6	0.2	0.4	0.3	0.4	0.2	0.3	-	0.2	0.4	0.4	-
C18:3	1.6	2.3	1.6	2.6	2.5	2.4	0.3	0.3	0.4	-	0.4	0.2	0.6	-
C20:4	0.2	0.9	-	0.8	0.8	0.7	3.4	5.5	3.8	4.2 ± 1.1	4.8	4.3	3.5	4.2 ± 0.7
C_B	0.6	0.8	-	0.6	0.5	-	6.2	6.6	4.3	5.7 ± 1.2	5.2	6.8	4.4	5.5 ± 1.2
% total unsaturated	43.5	61.6	39.3	65.0	64.3	63.6	56.7	58.3	54.5	56.5 ± 1.9	59.6	56.8	55.6	57.3 ± 2.1
% total unsaturated / C16:0	1.1	2.1	0.8	2.4	2.3	2.3	1.8	1.9	1.6	1.8 ± 0.2	2.1	1.8	1.7	1.9 ± 0.2

a. Values expressed as % of total fatty acids

b. Mean ± standard deviation

were due to experimental error, presumably during the methylation stage, since both of these methylation mixtures were black after reaction. Because of these 2 anomalous results, it was not possible to compare treated and untreated neutral lipid results.

There were no significant differences in the fatty acid composition of phospholipid fractions between treated and untreated muscles; even when the inherent interchicken variations were partially allowed for by dividing the total amount of unsaturated fatty acids by the amount of C16:0 (see section 3.22).

The phospholipids of both treated and untreated muscles were very much richer in C20:4 and C_B than raw muscles after long-term frozen storage (see section 3.22, Table VIb). This suggests that auto-oxidation had proceeded to some extent during frozen storage of these raw muscles; although the amounts of total unsaturated fatty acids were similar for both these raw muscles and the present cooked muscles. It is important to note that only general comparisons may be made between these 2 sets of results because of the effects of diet, age, sex, and frozen storage time on the lipid and fatty acid levels (see section 1.41).

There were no high correlation coefficients between the percentage neutral lipid and levels of C16:0, C18:2, and total unsaturated fatty acids, as was found for raw muscles (Table VId). In fact, the C18:2 content of these muscles (except for T1 and T3) was very constant, unlike for the raw muscles, when C18:2 was found to vary more between muscles to a greater extent than any other fatty acids (Table VIb). In addition, each of the other fatty acids of both neutral lipid and phospholipid showed relatively small interchicken variations. This seems surprising in view of the differing oxidation levels, as determined by TBA Numbers (Table XIj). It would appear, therefore, that only very small changes in fatty acid composition are required to cause measurable differences in TBA Number. Also, heat alone, may have been

expected to cause relatively large changes in fatty acid composition by increasing reaction rates. It therefore follows that relatively large interchicken variations would be expected in cooked muscles, because the raw muscle variations would be magnified on heating. However, other workers also have reported that cooking has very little measurable effect on the fatty acid composition of chicken muscles (see section 1.41).

Table XIe shows the fatty acid composition of the total lipid of these muscles which were calculated from the known neutral lipid and phospholipid levels (in order to compare with the rancid muscle results, Table XIg, and the cook-out juice results, Table XIh); but, again, there were no differences between treated and untreated muscles, when the anomalous results of T1 and T3 were taken into account.

3.752 Rancid Cooked Pectoralis Major Muscles

Table XIf shows that there was more C18:2 and a greater percentage of total unsaturated fatty acids in the neutral lipid of rancid treated muscles compared with untreated muscles. The difference in total unsaturated fatty acids between treated and untreated muscles was statistically significant at the 95% level, but the difference in C18:2 only approached significance. However, only a small number of muscles were tested. When the inherent interchicken differences were allowed for by comparing the amounts of total unsaturated fatty acids to the level of C16:0, each treated muscle was shown to contain a greater percentage of unsaturated acids than either of the untreated muscles. However, this difference only approached significance.

That treated muscles contain more C18:2 and total unsaturated fatty acids than untreated muscles, would be expected since polyphosphates protect cooked muscles against autooxidation (see below

TABLE XIe FATTY ACID COMPOSITION[a] OF TOTAL LIPID OF FRESHLY COOKED PECTORALIS MAJOR MUSCLES

FATTY ACID	TREATMENT						
	treated			untreated			
	T1	T2	T3	U1	U2	U3	M ± S.D.[b]
C14:0	1.2	1.0	1.5	1.0	1.0	1.0	1.0 ± 0.0
C14:2	2.8	4.1	1.3	2.9	2.2	2.0	2.4 ± 0.5
C16.0	36.6	30.3	40.6	27.5	29.7	30.8	29.3 ± 1.7
C16:1	1.2	1.7	0.9	2.6	2.8	3.0	2.8 ± 0.2
C18:0	9.8	8.9	11.0	9.1	8.6	8.3	8.7 ± 0.4
C18:1	32.6	30.1	33.1	34.2	32.7	34.3	33.7 ± 0.9
C18:2	7.5	14.3	6.6	15.2	14.7	14.6	14.8 ± 0.3
C20:0	1.8	0.2	0.5	0.2	0.4	0.3	-
C18:3	0.9	1.0	1.0	1.5	1.3	1.5	1.4 ± 0.1
C20:4	1.9	3.8	1.9	2.9	2.7	2.1	2.6 ± 0.4
CB	3.6	4.5	2.1	3.0	3.9	2.2	3.0 ± 0.9
% total unsaturated	50.5	59.5	46.9	62.3	60.3	59.7	60.8 ± 1.4
% total unsaturated / C16:0	1.4	2.0	1.2	2.3	2.0	1.9	2.1 ± 0.2

a. Values expressed as % of total fatty acids

b. Mean ± standard deviation

TABLE XIf

FATTY ACID COMPOSITION[a] OF NEUTRAL LIPID AND PHOSPHOLIPID OF RANCID COOKED PECTORALIS MAJOR MUSCLES

FATTY ACID	LIPID															
	Neutral								Phospholipid							
	treated				untreated				treated				untreated			
	T1	T2	T3	M±S.D.[b]	U1	U2	U3	M±S.D.	T1	T2	T3	M±S.D.	U1	U2	U3	M±S.D.
C14:0	1.2	1.2	1.2	1.2 ±0.0	-	1.2	1.4	1.3 ±0.1	0.4	-	0.3	-	0.3	-	0.6	-
C14:2	-	-	-	-	-	-	-	-	3.9	2.2	4.8	3.6 ±1.3	6.4	4.6	3.2	4.7 ±1.6
C16:0	27.4	28.6	27.6	27.9 ± 0.6	-	30.7	30.4	30.6 ± 0.2	33.4	36.1	33.4	34.3 ± 1.6	31.3	34.6	35.2	33.7 ± 2.1
C16:1	5.9	6.3	6.5	6.2 ± 0.3	-	7.0	6.7	6.9 ± 0.2	-	-	-	-	-	-	-	-
C18:0	6.5	7.2	6.4	6.7 ±0.4	-	7.7	7.6	7.7 ±0.1	11.3	12.9	10.6	11.6 ± 1.2	12.7	11.0	11.3	11.7 ± 0.9
C18:1	40.8	39.8	43.4	41.3 ± 1.9	-	41.6	40.9	41.3 ± 0.3	29.7	30.0	30.7	30.1 ± 0.5	31.3	29.1	31.3	30.6 ± 1.4
C18:2	13.9	12.8	11.6	12.8 ± 1.2	-	8.9	10.1	9.5 ± 0.6	13.4	11.8	12.2	12.5 ± 0.8	13.1	11.9	12.8	12.6 ± 0.6
C18:3	2.4	2.2	2.2	2.3 ±0.1	-	2.1	2.1	2.1 ± 0.0	0.3	0.3	0.3	-	0.3	0.3	0.4	-
C20:4	0.7	1.1	0.5	0.8 ±0.3	-	0.4	0.4	0.4 ± 0.0	3.1	2.2	3.3	2.9 ±0.6	2.4	2.9	2.7	2.7 ±0.3
C_B	1.1	0.6	0.6	0.8 ±0.3	-	0.4	0.3	0.4 ± 0.1	4.7	4.2	4.4	4.4 ±0.3	2.1	5.5	2.7	3.4 ±1.8
% total unsaturated	64.8	62.8	64.8	64.1 ± 1.2	-	60.4	60.5	60.5° ± 0.1	55.3	50.7	55.7	53.9 ± 2.8	55.6	54.3	53.1	54.3 ± 1.4
% total unsaturated C16:0	2.4	2.2	2.4	2.3 ±0.1	-	2.0	2.0	2.0 ± 0.0	1.7	1.4	1.7	1.6 ± 0.2	1.8	1.6	1.5	1.6 ±0.2

a. Values expressed as % of total fatty acids

b. Mean ± standard deviation

o Difference between treated and untreated significant at 95% level

for TBA Numbers). From these results it is clear that C18:2 is the only individual fatty acid which shows measurable changes due to autooxidation (and also due to diet), see section 3.23). Despite the presence of fatty acids which are more unsaturated, and therefore more susceptible to autooxidation than C18:2, it is suggested that C18:2 is the only acid which shows measurable differences because it is present in larger amounts than the other unsaturated fatty acids.

The TBA Numbers of treated freshly cooked muscles after 2 weeks storage at -20°C, were much lower than those of untreated muscles (Table XIj) and it may therefore be assumed that this difference would have been even larger between rancid treated and untreated muscles. Therefore, it appears that only very small changes in fatty acids produce large differences in TBA Numbers, (see also section 3.751).

No consistent differences in the fatty acid composition of the phospholipid fractions between treated and untreated muscles were apparent. This was surprising because phospholipids would be expected to be more susceptible to autooxidation than neutral lipids, because of their increased polyunsaturated fatty acid content. Therefore, differences between treated and untreated muscles, due to differences in the degree of autooxidation, would be expected. Previous results have suggested that phospholipids are protected against autooxidation in raw muscles by their close association with muscle proteins (see section 3.26). However, heat would be expected to break this association, and therefore expose the phospholipids to oxygen. However, phospholipids contain much greater amounts of C20:4 and C_B than neutral lipids, and these acids will undergo autooxidation more rapidly than the other unsaturated fatty acids. Since there is less than 6% of C20:4 and C_B present in phospholipids, their determination is subject to relatively large experimental error, and this could have obscured any differences between treated and untreated muscles. In fact, the mean amounts of C20:4 and C_B in treated muscles were higher than those in untreated muscles. Comparison of these levels, and the

levels of total unsaturated fatty acids, with C16:0 failed to show any statistically significant differences between treated and untreated muscles. Only these values for total unsaturated fatty acids are given in Table XIf because the values for C20:4 and C_B were very small, and subject to relatively large experimental errors as discussed above.

