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The effects of phosphate blends on the microbiology of a cooked meat system

John Allen Marcy
Iowa State University

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THE EFFECTS OF PHOSPHATE BLENDS ON THE MICROBIOLOGY OF A
COOKED MEAT SYSTEM

Iowa State University

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The effects of phosphate blends on the
microbiology of a cooked meat system

by

John Allen Marcy

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major: Food Technology

Approved:

Members of the Committee:

Signature was redacted for privacy.

In Charge of Major Work

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For the Major Department

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For the Graduate College

Iowa State University
Ames, Iowa

1987

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DEDICATION

This work is dedicated with love to my wife, Barb, who has supported me steadfastly throughout our time in Ames, especially when the times were trying, the hours long, and the money short.

INTRODUCTION

Inorganic phosphate use in hams and bacon was common in the United States by 1956 (Swift and Ellis, 1956), and has been allowed for use at 0.5% by weight in a large variety of products under the category of cooked sausage since 1982 (USDA, 1982). Regulation and subsequent use of phosphates is determined solely by their effect on the functional properties of the products to which they are added (Tompkin, 1983).

This group of chemicals is important to the producer and to the consumer from economic and product quality considerations. Like sodium nitrite, no other additive or group of additives has been shown to duplicate exactly the unique properties brought about by the inorganic phosphates when added to meat.

Due to recent concerns regarding the level of sodium in the diet (NAS, 1980; IFT, 1980; Sebranek et al., 1983), the inorganic phosphates have been utilized in formulating meat products with lower levels of sodium chloride. This has been done while maintaining product acceptability (Puolanne and Terrell, 1983; Thompson and Shimp, 1984). However, simply eliminating processed meats from the diet or requesting processed meats with lower salt levels will have only a minor impact on total sodium intake (Maurer, 1983).

Although phosphates will continue to be used in meat products because of their functional value, it is important to look broadly at the antimicrobial value of this group of additives. At the same time, a close look at particular phosphates and their specificity of action, if any, against groups or even single microorganisms in meats is long overdue. It is the intent of the work presented here to shed some light on the possibilities available for development.

Nomenclature and Structure of Phosphates Allowed in Meat Products in the United States

The inorganic phosphates allowed by the United States Department of Agriculture (USDA, 1982) for use in meat products are:

Disodium or dipotassium phosphate (orthophosphate, dibasic),
Monosodium or monopotassium phosphate (orthophosphate, monobasic),
Sodium metaphosphate, insoluble (Maddrell's salt),
Sodium polyphosphates, glassy (sodium hexametaphosphate, Graham's salt),
Sodium or potassium tripolyphosphate,
Sodium or potassium pyrophosphate (trisodium, tetrasodium or tetrapotassium), and
Sodium acid pyrophosphate.

The dibasic and monobasic, sodium or potassium salts of orthophosphoric acid are generally referred to as

orthophosphates and contain one phosphorus atom per molecule. The four pyrophosphates listed contain two phosphorus atoms per molecule. The acid pyrophosphate has two hydrogens available and is acidic in aqueous solution. Trisodium pyrophosphate has only one hydrogen and is neutral in solution, whereas tetrasodium and tetrapotassium pyrophosphate are fully neutralized with the alkaline metals and are alkaline in solution.

Phosphates containing three or more phosphorus atoms are referred to as polyphosphates if they form a straight chain and have the general formula of $M_{(n+2)}P_nO_{(3n+1)}$, where M stands for either hydrogen or a metal ion, such as sodium or potassium (Ellinger, 1972). Metaphosphates either have a cyclical or ring structure, or the general formula $M_nP_nO_{3n}$, as is the case with the extremely long-chain insoluble sodium metaphosphate, also known as Maddrell's salt (Van Wazer, 1958). The tripolyphosphates have three phosphorus atoms. Sodium polyphosphate, glassy is the correct name for what has been called, in the industry and the literature, sodium hexametaphosphate. It is a straight chain polyphosphate, usually containing between 10 and 15 phosphorus atoms (Ellinger, 1972). Sodium polyphosphate, glassy is also known as Graham's salt.

The inorganic phosphates will be abbreviated during the remainder of this work, with the abbreviations used shown in Table 1. The structures of some of the commonly used sodium phosphates are shown in Figure 1.

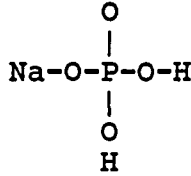
Functional Properties of Phosphates in Meat Products

In view of the fact that the regulation and use of inorganic phosphates are based upon the functional properties of these compounds in meats (Tompkin, 1983), this review includes information on those properties and some of the hypotheses concerning the mode of action of the phosphates in affecting such properties.

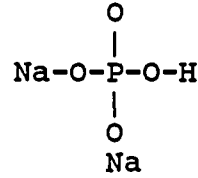
Table 1. Abbreviations of inorganic phosphates

PHOSPHATES	ABBREVIATION
Orthophosphates (general)	ortho-P
Sodium Acid Pyrophosphate	SAPP
Trisodium Pyrophosphate	Pyro-3
Tetrasodium Pyrophosphate	TSPP
Tetrapotassium Pyrophosphate	TKPP
Sodium Tripolyphosphate	STPP
Potassium Tripolyphosphate	KTPP
Sodium Polyphosphates, Glassy (sodium hexametaphosphate)	SPG

ORTHOPHOSPHATES