Table XIg shows the fatty acid composition of the total lipid of rancid cooked muscles. The values for T1, 2, 3 and U1, 2, 3, were calculated from the known levels of neutral lipid and phospholipid (Table XIc); and the values for T4,5,6 and U4,5,6 were determined directly on the total lipid. There were no consistent differences between treated and untreated muscles, although the mean values for C18:2, C20:4 and C_B were higher for treated muscles. Also, the mean value for the amount of total unsaturated fatty acids was greater for treated muscles, when the interchicken variability was partially overcome by dividing the levels by the amount of C16:0, but not when this was not taken into account.

3.753 Comparison Between Freshly Cooked and Rancid Muscles.

By comparing Tables XId and XIf it is seen that the greatest changes in the fatty acids of neutral lipid between freshly cooked and rancid muscles occurred for C18:2, which decreased with rancidity. However, because comparisons for T1 and T3 were not possible (because no C18:2 was detected in these freshly cooked muscles), no statistical weight could be given to these observations.

The greatest changes in the fatty acids of phospholipid between fresh and rancid muscles, occurred for C20:4 and C_B, but there were no consistent differences between treated and untreated samples; although the mean loss for treated muscles was less than that for untreated muscles. That treated muscles lose lower amounts of unsaturated fatty acids on becoming rancid, is to be expected from the TBA Numbers (Table XIj). However, once again it may be seen that large

differences in TBA Numbers are caused by only slight changes in fatty acid composition. In addition, all rancid phospholipid fractions contained lower amounts of C18:2 and total unsaturated fatty acids than the freshly cooked fractions. The mean difference for the treated muscles was lower than for the untreated muscles, but this difference was not statistically significant.

By comparing Tables XIe and XIg it is seen that the differences in fatty acid composition of the total lipid of T1,2,3 and U1,2,3 between freshly cooked and rancid muscles showed the same general trends as those for the neutral lipid and phospholipid fractions.

3.754 Cook-out Juices.

Table XIh shows the fatty acid composition of the cook-out juices of pectoralis major muscles, and it may be seen that there were relatively large interchicken variations for each of the fatty acids. There were no consistent differences between treated and untreated samples, although the mean levels of individual and total amounts of unsaturated fatty acids were greater for treated samples.

There were no consistent relationships between these results and those of the cooked muscles, i.e. large amounts of an acid in a particular cook-out juice, did not always correspond to low amounts of this acid in the cooked muscle. However, it was not possible to make comparisons because, for instance, a low level of an acid in the cook-out juice does not necessarily mean that the cooked muscle had retained a high level of this acid.

Table XIi shows the fatty acid composition of the cook-out juices from leg muscles. There were relatively large interchicken variations, as for the pectoralis major samples. However, in this case the mean unsaturated fatty acid values for treated samples were not higher than those for the untreated samples. This corresponds to the fact that no added phosphate is present in the leg muscles from treated

TABLE XIg

FATTY ACID COMPOSITION[a] OF TOTAL LIPID OF RANCID COOKED PECTORALIS MAJOR MUSCLES

FATTY ACID	TREATMENT													
	treated							untreated						
	T1	T2	T3	T4	T5	T6	M±S.D.[b]	U1[c]	U2	U3	U4	U5	U6	M±S.D.[b]
C14:0	0.8	0.4	0.8	-	-	-	-	-	0.6	1.0	-	-	-	-
C14:2	2.0	1.4	2.3	3.8	3.5	4.3	2.9 ±1.1	-	2.4	1.6	2.9	2.0	1.6	2.1 ±0.6
C16:0	30.5	33.4	30.4	26.4	30.2	30.1	30.2 ± 2.2	-	32.8	32.7	30.6	32.1	29.4	31.5 ± 1.5
C16:1	2.9	2.3	3.4	2.4	3.4	3.2	2.9 ± 0.5	-	3.3	3.5	2.9	3.3	3.6	3.3 ± 0.3
C18:0	9.0	10.9	8.4	12.4	11.1	11.1	10.5 ± 1.5	-	9.5	9.4	11.0	10.1	10.1	10.0 ± 0.6
C18:1	35.1	33.5	37.4	30.2	33.0	33.5	33.8 ± 2.4	-	35.0	36.2	36.3	35.6	38.3	36.3 ± 1.2
C18:2	13.6	12.2	11.9	13.7	11.8	11.2	12.4 ± 1.0	-	10.5	11.4	10.8	11.5	11.8	11.2 ± 0.5
C18:3	1.3	1.0	1.3	1.2	1.6	1.2	1.3 ± 0.2	-	1.1	1.3	1.4	1.3	1.5	1.3 ± 0.2
C20:4	1.9	1.8	1.8	5.5	3.0	2.8	2.8 ± 1.4	-	1.7	1.5	1.7	1.7	2.0	1.7 ± 0.2
C_B	2.9	2.9	2.4	4.5	2.5	2.4	2.9 ± 0.8	-	3.1	1.5	1.6	2.2	1.5	2.0 ± 0.7
% total unsaturated	59.7	55.1	60.5	61.3	58.9	58.6	59.0 ± 2.0	-	57.1	57.0	57.6	57.6	60.3	57.9 ± 1.4
% total unsaturated / C16:0	2.0	1.7	2.0	2.3	2.0	2.0	2.0 ± 0.2	-	1.7	1.7	1.9	1.8	2.0	1.8 ± 0.1

a. Values expressed as % of total fatty acids

b. Mean ± standard deviation

c. Not calculated because the neutral lipid results were not known.

TABLE XIh FATTY ACID COMPOSITION[a] OF TOTAL LIPID OF COOK-OUT JUICES OF PECTORALIS MAJOR MUSCLES

FATTY ACID	TREATMENT												
	treated							untreated					
	T1	T2	T3	T4	T5	T6	M±S.D.[b]	U1	U2	U3	U4	U5	U6	M±S.D.[b]
C14:0	3.6	2.8	1.6	5.1	1.5	2.2	2.8 ±1.4	4.2	4.3	8.0	1.5	1.3	1.6	3.5 ± 2.6
C16:0	17.4	24.8	25.5	23.6	26.9	24.4	23.8 ± 3.3	35.0	27.5	27.8	27.3	25.5	24.1	27.9 ± 3.8
C16:1	6.4	6.9	6.1	4.4	6.3	6.5	6.1 ± 0.9	5.2	6.7	6.1	5.3	7.0	5.9	6.0 ± 0.7
C18:0	5.2	6.2	6.1	10.7	6.0	5.4	6.6 ± 2.0	9.1	6.8	9.9	6.8	5.7	5.0	7.2 ± 1.9
C18:1	28.3	37.5	42.6	27.1	39.6	37.0	35.4 ± 6.3	35.6	35.3	37.0	40.8	44.4	43.0	39.4 ± 3.9
C18:2	6.0	9.7	11.7	9.4	13.4	12.8	10.5 ± 2.7	3.6	8.9	1.9	13.3	12.7	11.3	8.6 ± 4.8
C20:0	1.3	0.3	0.5	1.2	0.7	1.3	1.8 ± 1.1	0.3	0.4	-	0.4	0.6	1.4	-
C18:3	4.0	2.5	2.5	6.2	3.1	4.7	3.8 ± 1.4	3.5	2.8	2.9	2.8	2.6	3.0	2.9 ± 0.3
C_A	12.4	3.4	1.4	3.7	0.6	1.6	3.9 ± 4.4	0.3	1.0	-	0.5	-	1.3	-
C20:4	1.9	1.0	0.6	1.8	0.6	1.1	1.2 ± 0.6	1.5	1.3	1.9	0.5	0.4	-	-
C_B	13.6	4.9	1.6	7.1	1.6	2.9	5.3 ± 4.6	1.8	5.3	4.6	1.2	-	3.6	3.3 ± 1.8
% total unsaturated	72.6	65.9	66.5	59.7	65.2	66.6	66.1 ± 4.1	51.5	61.3	54.4	64.4	67.2	68.1	61.2 ± 6.8
% total unsaturated / C16:0	4.2	2.7	2.6	2.5	2.4	2.7	2.9 ± 0.7	1.5	2.2	2.0	2.4	2.6	2.8	2.3 ± 0.5

a. Values expressed as a % of total fatty acids

b. Mean ± standard deviation

TABLE XIi FATTY ACID COMPOSITION[a] OF TOTAL LIPID OF COOK-OUT JUICES OF LEG MUSCLES

FATTY ACID	TREATMENT													
	treated							untreated						
	T1	T2	T3	T4	T5	T6	M±S.D.[b]	U1	U2	U3	U4	U5	U6	M±S.D.[b]
C16:0	27.8	26.9	26.2	26.0	23.6	23.4	25.7 ± 1.8	25.5	25.7	27.1	26.0	26.2	26.3	26.1 ± 0.6
C16:1	6.8	7.1	5.8	6.0	4.9	5.1	6.0 ± 0.9	6.7	5.6	7.9	6.7	7.1	7.8	7.0 ± 0.8
C18:0	5.6	5.8	6.4	5.9	9.5	6.5	6.6 ± 1.5	5.3	6.1	5.1	5.6	6.6	4.4	5.5 ± 0.8
C18:1	39.9	36.9	38.4	36.5	28.2	33.4	35.6 ± 4.2	40.7	36.6	40.8	41.9	38.1	42.6	40.1 ± 2.3
C18:2	13.9	14.2	12.6	14.6	15.6	14.8	14.3 ± 1.0	15.6	14.4	14.9	15.2	11.5	13.5	14.2 ± 1.5
C20:0	0.6	1.5	1.1	0.9	1.2	2.2	1.3 ± 0.6	-	1.1	-	0.3	0.8	0.4	-
C18:3	3.0	3.6	3.6	3.6	3.4	4.1	3.6 ± 0.4	3.1	3.6	2.8	2.8	3.4	2.5	3.0 ± 0.4
C20:4	0.5	0.6	0.6	0.7	0.3	-	-	0.7	0.5	0.5	0.4	0.8	0.3	-
C_B	2.3	3.5	5.5	6.0	13.4	10.7	6.9 ± 4.3	2.4	6.3	0.9	1.4	5.7	2.3	3.2 ± 2.3
% total unsaturated	66.4	65.9	66.5	67.4	65.8	68.1	66.7 ± 0.9	69.2	67.0	67.8	68.4	66.6	69.0	68.0 ± 1.1
% total unsaturated / C16:0	2.4	2.4	2.5	2.6	2.8	2.9	2.6 ± 0.2	2.7	2.6	2.5	2.6	2.5	2.6	2.6 ± 0.1

a. Values expressed as % total fatty acids

b. Mean ± standard deviation

chickens (Table X1p), and that there are no differences in the TBA Numbers between treated and untreated leg muscles (Table X1j).

It is difficult to account for these large interchicken variations in the fatty acid composition of cook-out juices. However, the results show that large amounts of C_A and C_B were sometimes present in the cook-out juices, and it is assumed that these arose from the phospholipid fraction, which is richer in these acids than the neutral lipid (although both raw and cooked muscles contained only very small amounts of C_A and its level has not been given in this experiment, or in section 3.2). Therefore, it would seem that protein denaturation during heating, which would result in the cleavage of any phospholipid-protein associations, plays an important part in determining the amount and types of fatty acids lost during cooking.

3.76 TBA NUMBERS

Table X1j shows that, as expected, the TBA Numbers of cooked treated pectoralis major muscles were significantly lower than those of untreated muscles; indicating that polyphosphates inhibit autooxidation in cooked muscles. There was no significant difference between treated and untreated leg muscles, confirming previous results (section 3.521, Table 1Xb).

The TBA Numbers of untreated pectoralis major muscles were very much higher than those of untreated muscles after 18 weeks frozen storage during holding for 0 days at 4°C (Table V11a).

Also, the TBA Numbers of the treated and untreated leg muscles were similar to those of these 18 week muscles after holding for 3 days at 4°C (Table V11a). However, it must be remembered that the present freshly cooked pectoralis major and leg muscles had been stored at -18°C for 2 and 6 weeks respectively, prior to TBA analysis, and were thawed for approximately 1½ hours before testing. Therefore, these higher TBA Numbers were probably a consequence of autooxidation during this thawing

TABLE XIj

TBA NUMBERS OF COOKED PECTORALIS MAJOR AND LEG MUSCLES, DURING HOLDING FOR 2 AND 6 WEEKS RESPECTIVELY AT -20°C

MUSCLE	TREATMENT													
	treated							untreated						
	T1	T2	T3	T4	T5	T6	M ±S.D.a	U1	U2	U3	U4	U5	U6	M ±S.D.a
Pectoralis Major	0.41	1.04	0.53	-	-	-	0.66 ± 0.33	3.09	4.10	5.10	-	-	-	4.10 ‡ ± 1.01
Leg	7.62	7.55	6.37	7.92	9.29	8.93	7.95 ± 1.05	6.16	5.94	8.10	7.86	8.21	8.64	7.49 ± 1.14

a. Mean ± standard deviation

‡ Difference between treated and untreated significant at 99% level

period, since TBA Numbers of cooked untreated muscles have been found to increase rapidly during holding. Since, cooked treated muscles are protected against oxidation, then it is not surprising that the TBA Numbers of the present treated muscles and those of chickens after 18 weeks of frozen storage were similar.

3.77 CORRELATION COEFFICIENTS BETWEEN TBA NUMBERS AND FATTY ACID LEVELS OF PECTORALIS MAJOR MUSCLES.

The TBA tests were performed on freshly cooked pectoralis major muscles after 2 weeks storage at -20°C. However, the TBA Numbers of both treated and untreated pectoralis major muscles were found in a previous experiment to have changed by only a small extent during 3 months storage at -18°C, section 3.522, Table 1Xe). Therefore, it was thought that it was valid to compare the present TBA Numbers with the fatty acid levels of the freshly cooked muscles. Also, it was thought that useful trends would be revealed by comparing these TBA Numbers with the fatty acid levels of the rancid cooked muscles. However, it must be remembered that TBA Numbers fluctuate inconsistently during the course of autooxidation (see for example Table 1Xb), and therefore, these correlations will only be approximate.

The correlation coefficients for treated and untreated muscles were calculated separately because their TBA Numbers were significantly different.

3.771 Freshly Cooked Muscles

Table X1k shows that the highest coefficients were obtained between TBA Numbers and the levels of total unsaturated fatty acids and C20:4 of the phospholipids, for both treated and untreated muscles. It should be noted that the numerical values of the coefficients for total unsaturated fatty acids divided by the levels of C16:0 (which helps to account for inherent interchicken variations, see section 3.22), were lower than those for the levels of total unsaturated fatty acids alone,

TABLE X1k CORRELATION COEFFICIENTS[a] BETWEEN TBA NUMBERS AND FATTY ACID LEVELS OF FRESHLY COOKED PECTORALIS MAJOR MUSCLES

TREATMENT	LIPID	FATTY ACID					
		% total unsaturated	% total unsaturated C16:0	C18:1	C18:2	C20:4	C_B
treated[b]	phospholipid[c]	+0.70	+0.63	-0.70	+0.23	+1.00 ‡	+0.49
untreated	neutral[c]	-1.00 ‡	-0.87	-0.78	-0.00	-	-
	phospholipid[c]	-0.98	-0.96	+0.27	-0.94	-0.99	-0.32
	total lipid[c]	-0.96	-0.96	+0.05	-0.93	-0.96	-0.47

a. Based on data in Tables X1d, X1e and X1j

b. Neutral lipid and total lipid coefficients not calculated because the neutral lipid of T1 and T3 had anomalous fatty acid values

c. 0.997 required for significance at 95% level

‡ Significant at 99% level

TABLE XIL CORRELATION COEFFICIENTS[a] BETWEEN TBA NUMBERS AND FATTY ACID LEVELS OF RANCID COOKED PECTORALIS MAJOR MUSCLES

TREATMENT	LIPID	FATTY ACID					
		% total unsaturated	% total unsaturated C16:0	C18:1	C18:2	C20:4	C_B
treated	neutral[b]	-0.98	-0.98	-0.58	-0.15	-	-
	phospholipid[b]	-0.97	-0.98	-0.05	-0.81	-0.94	-0.90
	total lipid[b]	-0.95	-0.98	-0.69	-0.51	-0.65	+0.34
untreated[c]	phospholipid[b]	-1.00[o]	-0.98	-0.00	-0.24	+0.60	+0.17

a. Based on data in Tables Xlf, Xlg and Xlj

b. 0.997 required for 95% significance

c. Neutral lipid and total lipid coefficients not calculated because only 2 muscles analysed for neutral lipid

o Significant at 95% level.

but were still relatively high. The coefficient for C20:4 of the phospholipids of treated muscles was statistically significant, and this value for the untreated muscles approached significance, as did their value for the total amount of unsaturated fatty acids. However, all the coefficients, except for C18:1, for treated muscles were positive, and those for untreated muscles, negative, except for C18:1.

One would expect negative coefficients between TBA Numbers and the levels of unsaturated fatty acids, i.e. as the amount of unsaturated fatty acid decreases due to autooxidation, then the TBA Number should increase. It appears therefore, that this is the case for untreated muscles, but not for treated. The TBA number (Table XIj) showed that autooxidation had proceeded to a much greater extent in untreated compared to treated muscles. Therefore, since it appears that it is not possible to detect changes in fatty acids during the early stages of autooxidation, (sections 3.751, 3.752 and 3.753), it is perhaps not surprising that the fatty acids of treated muscles were not negatively correlated with their TBA Numbers. The converse is true, of course, for untreated muscles where autooxidation had proceeded.

However, it could also be the case that, if high amounts of unsaturated fatty acids are lost in the cook-out juices, then this would leave lower amounts of autooxidation-susceptible fatty acids in the cooked muscles. This would result in low TBA Numbers due to reduced autooxidation. In this case therefore, positive correlation coefficients would be expected between TBA Numbers and the levels of unsaturated fatty acids in the cooked muscles. Therefore, because positive correlation coefficients were found for treated muscles it could mean that polyphosphates lead to a greater amount of unsaturated acids being lost during cooking. However, the fatty acid results for the total lipid of freshly cooked muscles (Table XIe) and the cook-out juices (Table XIh) did not show this, although it must be remembered that the fatty acid composition of freshly cooked T1 and T3 could not be compared with the cook-out juice

results (see section 3.751). When the total percentage of unsaturated fatty acids divided by C16:0 for the total lipid of rancid cooked muscles (Table XIg) were compared with these levels in the cook-out juices, it was seen that the treated muscles lost 2.2, 1.0 and 0.6%, and the untreated muscles 0.5 and 0.3% during cooking. However, these values do not necessarily mean that lower amounts of unsaturated fatty acids were lost from untreated muscles compared to treated, since the levels of unsaturated acids in rancid muscles will have been affected by autooxidation. These points are further discussed in section 3.773.

The coefficients for the neutral lipids of treated muscles were not calculated because of the anomalous fatty acid values which were obtained for T1 and T3. All of these coefficients for untreated muscles, however, were negative. The coefficients for C20:4 and C_B were not calculated because only trace amounts of these acids were present. The coefficient for the level of total unsaturated fatty acids was statistically significant, and approached significance when divided by C16:0.

The coefficients for the total lipid fatty acids of untreated muscles showed the same general trends as for the neutral lipid and phospholipid fatty acids, with the values for the levels of total unsaturated fatty acids alone and divided by C16:0, and for C18:2 and C20:4, approaching significance.

3.772 Rancid Cooked Muscles

Table XIL shows that all of the correlation coefficients between TBA Numbers and the fatty acid levels of the neutral lipid and phospholipid of treated muscles were negative, and approached significance in the case of all but C18:1 and C18:2. The fact that high negative coefficients were found indicates that as autooxidation proceeds, then the TBA Numbers may be correlated with decrease of unsaturated fatty acids. This bears out the discussion above for freshly cooked muscles,

where the TBA Numbers of treated muscles were not negatively correlated with fatty acids, presumably because the decrease in fatty acids during the early stages of autooxidation are too small to produce statistically significant trends. However, this discussion also suggested that TBA Numbers were positively correlated with fatty acid levels in freshly cooked treated muscles, because polyphosphates may cause a greater amount of unsaturated fatty acids to be lost on cooking. If this were the case, however, then negative correlations in the rancid muscles would still be expected since the unsaturated fatty acids remaining in the cooked muscles would have undergone considerable oxidation during the course of rancidity.

The correlation coefficients for the total lipid fatty acids of treated muscles showed the same general trends as those for neutral lipid and phospholipid acids, except for C_B where a small positive correlation was found.

For untreated muscles, only the correlation coefficients between TBA Numbers and the fatty acids of phospholipids were calculated. This was because only 2 of these muscles were analysed for neutral lipid fatty acids, and it is not possible to correlate 2 sets of results. As with the freshly cooked untreated muscles, there were high negative correlations between TBA Numbers and the levels of total unsaturated fatty acids alone and when divided by C16:0. In fact, this coefficient for the total unsaturated fatty acids was statistically significant. However, positive correlations were obtained for C20:4 and C_B, but the numerical value of these were relatively small.

The 18- carbon fatty acids of neutral lipid and the 20 plus polyunsaturated fatty acids of phospholipids, were found to be the most important indicators of autooxidation, as they were for the raw muscles (see section 3.25).

It should be remembered that these coefficients were calculated between rancid muscle fatty acid levels and the TBA Numbers of the freshly

cooked muscles. It is therefore not possible to arrive at definite conclusions from these results. This point is discussed in the introductory paragraph (section 3.77).

All of the coefficients for both freshly cooked and rancid cooked muscles, were numerically higher than those obtained for raw muscles (Table VId). This again indicates that during the early stages of autooxidation, as was the case for the raw muscles, then the changes in fatty acids are too small to produce the expected correlations between their levels and TBA Numbers.

3.773 Cook-Out Juices

Table XIm shows the correlation coefficients between TBA Numbers and the fatty acids of the total lipid of the cook-out juices of pectoralis major muscles. It is difficult to discuss these results in detail because of the large interchicken variations in the fatty acid composition (Table XIh), and also, of course, the TBA tests were performed on the cooked muscles and not on the cook-out juices. However, it may be significant that the correlation coefficients for the total percentage of unsaturated fatty acids alone, and when divided by C16:0, were negative for treated samples and positive for untreated samples. This suggests that, low TBA Numbers in the cooked treated muscles are a result of high levels of unsaturated fatty acids in the treated cook-out juices, and vice versa for the untreated samples. Therefore, the discussions in sections 3.771 and 3.772 seem valid i.e. that polyphosphates lead to greater amounts of unsaturated fatty acids being lost during cooking, which reduces the autooxidation in these freshly cooked muscles leading to low TBA Numbers.

Table XIn shows the correlation coefficients between TBA Numbers and the fatty acids of the total lipid of the cook-out juices of leg muscles. In this case, since there was no statistically significant difference in the TBA Numbers between treated and untreated muscles,

TABLE XIm

CORRELATION COEFFICIENTS[a] BETWEEN TBA NUMBERS AND FATTY ACID LEVELS OF THE COOK-OUT JUICES FROM PECTORALIS MAJOR MUSCLES

TREATMENT	FATTY ACID						
	total % unsaturated	total % unsaturated C16:0	C18:1	C18:2	C_A	C20:4	C_B
treated [b]	-0.71	-0.60	+0.34	+0.34	-0.51	-[c]	-0.42
untreated[b]	+0.29	+ 0.70	+0.77	-0.23	-[c]	+0.65	+0.76

a. Based on data in Tables XIh, XIj

b. 0.997 required for significance at 95% level.

c. Not calculated because only small amounts of this fatty acid present.

TABLE XIn

CORRELATION COEFFICIENTS[a] BETWEEN TBA NUMBERS AND FATTY ACID LEVELS OF THE COOK-OUT JUICES FROM LEG MUSCLES

	FATTY ACID[b]				
	total % unsaturated	total % unsaturated C16:0	C18:1	C18:2	C_B
treated [c]	+0.19	+0.79	-0.84	+0.96	+0.78
untreated[c]	+0.02	-0.61	+0.54	-0.48	-0.39
treated + untreated [d]	-0.06	+0.03	-0.37	+0.08	+0.39

a. Based on data in Tables XIi and XIj

b. Values for C_A and C20:4 not calculated because only trace amounts of these fatty acids present.

c. 0.811 required for significance at 95% level.

d. 0.576 required for significance at 95% level.

the coefficients were calculated for treated and untreated muscles together, as well as separately. The correlation values for the total percentage of unsaturated fatty acids divided by C16:0 approached significance for both treated and untreated samples separately. However, the treated value was positive, and the untreated value, negative. For treated and untreated together, a very small positive value was obtained. The coefficients for the other fatty acids of treated and untreated samples separately, were also of opposite signs. It is difficult to account for these differences between treated and untreated results, especially since the signs of both sets of coefficients were opposite to those obtained for the cook-out juices of pectoralis major muscles. Since the leg muscles from treated chickens were found to contain no added phosphorus or sodium (Table X1p) and to have similar TBA Numbers to the leg muscles from untreated chickens (Table X1j) it may have been expected that these correlations between TBA Numbers and fatty acids would be identical for both treated and untreated samples. It can only be assumed that the large inter-chicken variations in the cook-out juice fatty acids (Table X1i) resulted in unequal distribution of the results between the 2 sets of samples. Unfortunately, the fatty acid composition of the freshly cooked and rancid leg muscles were not determined, and so these correlation coefficients could not be compared with the present ones.

3.78 CORRELATION COEFFICIENTS BETWEEN TBA NUMBERS AND LIPID LEVELS OF PECTORALIS MAJOR MUSCLES

Table X1o shows the correlation coefficients between TBA Numbers and the lipid levels of both freshly cooked and rancid treated and untreated pectoralis major muscles. The coefficients between TBA Numbers and the total lipid and phospholipid levels of untreated muscles were of the same sign as those obtained by Wilson et al[138] (see Table V1g for values).

TABLE XIo

CORRELATION COEFFICIENTS[a] BETWEEN TBA NUMBERS AND LIPID LEVELS OF FRESHLY COOKED AND RANCID PECTORALIS MAJOR MUSCLES

TREATMENT	STATE OF MUSCLE	LIPID		
		Total lipid as % tissue	Neutral lipid as % tissue	Phospholipid as % tissue
treated	freshly cooked	+0.16	-0.46	+0.87
	rancid[b]	-0.94	-0.92	-1.00‡
untreated	freshly cooked[b]	-0.25	-0.11	-0.40
	rancid[b]	-0.43	-[c]	-[c]

a. Based on data in Tables XIc and XIj

b. 0.997 required for significance at 95% level

c. Not calculated because the neutral lipid and phospholipid content of only 2 muscles calculated.

‡ Significant at 99% level

The coefficient between TBA Numbers and the phospholipid content (% of tissue) of freshly cooked treated muscles was positive and approached significance, whilst that for untreated muscles was negative and relatively small. That this coefficient for treated muscles was positive, indicates that phospholipids play the major role in autooxidation of freshly cooked muscles, compared with the finding that neutral lipids play the major role in both treated and untreated raw muscle autooxidation (section 3.26). Two points arise from these findings. First, that there is a difference between treated and untreated cooked muscle autooxidation, and second that there is a difference between raw and cooked muscle autooxidation.

This first point seems to indicate that polyphosphates react in some way with phospholipids, which was also suggested by the results obtained from raw muscles in section 3.26. However, there was no significant difference in the phospholipid content of treated and untreated cooked muscles, unlike for the raw muscles when the treated muscles contained significantly less phospholipid than the untreated muscles, (Table VIa). However, the mean value for the treated cooked muscles was lower than that for the untreated cooked muscles. This evidence for polyphosphate interaction with phospholipids which leads to changes in the TBA Numbers of both raw and cooked muscles, is tenuous, being based entirely on statistically insignificant correlations between TBA Numbers and phospholipids (see also the discussion in section 3.26). Also, of course, any interaction between phospholipids and polyphosphates would be expected to shield the phospholipids against autoxidation, and it is surprising, therefore, that as the phospholipid content of treated cooked muscles increases, then so do the TBA Numbers. However, it must be remembered that these positive correlations also indicate that phospholipids play a major role in cooked tissue autooxidation (presumably because any phospholipid-protein associations which prevent phospholipids undergoing

autooxidation in raw tissues, are destroyed during cooking, thus exposing phospholipids to oxygen; see section 3.26). This fact will undoubtedly overshadow any decrease in autooxidation of treated cooked tissue due to phospholipid-polyphosphate associations. Also, of course, any phospholipid-polyphosphate associations will also be expected to have been destroyed by heat.

This discussion only serves to illustrate the difficulty in interpreting any correlation coefficients when more than 2 variables are operating. It is important to stress at this point that correlation coefficients only serve to indicate trends, and in themselves do not provide experimental evidence, but may be used to support results from independent experiments (see for example, Neville and Kennedy[140]).

For rancid cooked muscles, a statistically significant negative correlation was obtained between TBA Numbers and the phospholipid content of treated muscles. The correlations for total lipid and neutral lipid were also negative and approached significance. These correlations between the TBA Numbers and neutral lipid and phospholipid contents of untreated muscles were not calculated because only 2 of these muscles were analysed for these lipid fractions. The untreated value for the total lipid was also negative, however. This indicates that increasing TBA Numbers are associated with decreasing lipid levels in rancid cooked muscles.

It must be remembered that these correlations were between the TBA Numbers of freshly cooked muscles and the lipid levels of rancid cooked muscles. Since TBA Numbers have been found to fluctuate inconsistently during autooxidation, these coefficients may be subject to error (see also, section 3.77). Nevertheless, from these coefficients, it appears that as autooxidation proceeds, high TBA Numbers are associated with low levels of total lipid, neutral lipid and phospholipid.

However, these lipid levels varied inconsistently between freshly cooked and rancid muscles, i.e. not all rancid muscles had lower lipid levels than the freshly cooked muscles (Table X1c).

By comparing the results of freshly cooked and rancid muscles, it appears that during the 5 day storage period, TBA Numbers change from being dependant on the phospholipid levels, to being independent of any one lipid fraction. This is presumably because lipases are active during this storage period. Therefore, the rancid lipid levels will be partially determined by the extent of enzymatic breakdown, and will not be a reflection of the oxidative status of the muscle only. Also, of course, during the later stages of autooxidation, the unsaturated fatty acids of neutral lipid would be expected to have oxidised, as well as the highly susceptible fatty acids of phospholipids.

3.79 IONIC COMPOSITION

There is a paucity of information on the ionic composition of chicken tissues, especially after polyphosphate treatment. The figures of Adams,[141] which are quoted in Tables X1p and X1q for phosphorus, sodium, calcium and iron, were calculated from literature values obtained from a variety of sources for composite samples of light meat and dark meat from chickens cooked by roasting. No values are available from broilers which were steamed as in the present work.

3791 Phosphorus and Sodium

Table X1p shows the phosphorus and sodium contents of all thaw losses, muscles and their cook-out juices. The values for untreated pectoralis major and leg muscles were of the same order as those calculated by Adams,[141] for composite samples of light meat and dark meat. These values are given in this Table.

The phosphorus contents of pectoralis major muscles were similar to those of batch B chickens (see Table V111b), but higher values were obtained for both treated and untreated leg muscles in the present

TABLE XIp PHOSPHORUS AND SODIUM CONTENTS[a] OF THAW LOSSES, COOKED MUSCLES AND THEIR COOK-OUT JUICES

SAMPLE	ION	TREATMENT													
		Treated							Untreated						
		T1	T2	T3	T4	T5	T6	M±S.D.[b]	U1	U2	U3	U4	U5	U6	M±S.D.[b]
Thaw Loss	P	0.101	0.144	0.101	0.068	0.125	0.143	0.115 ±0.030	0.029	0.032	0.036	0.028	0.035	0.037	0.033 ±0.004‡‡
	Na	0.117	0.166	0.129	0.098	0.154	0.183	0.141 ±0.032	0.051	0.059	0.075	0.063	0.068	0.064	0.063+ ±0.008‡‡
Cooked Pectoralis Major Muscle	P	0.404	0.340	0.358	0.477	0.368	0.408	0.393 ±0.049	0.219	0.255	0.210	0.250	0.215	0.262	0.235+ ±0.023‡‡ (0.265)[c]
	Na	0.245	0.162	0.198	0.280	0.199	0.230	0.219 ±0.041	0.047	0.046	0.048	0.045	0.047	0.064	0.050+ ±0.007‡‡ (0.064)[c]
Cook-out juice from Pectoralis Major Muscle	P	0.261	0.247	0.280	0.284	0.265	0.306	0.274 ±0.021	0.164	0.188	0.170	0.170	0.199	0.165	0.176+ ±0.014‡‡
	Na	0.230	0.178	0.234	0.228	0.202	0.263	0.222 ±0.029	0.051	0.051	0.060	0.052	0.064	0.060	0.056+ ±0.006‡‡
Cooked Leg Muscle	P	0.206	0.193	0.206	0.202	0.190	0.194	0.199 ±0.007	0.169	0.177	0.174	0.183	0.177	0.175	0.176+ +0.005‡‡ (0.230)[c]
	Na	0.081	0.069	0.086	0.066	0.073	0.062	0.073 ±0.006	0.069	0.071	0.067	0.066	0.069	0.071	0.069 ±0.002 (0.086)[c]
Cook-out Juice from Leg Muscle	P	0.143	0.140	0.165	0.153	0.122	0.119	0.140 ±0.018	0.133	0.153	0.142	0.119	0.126	0.123	0.133 ±0.013
	Na	0.077	0.073	0.090	0.081	0.073	0.071	0.077 ±0.007	0.075	0.078	0.071	0.059	0.063	0.075	0.070 ±0.008

a. Values expressed as a % of sample weight

b. Mean± standard deviation

c. Figures in brackets are values of Ad$_a$ms,[141]

+ ‡‡ Difference between treated and untreated significa at 99.9% level

experiment compared to the previous analyses.

As expected, the treated pectoralis major muscles contained significantly more phosphorus and sodium than the untreated muscles. This was true also of the treated thaw losses and the pectoralis major cook-out juices. This indicates that sodium polyphosphates, which are injected into the breast muscles, result in increased levels of both ions in the cooked pectoralis major muscles and their cook-out juices. In addition, treated chickens lose more of both these ions on thawing, than untreated chickens.

The treated leg muscles contained significantly more phosphorus than the untreated muscles. The actual numerical difference, however, was small, and also there was no significant difference in TBA Numbers between treated and untreated leg muscles (see Table Xlj); and very small phosphorus uptake has been reported to decrease TBA Numbers (see section 1.43). Also, there was no significant difference in phosphorus content between treated and untreated cook-out juices from leg muscles. These points suggest that the difference in phosphorus content between treated and untreated leg muscles was only statistically significant because the interchicken variation was very small. Also, previous results (Table Vlllb) showed that there were no statistically significant differences in phosphorus content between treated and untreated raw or cooked leg muscles (see also section 3.42).

There was no significant difference in the sodium content between treated and untreated leg muscles, although the mean value for treated muscles was higher than that for untreated muscles.

The results presented here and in Table Vlllb conclusively show that very little, if any, added phosphorus and sodium is present in the leg muscle of chicken treated with sodium polyphosphate by injection into the breast muscles of the eviscerated carcase; despite claims that even distribution is achieved by this procedure (see section 1.2). The only other results of this nature which have been found in the literature

are those of Truman and Dickes,[21] who analysed raw muscles. Their results would seem to agree with the present findings that added phosphate is not present in the leg muscles of treated chickens; but they did find that leg muscles from treated chickens contained more sodium than those from untreated chickens (see section 1.2 for the values). However, these results were obtained from only 2 treated and 2 untreated chickens of unspecified origin and therefore must be treated with caution. Also Grey et al,[18] stated that they found no added phosphorus in the leg muscles from commercially treated chickens, but they gave no values, nor did they analyse for sodium.

It seems unlikely that chickens so treated with polyphosphates would be cut up and sold as breast and leg portions declared as containing added phosphates; but this possibility does exist.

It should be noted that all of the cook-out juices contained relatively high concentrations of both phosphorus and sodium, indicating that these ions are lost during cooking of both treated and untreated muscles. By analysing the cook-out juices in this way, the question of whether or not ions are leached out during cooking may be resolved without having to express the muscle results on a dry weight basis c.f. discussion in section 3.42.

3.792 Magnesium, Calcium and Iron

Table XIq shows the magnesium, calcium and iron contents of all thaw losses, muscles and their cook-out juices. The calcium and iron contents of untreated pectoralis major and leg muscles were of the same order as those calculated by Adams,[141] for composite samples of light meat and dark meat, and these values are given in this Table.

All tissues and juices contained only small amounts of iron, and these results were of little value. It is possible that the determination of iron was hindered by the presence of other ions, especially phosphorus, although this possibility was taken into account

TABLE XIq MAGNESIUM, CALCIUM AND IRON CONTENTS[a] OF THAW LOSSES, COOKED MUSCLES AND THEIR COOK-OUT JUICES

SAMPLE	ION	TREATMENT													
		Treated							Untreated						
		T1	T2	T3	T4	T5	T6	M±S.D.[b]	U1	U2	U3	U4	U5	U6	M±S.D.[b]
Thaw Loss	Mg	55	55	40	40	55	60	51± 9	35	35	45	35	40	40	38± 4‡
	Ca	60	80	75	65	85	105	78±16	60	60	60	65	55	55	59± 4‡
	Fe	10	5	10	5	10	15	9± 4	10	10	15	15	10	10	12± 3
Cooked Pectoralis Major Muscle	Mg	235	268	232	318	253	279	264±32	312	343	268	315	293	356	315±32[x]
	Ca	54	54	46	64	47	56	54± 7	69	104	58	60	47	75	69±20 (109)[c]
	Fe	36	13	15	32	16	14	-	35	15	23	15	12	19	20± 8 (13)[c]
Cook-out juice from Pectoralis Major Muscle	Mg	175	220	195	225	220	210	208±19	235	265	250	240	295	240	254±23‡
	Ca	30	25	30	30	30	30	29± 2	30	30	35	30	40	35	33± 4[o]
	Fe	5	5	5	5	5	5	-	5	5	5	5	5	5	-
Cooked Leg Muscle	Mg	248	230	231	236	245	259	242±11	214	237	229	247	235	230	232±11
	Ca	66	61	62	67	61	61	63± 3	61	59	61	49	63	66	60± 6 (127)[c]
	Fe	17	15	31	17	15	15	-	15	24	31	33	31	16	25± 8 (17)[c]
Cook-out juice from leg Muscle	Mg	175	165	185	190	160	180	176±12	165	185	180	145	155	175	168±15
	Ca	30	25	35	35	30	25	30± 4	30	30	25	25	30	25	28± 3
	Fe	5	5	5	10	5	5	-	10	5	5	5	5	5	-

a. Values expressed as ppm (microg per g of sample)

b. Mean ± standard deviation

c. Figures in brackets are values of Adams[141]

o Difference between treated and untreated significant at 95% level

x " " " " " " " " 98% level

‡ " " " " " " " " 99% level

during the analyses. However, small amounts (< 20ppm) were also found in cooked chicken tissues by Adams,[141] (see Table XIq) and by Rognerud,[142]

The mean values for the magnesium and calcium contents of treated thaw losses were significantly higher than those of the untreated thaw losses; indicating that polyphosphates remove these ions from chicken tissues. This effect was further illustrated by the fact that the mean values for the magnesium and calcium contents of treated pectoralis major muscles were less than those for untreated muscles. This difference was statistically significant in the case of magnesium, but not in the case of calcium. However, there were only relatively small amounts of calcium in the muscles and the interchicken variation was large; and therefore lack of statistical significance may have been expected.

Also, each of the treated cook-out juices from pectoralis major muscles contained less magnesium than the untreated muscles, and the difference between the mean value was statistically significant. The mean value for the calcium content of these treated juices was significantly lower than the value for the untreated juices.

There were no significant differences in magnesium or calcium contents between treated and untreated leg muscles or their cook-out juices. In fact, the treated mean values were all higher than the untreated values.

These results indicate therefore, that added polyphosphates react with magnesium and calcium in some way which results in the loss of these ions during thawing, leading to reduced levels in the cooked pectoralis major muscles and their cook-out juices. On this basis it is not surprising that there were no significant differences in the magnesium and calcium contents of treated and untreated leg muscles and their cook-out juices, because no, or very little, added phosphorus (or sodium) was found in these muscles (see Table XIp).

Treated thaw losses contained about 25% more of both calcium and magnesium than the untreated samples, indicating that these ions are lost to the same degree during thawing. This seems to contradict the findings of Inklaar,[101] and Baldwin and deMan,[102] (cited in section 1.612), that about 3 times more calcium than magnesium is firmly bound to muscle proteins, and is therefore unavailable for reaction with added phosphates. However, factors other than that they tested beef muscle, make direct comparisons between their results and the present ones impossible. First, the amounts of ions present in the free, and soluble and insoluble protein-bound states were not calculated here, and therefore the effect of polyphosphates on this equilibrium could not be determined. The importance of specifying the state of the ions is illustrated by comparing the work of Baldwin and deMan, who did, and Inklaar, who did not (see section 1.612). Second, individual polyphosphate species have different relative affinities for these metals (see sections 1.612 and 1.613), and therefore the difference in ionic composition between treated and untreated samples depends not only on the relative availabilities of magnesium and calcium; but on the levels of the individual polyphosphates - which were not known exactly. Consequently, the most that can be said of these thaw loss results is that, Puron 604 results in calcium and magnesium being lost to the same extent during thawing, from chickens injected, stored and analysed as specified here.

The treated pectoralis major muscles contained 22% less calcium than the untreated muscles, and 16% less magnesium; and the cook-out juices from treated pectoralis major muscles 12% less calcium and 18% less magnesium than the untreated juices. Calcium and magnesium, therefore, were lost to the same degree from treated muscles plus cook-out juices. These losses were very high, and since metals are known to be prooxidants (see section 1.42), it seems that this removal of calcium and magnesium by polyphospnates must, at least in part, explain their

antioxidant properties. In addition, these findings reinforce the explanations of the effect of polyphosphates on WHC which depend on polyphosphate chelation of calcium and magnesium (see section 1.612).

Thawed raw treated muscles must also contain less calcium and magnesium than untreated muscles, although the raw samples were not analysed. However, polyphosphates have been shown to have little or no antioxident effect on raw muscles. It can only be assumed that heat greatly increases the prooxidant properties of metals.

These ionic composition results are very important since they conclusively show that polyphosphate treated chicken muscles contain less calcium and magnesium than untreated muscles after thawing. To the author's knowledge this is the first time that the ionic composition of commercially treated chicken muscles have been compared to untreated muscles in this manner.

It must be remembered that literature values are usually quoted on a wet weight basis, and therefore, it is not possible to determine if ions are leached out during cooking, because of the effect of moisture losses during cooking (see section 3.42; and, for example, the discussion of Zenoble and Bowers[143]). Analysis of the cook-out juices overcomes this problem and provides a more accurate determination than would values calculated from a comparison of raw and cooked dry weight values.

3.710 Correlation Coefficients between Phosphorus Content and Sodium, Magnesium and Calcium Contents

Table XIr shows the correlation coefficients between phosphorus content and sodium, magnesium and calcium contents of all thaw losses, cooked muscles and cook-out juices.

These coefficients were primarily calculated in order to see if loss of calcium and magnesium during cooking was directly related to the loss of phosphorus i.e. to see if calcium and magnesium were lost from treated muscles as a result of chelation with phosphate anions, as was suggested in section 3.43.

TABLE XIr

CORRELATION COEFFICIENTS[a] BETWEEN PHOSPHORUS CONTENT AND SODIUM, MAGNESIUM AND CALCIUM CONTENTS OF THAW LOSSES; COOKED MUSCLES AND THEIR COOK-OUT JUICES.

ION	SAMPLE									
	Thaw Losses		Pectoralis Major Muscle		Cook-out Juice from Pectoralis Major Muscle		Leg Muscle		Cook-out Juice from Leg Muscle	
	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated
Sodium	+0.98‡‡	+0.67	+0.97‡	+0.48	+0.90x	+0.34	+0.62	-0.34	+0.90x	+0.64
Magnesium	+0.81o	+0.82o	+0.71	+0.90x	+0.08	+0.97‡	-0.24	+0.99‡‡	+0.59	+0.80
Calcium	+0.79	+0.79	+0.82o	+0.64	+0.42	+0.50	+0.68	-0.68	+0.75	+0.40

a. Based on data in Tables XIp and XIq

o Significant at 95% level

x Significant at 98% level

‡ Significant at 99% level

‡‡ Significant at 99.9% level

If the above were true, then positive correlation coefficients would be expected between the phosphorus content and the calcium and magnesium contents of treated cook-out juices. This was true for both treated and untreated pectoralis major and leg cook-out juices; in fact, the only statistically significant coefficients were obtained between the phosphorus content and magnesium content of untreated juices. This theory, therefore, may not be proved from these results. It must be remembered, however, that the interchicken variations in phosphorus, magnesium and calcium contents may have invalidated these calculations. The correlation coefficients between the phosphorus content and calcium and magnesium contents of the thaw losses and cooked muscles also failed to distinguish between treated and untreated samples.

The coefficients between the phosphorus content and sodium content of treated thaw losses, muscles and cook-out juices, were positive and statistically significant. This is not surprising since high losses of the anion component of the injection would be expected to correspond with high losses of the cation component.

3.711 Correlation Coefficients between TBA Numbers and Phosphorus, Magnesium and Calcium Contents of Cooked Muscles

Table XIs shows the correlation coefficients between TBA Numbers and phosphorus, magnesium and calcium contents of pectoralis major and leg muscles. The TBA Numbers of only T1,2 and 3 and U1,2 and 3 pectoralis major muscles were calculated, and therefore these coefficients were calculated for these 6 muscles only. However, the TBA Numbers of all the leg muscles were determined, and these coefficients were calculated for all 12 muscles.

These correlation coefficients were calculated in order to see if there was any relationship between the extent of autooxidative rancidity (as determined by the TBA Numbers) and the levels of added phosphate (see also section 3.43). In addition, it was thought that useful results would be obtained by comparing TBA Numbers with the

TABLE X1s

CORRELATION COEFFICIENTS[a] BETWEEN TBA NUMBERS AND PHOSPHORUS, MAGNESIUM AND CALCIUM CONTENTS OF PECTORALIS MAJOR AND LEG MUSCLES

ION	SAMPLE			
	Pectoralis Major Muscle[b]		Leg Muscle[c]	
	treated	untreated	treated	untreated
Phosphorus	-0.83	-0.19	-0.76	+0.33
Magnesium	+0.97	-0.58	-0.69	+0.32
Calcium	+0.34	-0.23	-0.23	+0.18

a. Based on data in Tables X1j, X1p and X1q

b. 0.997 required for significance at 95% level

c. 0.811 required for significance at 95% level

levels of magnesium and calcium.

It may be seen from Table XIs that the correlation coefficients between TBA Numbers and phosphorus levels of treated pectoralis major and leg muscles were negative and numerically high, although they were not statistically significant. Thomson,[86] also found a negative correlation between TBA Numbers and phosphorus levels of treated, cooked fryer chickens. Since polyphosphates decrease the TBA Number of treated, cooked chicken, then these negative correlations are as would be expected i.e. as the amount of added phosphate increases, then the TBA Number decreases. It is interesting to note that treated leg muscles exhibited this behaviour, although there was no significant difference in TBA Numbers between treated and untreated leg muscles (see also section 3.791).

These correlation coefficients between TBA Numbers and phosphorus levels for untreated leg and pectoralis major muscles were numerically small, and impossible to interpret. This is because there is no justification for expecting a significant correlation between these variables in untreated chicken muscle, and the values are only given here so that they may be compared with the treated results.

The correlation coefficients between TBA Numbers and magnesium and calcium contents are more difficult to interpret. In untreated muscles, a positive correlation would be expected since high concentrations of metal ions, which are pro-oxidants, would be expected to result in high TBA Numbers. Also, the same would be expected of treated leg muscles because there was no significant difference in magnesium or calcium contents between treated and untreated leg muscles. Such positive correlations were found for untreated leg muscles, but not for treated leg muscles or untreated pectoralis major muscles. In any case these coefficients were not statistically significant.

For treated muscles, the same reasoning as for untreated muscles applies, but with the additional variables of the effect of added phosphates on the TBA Numbers and on the metal ion concentration. Such positive correlations were obtained for treated pectoralis major muscles, but again these were not statistically significant. Further discussion of these results would be pointless in view of the variables mentioned above.

3.8 COMPARISON OF TBA RESULTS FROM EACH EXPERIMENT

Since the TBA Numbers of both raw and cooked muscles were determined on muscles from chickens after ½ to 47 months of frozen storage, it was thought that a comparison of each set of results would yield important information about the nature of the TBA test itself.

Table XIIa shows all of the mean TBA Numbers of raw and cooked muscles after 0 days of holding, i.e. "immediately" after excision of the raw muscles, and after cooking of cooked muscles, within the time limit of sample preparation and after no more than 2 weeks storage of the excised muscle at -18°C. It may be seen that each set of values were similar to each other, except for untreated cooked pectoralis major muscles after ½ month of frozen storage. This anomaly is accounted for in section 3.76. Three important points arise from this observation:

1. Freshly excised and freshly cooked muscles have similar TBA Numbers. This indicates that autooxidation does not proceed to any great degree during the cooking process used in this work, nor during the cooling, mincing and sample preparation stages (at least 1 hour in total).

2. Raw and cooked muscles from chickens after short-term frozen storage have similar TBA Numbers to those from chickens after long-term frozen storage, during 0 days of holding. This indicates that the TBA test when performed during 0 days of holding, is not suitable for determining the oxidative status of muscles (see section 3.61, where freshly excised muscles from chickens after 47 months frozen storage gave off rancid odours).

3. The above 2 points apply to muscles from both treated and untreated chickens. This indicates that the TBA test is not suitable for distinguishing between treated and untreated muscles, when performed during 0 days of holding of either the raw or cooked muscles.

TABLE XIIa

MEAN TBA NUMBERS OF RAW AND COOKED PECTORALIS MAJOR AND LEG MUSCLES FROM CHICKENS AFTER VARIOUS PERIODS OF STORAGE AT -18oC, DURING 0 DAYS HOLDING.

Time of storage of chickens at -18°C(Months)	STATE OF MUSCLE	MUSCLE			
		Pectoralis Major		Leg	
		treated	untreated	treated	untreated
½	Cooked	0.66±0.33	4.10±1.01	-	-
4½	raw	0.58±0.25	0.73±0.28	0.45±0.06	0.55±0.25
	cooked	0.72±0.14	0.66±0.11	-	-
21	raw	0.48	0.94	-	-
	cooked	1.25	0.48	-	-
36	raw	0.35±0.16	0.82±0.35	1.22±0.67	0.65±0.19
47	raw	0.48±0.01	0.69±0.13	-	-

Table XIIb shows all of the TBA Numbers of raw and cooked pectoralis major muscles during 1 to 9 days of holding at 4°C. It should be noted that the TBA Numbers of muscles after 21 months frozen storage, during holding at 4°C (Table Vc) are not given in this Table. This is because of their unrepresentative nature (see section 3.1).

Two important points are illustrated in this Table:

1. For cooked muscles, the TBA Numbers of treated muscles were always significantly lower than those of untreated muscles during 1 to 9 days holding (cf. point 3 above).

2. For raw muscles, the TBA Numbers of muscles from chickens after 6 months frozen storage were much higher than those of muscles from chickens after 47 months frozen storage, during 8 days of holding. This was presumed to be because autooxidation had proceeded during 47 months frozen storage leading to reduced levels of malonaldehyde in these chicken tissues (see section 3.61). Thus, TBA analysis of muscles during holding for 1 or more days, distinguished between muscles from chickens after short-term and long-term frozen storage.

It may be seen therefore, that TBA tests should be performed throughout a period of holding in order to gain the maximum amount of information in experiments of the present type. In fact, if TBA tests had been performed on only freshly cooked muscles, in this work, then it would have been concluded that polyphosphate treatment had little effect on autooxidation of cooked chicken tissue. This finding is in addition to the known limitations of the TBA test during the very early and very late stages of autooxidation (see section 2.31).

A further point which is illustrated in Table XIIb is that the TBA Numbers of some individual raw and treated cooked samples fluctuated during holding, and did not steadily increase to a plateau, as may have been expected. These fluctuations in untreated raw and cooked meat muscles have also been reported by Keskinel et al;[38] and

TABLE XIIb

TBA NUMBERS OF RAW AND COOKED PECTORALIS MAJOR MUSCLES FROM CHICKENS AFTER VARIOUS PERIODS OF STORAGE AT -18°C, DURING 1 TO 9 DAYS HOLDING AT 4°C

Time of Storage of chickens at -18°C(Months)	Days Held at 4°C	TREATMENT														
		Raw								Cooked						
		treated				untreated				treated				untreated		
		1	2	3	M±S.D.[a]	4	5	6	M±S.D.[a]	1	2	3	M±S.D.	4	5	6	M
4½	2	-	-	-	-	-	-	-	-	0.80	0.80	2.60	1.40 ±1.04	6.64	5.81	8.30	
6	1	-	-	-	-	-	-	-	-	0.90	1.25	1.33	1.16 ±0.23	5.27	8.64	8.57	
	2	1.05	1.04	1.30	1.13 ±0.13	1.33	1.56	1.12	1.34 ±0.22	-	-	-	-	-	-	-	
	5	2.55	3.75	2.82	3.04 ±0.63	2.68	1.40	1.93	2.00 ±0.64	-	-	-	-	-	-	-	
	7	-	-	-	-	-	-	-	-	0.79	1.11	1.99	1.30 ±0.62	8.89	11.52	12.38	±
	8	2.66	4.57	3.68	3.64 ±0.96	2.25	2.82	1.25	2.11 ±0.79	-	-	-	-	-	-	-	
	9	3.03	4.04	4.03	3.70 ±0.58	1.90	3.93	1.40	2.41 ±1.34	0.54	1.62	1.95	1.37 ±0.74	9.11	12.06	12.60	
47	4	0.59	0.58	-	0.59 ±0.01	1.62	0.81	-	1.22 ±0.57	-	-	-	-	-	-	-	
	8	0.80	0.29	-	0.55 ±0.36	1.33	0.60	-	0.97 ±0.52	-	-	-	-	-	-	-	

a. Mean ± standard deviation

in treated and untreated cooked chicken muscles by Thomson,[86] Also, Jacobson and Koehler,[40] (see Table II for values) and Arafa and Chan,[144] have observed these fluctuations during holding of untreated cooked chicken tissues. It seems reasonable to assume, as Dawson and Schierholz,[145] did, that the products responsible for TBA reaction are produced and recombined in meat systems in an "erratic fashion".

3.9 SUMMARY OF ALL THE RESULTS

3.91 PHOSPHORUS COMPOSITION

The phosphorus contents of raw and cooked pectoralis major, pectoralis minor, and leg muscles were determined (Table VIIIb); and of thaw losses, cooked pectoralis major and leg muscles, and their cook-out juices (Table XIp). There were clear differences between treated and untreated samples:

All treated thaw losses, raw and cooked pectoralis major and pectoralis minor muscles, and the cook-out juices from pectoralis major muscles, contained significantly more phosphorus than the untreated samples. Also, one batch of cooked treated leg muscles contained significantly more phosphorus than the untreated muscles, but this difference was very small compared to the differences between treated and untreated breast muscles. Also, there was no significant difference in phosphorus content between the treated and untreated cook-out juices from these leg muscles (Table XIp). For the other batch of leg muscles, there were no significant differences between either the raw or cooked treated and untreated muscles (Table VIIIb).

Therefore, polyphosphate treatment of eviscerated chickens by injection into the breast muscles prior to chilling and freezing, results in increased levels of phosphorus in the thaw losses and in the raw and cooked pectoralis major and pectoralis minor muscles. Also, the cook-out juices from treated pectoralis major muscles contain significantly more phosphorus than those from untreated chickens. However, little or no added phosphorus was found in the leg muscles from treated chickens.

3.92 SODIUM COMPOSITION

The sodium contents of thaw losses, cooked pectoralis major and leg muscles, and their cook-out juices were determined (Table XIp). The differences between treated and untreated samples were the same as for the phosphorus contents, except for the leg muscles, when there was

no significant difference in sodium content between treated and untreated muscles.

Therefore, chickens injected with sodium polyphosphate, as detailed above, have increased levels of sodium in their thaw losses and cooked pectoralis muscles and cook-out juices, but not in their leg muscles and cook-out juices.

3.93 MAGNESIUM, CALCIUM AND IRON COMPOSITIONS

The magnesium, calcium and iron contents of thaw losses, cooked pectoralis major and leg muscles, and their cook-out juices were determined (Table XIq). The mean values for the magnesium and calcium contents of treated thaw losses were significantly higher than those of untreated thaw losses; and these mean values for treated cooked pectoralis major muscles and cook-out juices were significantly lower than the mean untreated values. There were no significant differences between treated and untreated leg muscles and cook-out juices for either the calcium or magnesium contents. The levels of iron in all of these samples were very low and of little value for comparing treated and untreated samples.

Therefore, polyphosphate treatment was found to result in calcium and magnesium being lost from frozen chickens during thawing. The treated pectoralis major muscles and their cook-out juices contained less calcium and magnesium than the untreated muscles, but the treated leg muscles and cook-out juices did not.

3.94 HYDROLYSIS OF POLYPHOSPHATES IN CHICKEN TISSUES

Attempts were made to study the rate of hydrolysis of sodium tripolyphosphate after mixing with excised chicken muscles, and during subsequent storage at $4^{\circ}C$ (section 3.31). However, the TLC method used to detect and measure the amount of polyphosphate species present, lacked sensitivity, and the results only pointed to the fact that considerable hydrolysis occurred immediately after mixing and was probably complete after 23 hours of refrigerated storage.

A further experiment was conducted using ^{31}P-FTNMR to detect and determine polyphosphate species (section 3.32). Samples of pectoralis major muscles from treated chickens after 5, 15 and 43 months storage at -18°C were analysed. The fraction of polyphosphates hydrolysed in the 5 month muscles ranged from 0 to 4%; in the 15 month muscles, 9 to 32%; and in the 43 month muscles, 66 to 100% (Table VIIa). There was an anomalous result in the 5 month samples, where 40% hydrolysis occurred, but this was explained (see section 3.32).

Therefore, this first experiment found that considerable hydrolysis of sodium tripolyphosphate occurred in excised chicken muscle immediately after mixing, and was probably complete within 23 hours at 4°C. The second experiment showed that hydrolysis of a commercial mixture of sodium polyphosphates (Puron 604) progressed through 15 to 43 months of frozen storage, but that very little hydrolysis had occurred after 5 months of frozen storage.

3.95 UPTAKE OF CHILL WATER

Of the 3 separate batches of chickens used in these experiments, the uptake of chill water was calculated in 2 of them, as the difference in pre-chill and post-chill (and drain) weights, (section 3.71). There was no significant difference in chill water uptake between 6 treated and 6 untreated batch C chickens (Table XIa). However, for the total number of 11 treated and 11 untreated batch C chickens, the mean value for the treated chickens was significantly less than that for the untreated chickens. Also, the mean value for the total number of 12 treated batch A chickens was significantly less than the mean value for the total number of 12 untreated chickens. In addition, mean values showed that the treated batch A chickens took up less chill water, and received a higher level of polyphosphate injection, than the treated batch C chickens.

Therefore, polyphosphate injection by pre-chill injection,

was found to result in a decrease of chill water uptake, when the mean values of the entire batch of treated and untreated chickens were compared. Also, there was some evidence of the amount of chill water uptake decreasing with increasing levels of injection.

3.96 COOK LOSSES

The cook losses of excised pectoralis major and leg muscles after steam cooking were determined (Tables 1Xa, Xlb). Also, the cook losses of 1 treated and 1 untreated pectoralis major muscle after roasting with no added fat were determined (Table Va). There were no significant differences in cook losses between treated and untreated samples for either the pectoralis major or leg muscles after steam cooking. However, after roasting, the treated pectoralis major muscle had a considerably higher cooked yield than the untreated muscle. It was not possible, however, to take this as proof that polyphosphates reduce cook losses during roasting, because only 1 muscle from each treatment was tested.

Therefore, polyphosphate treatment was found to have no effect on cook losses after steam cooking, but there was some evidence that roasting losses may be reduced by polyphosphates.

3.97 MOISTURE CONTENT

The moisture content of raw pectoralis major, pectoralis minor, and leg muscles were determined (Table Vlllc). The treated pectoralis major muscles contained significantly more water than the untreated muscles, but there were no significant differences between treated and untreated pectoralis minor or leg muscles.

Therefore, polyphosphate treatment by injection into the breast muscles was found to result in increased levels of water in the raw pectoralis major muscles, but not in the pectoralis minor or leg muscles.

3.98 AMOUNT AND TOTAL HAEM CONTENT OF THAW LOSSES

The volume of thaw losses from treated and untreated chickens was determined (Tables Va and XIa). One treated chicken after 21 months of frozen storage was found to have less fluid on thawing than 1 untreated chicken (Table Va). However, there was no significant difference in the mean thaw loss values between 6 treated and 6 untreated chickens after 2 weeks frozen storage (Table XIa).

The total haem content of the thaw losses from treated and untreated chickens was determined (sections 3.62 and 3.72, Table XIa). Preliminary studies on 2 treated and 2 untreated chickens after 47 months frozen storage, indicated that the treated thaw losses contained more than twice as much total haem than the untreated thaw losses (section 3.62). However, analysis of the thaw losses from 6 treated and 6 untreated chickens after 2 weeks storage at $-18^{o}C$, showed that there was no significant difference between treated and untreated thaw losses (Table XIa).

Therefore, polyphosphate treatment was found to have no effect on the volume of thaw loss from chickens after 2 weeks frozen storage; but there was some evidence to suggest that it reduced thaw loss in chickens after 21 months frozen storage. Also, polyphosphate treatment was found to result in increased amounts of total haem in the thaw losses of chickens after 47 months frozen storage, but not after 2 weeks frozen storage.

3.99 LIPID COMPOSITION

The lipid composition of both raw (Table VIa) and cooked (Table XIc) muscles were determined. For raw muscles, it was found that neutral lipid showed greater interchicken variation than phospholipid. Also, there was a direct relationship between the neutral lipid and total lipid levels, indicating that higher lipid levels arise primarily from the deposition of neutral lipid. Leg muscles contained more total lipid

and a higher proportion of neutral lipid than the pectoralis major muscles. For both freshly cooked and rancid cooked muscles there were no consistent interchicken differences.

The only significant difference between polyphosphate-treated and untreated muscles occurred for the phospholipid contents (% wet tissue) of raw pectoralis major muscles; when the treated muscles contained significantly less phospholipid than the untreated muscles.

Therefore, on the whole there were no significant differences in lipid composition between treated and untreated raw or cooked muscles.

3.910 FATTY ACID COMPOSITION

The fatty acid composition of raw pectoralis major (Table Vlb) and leg (Table Vlc) muscles, freshly cooked and rancid cooked pectoralis major muscles (Tables Xld and Xlf), and of the cook-out juices of pectoralis major (Table Xlh) and leg (Table Xli) muscles were determined. The main fatty acids in both neutral lipid and phospholipid fractions were C16:0, C18:1 and C18:2, with phospholipids also containing large amounts of C18:0, and more C20:4 and other long-chain fatty acids than the neutral lipids. This was true of both raw and cooked muscles, although the interchicken variation in the raw muscles was small, and that of the cooked muscles relatively large. The total lipid of the cook-out juices usually contained large amounts of C16:0, C18:1 and C18:2, but there were very large interchicken variations.

There were no statistically significant differences between polyphosphate-treated and untreated raw or freshly cooked muscles, or cook-out juices. However, for rancid cooked pectoralis major muscles, the neutral lipid of treated muscles contained significantly more total unsaturated fatty acids than that of untreated muscles.

Therefore, on the whole, there were no significant differences in fatty acid composition between treated and untreated muscles.

3.911 TBA NUMBERS

The TBA Numbers of treated and untreated raw (Tables Vc, Vle, Vlla, lXc, Xa) and cooked (Tables Vc, Vlla, lXb), pectoralis major muscles were determined during 0 to 11 days of holding at 4°C. These results are summarised in section 3.8.

There were no significant differences in TBA Numbers between raw treated and untreated pectoralis major muscles. However, the TBA Numbers of cooked treated pectoralis major muscles were significantly lower than those of untreated muscles throughout 1 to 11 days of holding, but not after 0 days. For leg muscles (Tables Vle, Vlla, lXb, lXc, Xa), there were no significant differences between treated and untreated raw or cooked muscles (presumably because little or no added phosphate was present in leg muscles, see section 3.42 and 3.791).

Therefore, polyphosphate treatment was found to produce low TBA Numbers in cooked pectoralis major muscles after 1 or more days of holding, and to have no effect on the TBA Numbers of raw pectoralis major muscles and raw or cooked leg muscles.

3.912 ADDITIONAL RESULTS

Correlation coefficients between various sets of results were calculated, and a number of important points arose from these calculations. There was some evidence to suggest that:

a) Dietary C18:2 is deposited in chicken tissues to a larger extent than other dietary fatty acids; and also that neutral lipids exhibit most pronounced changes in the 16- and 18- carbon fatty acids, and phospholipids in the long-chain fatty acids, as a result of diet (section 3.23).

b) The 16- and 18- carbon fatty acids of neutral lipid, and the poly-unsaturated 20 plus- carbon fatty acids of phospholipids are the most important indicators of the degree of autooxidation in raw (section 3.25) and cooked (section 3.772) muscles. However, fatty acid levels were not

significantly correlated with TBA Numbers during the early stages of raw and cooked muscle autooxidation; but these values approached significance during the later stages of autooxidation - as in rancid cooked muscles (section 3.772).

c) The neutral lipids play the major role in raw muscle autooxidation (section 3.26), and the phospholipids in cooked muscle autooxidation, although autooxidation in rancid cooked muscles is independent of any one lipid level (section 3.78).

d) TBA Numbers of treated cooked pectoralis major muscles decrease as the level of polyphosphate treatment increases (section 3.711).

e) Polyphosphate treatment results in increased levels of unsaturated fatty acids being lost in the cook-out juices of treated muscles compared to untreated samples (sections 3.771, 3.772 and 3.773).

4. CONCLUSION

4.1 GENERAL INFORMATION

The bulk of this work was concerned with the determination of the degree of autooxidation of chicken tissues (as measured by the TBA test), and the effect of the fatty acid composition of muscles on these values. Unfortunately, it was apparent that large interchicken differences in TBA Numbers were not a result of measurable differences in fatty acid composition. However, the fatty acid composition of rancid cooked muscles showed much greater interchicken variation than that of freshly cooked and raw muscles; and some correlation coefficients between TBA Numbers and fatty acid levels approached significance. These correlations were negative for unsaturated fatty acids, which means that TBA Numbers increase as the levels of unsaturated fatty acids decrease, as would be expected.

Thus, it appears that the changes in fatty acid composition caused by autooxidation are too small to be detected by the GLC procedure used in this work. The TBA test, on the other hand, proved to be a sensitive method for determining the carbonyl by-products of autooxidation.

It was suggested that autooxidation seems to depend more on the fatty acids of neutral lipids than those of phospholipids in raw muscle because the phospholipids, which are highly susceptible to autooxidation because of their high polyunsaturated fatty acid content, are in close association with muscle proteins and are therefore unavailable for reaction. In cooked muscles, however, phospholipids were found to play the major role in autooxidation; and it was assumed that this was because the phospholipid-protein associations were destroyed by heat. In rancid cooked muscles autooxidation was found to be independent of any one lipid level. It was assumed that this was because, in addition to autooxidation, lipase action would have affected the lipid levels during the 5 day holding period. Also, of course, during the late

stages of autooxidation, the less susceptible unsaturated fatty acids of neutral lipid would have been oxidised, as well as the highly susceptible polyunsaturated fatty acids of phospholipids.

4.2 MODE OF ACTION OF POLYPHOSPHATES

It is convenient at this stage to summarise the effects of polyphosphates in frozen chickens, before going on to discuss the possible reasons for these effects.

Polyphosphate treatment of eviscerated chicken carcases by injection into the breast muscles, prior to chilling and freezing, has been found to affect the chicken in the following ways:

1. To have a variable effect on the amount of chill water uptake and thaw and cook losses. The bulk of the present work suggested that polyphosphates have no effect on any of these levels, but a few results showed that each of these levels were reduced due to treatment. Grey et al,[18] reported these same inconsistent results. However, it must be stressed that they found that treatment resulted in a net increase in the weight of the carcase at all stages of processing and cooking, compared to untreated samples.

2. To result in increased amounts of haem compounds in the thaw losses of chickens after long-term storage, but not after short-term storage.

3. To have no measurable effect on the lipid and fatty acid composition of freshly cooked chicken muscles. However, there was some evidence to suggest that treatment resulted in decreased levels of phospholipid in raw muscles, and increased levels of unsaturated fatty acids in rancid cooked muscles.

4. To result in reduced TBA Numbers in cooked muscles after the first day of holding, but to have no effect on raw muscle values.

5. To result in increased moisture levels in raw pectoralis major muscles, but to have no effect on the moisture content of pectoralis minor or leg muscles.

6. To result in increased levels of calcium and magnesium in thaw losses, and decreased levels in cooked muscles and cook-out juices.

It should be noted that leg muscles from treated chickens were found to contain very little or no added phosphate. Therefore, there were no differences between the leg muscles from treated and untreated chickens, and each of the above points apply only to the pectoralis major muscles.

Perhaps the most important finding of this work was that polyphosphate treatment was found to result in increased levels of calcium and magnesium in thaw losses, and decreased amounts in cooked muscles and their cook-out juices (and therefore in raw muscles). This finding proved that polyphosphates actually remove these metal ions from chicken muscles; whereas previous work by other workers has only indicated that polyphosphates alter the calcium and magnesium equilibrium in meat tissues.

Therefore, it seems that added polyphosphates are able to form stronger complexes than proteins with metals, resulting in loss of these metals from chicken muscles. However, the present work tells us nothing about the mechanism of this reaction; nor whether polyphosphates remove metal ions simply by virtue of their well-known metal chelation properties, or because of their structural similarity to ATP.

There was some indirect evidence to suggest that polyphosphates are associated in some way with the phospholipids of raw and cooked chicken tissues. This seems feasible since the polar phospholipids are themselves associated with muscle proteins, and would perhaps be affected by the removal of calcium and magnesium ions which bridge muscle proteins.

A further important point to arise from this work was that added polyphosphates were found to undergo considerable hydrolysis immediately after mixing with excised chicken muscle, and also after 15 and 43 months of frozen storage of the commercially injected chickens. This rapid hydrolysis on mixing has been found in other types of meat, but chicken tissues have not been studied in this way before. Also, no polyphosphate analyses have previously been carried out on chickens after prolonged frozen storage. Orthophosphate, the ultimate hydrolysis product, is not effective as an antioxidant, but cooked treated muscles after prolonged frozen storage have been found to have lower TBA Numbers than untreated muscles. Therefore, injected polyphosphates must have some immediate and irreversible effect on chicken tissues. Metal-chelation leading to loss of metal ions from the treated thawed chicken, could well be such an immediate and irreversible reaction.

It must be remembered at this point that loss of calcium and magnesium from chicken tissues could both increase WHC and inhibit autooxidation, as explained in section 1.6.

Therefore, each of the 3 points above explain the effects of polyphosphates by their ability to chelate and remove calcium and magnesium from chicken tissues. However, this cannot be the sole reason for the effectiveness of polyphosphates because other workers have found that individual polyphosphate species and other metal-chelation agents such as EDTA do not affect meat tissues in proportion to their metal-chelation strength.

Lipid and fatty acid analyses failed to distinguish between treated and untreated samples. However, correlation coefficients between TBA Numbers and fatty acid levels indicated that polyphosphate treatment may result in increased amounts of unsaturated fatty acids being lost from cooked treated muscles compared to untreated muscles.

This effect would obviously help to account for the fact that cooked treated muscles have lower TBA Numbers than cooked untreated muscles. However, it was not possible from the present work to determine the mechanism by which this reaction could take place; unless it is connected with the suggestion that polyphosphates associate in some way with phospholipids.

This work has therefore proved that added polyphosphates remove calcium and magnesium from thawed chicken carcases, and this fact alone may partly explain the mode of action of polyphosphates. There was some evidence to suggest that added polyphosphates may also be associated with the phospholipids of raw and cooked tissues, and to result in loss of unsaturated fatty acids during cooking.

APPENDIX I

BACTERIOLOGICAL EXAMINATION

In experiment 3.5 it was decided to check for the presence of micro-organisms in the cooked pectoralis major muscles held at 4°C when it was noticed that there were no objectionable odours after 7 days of holding, and that the TBA Numbers of treated muscles remained very low. The method is outlined in section 2.8, and the qualitative nature of these results is also explained in this section.

Preliminary qualitative methods showed that after 8 days of holding at 4°C samples of all 3 treated muscles showed colony growth after incubation on nutrient Agar plates. No growth was apparent for any of the untreated samples. After 10 days of holding each treated muscle again showed colony growth and so did number 4, and to a lesser extent number 6 of the untreated samples. It has been suggested that micro-organisms destroy the carbonyl compounds measured in the TBA test,[145] and, therefore, contaminated samples might be expected to have reduced TBA Numbers. However, although the TBA Number of sample number 4 was always considerably lower than that of number 5 during 9 days of holding, that of number 6 was higher (Table 1Xb). No bacteriological tests were carried out on the raw muscles, and so the slightly higher TBA Numbers of treated samples compared to untreated samples (Table 1Xc) could not be checked against microbial growth, to see if micro-organisms do, in fact, reduce the carbonyl content of muscle.

Raw leg muscles after 7 days of holding at 4°C showed no overall differences in micro-organism content between muscles from treated and untreated chickens. These results would seem to bear out the results of section 3.42 and 3.791 that little or no polyphosphate reaches the leg muscle of treated chickens. However, Foster and Mead,[96] (see section 1.53 for experimental details), found

that whilst polyphosphate inhibited micro-organism growth in raw breast muscle, it had little effect on raw leg muscle.

This growth promotion effect of polyphosphate on cooked pectoralis major muscle, is in direct conflict with the reasonably well established antimicrobial effects of polyphosphates (see section 1.53). However, this antimicrobial effect has been reported in raw chicken muscles, and no results for cooked muscles have been found in the literature. This is understandable, since microbial spoilage is of much greater importance in raw meat because the cooking process destroys all vegetative micro-organisms. Unfortunately, no tests on raw pectoralis major muscles were carried out here because detailed microbiological work was beyond the scope and time limit of this research. However, raw treated pectoralis major muscles had objectionable rancid odours and a "sweaty" appearance after 9 days of holding at $4^{o}C$, whilst untreated samples were of normal texture and had only slight off-odours after this time. In addition, all of the literature quoted in section 1.53 tested the microbial status of chicken tissues after immersion of the whole bird in polyphosphate solutions, or of intimate mixtures of polyphosphate and chicken muscle. It is conceivable that treatment by injection, as in this research, may affect the micro-organisms in a different way.

This is undoubtedly an area which warrants further research; especially in view of the fact that micro-organisms may destroy the carbonyl compounds which are measured in the TBA test, and this could help to account for the wide range of TBA Numbers found for similarly treated samples, as suggested by Moerck and Ball.[146]

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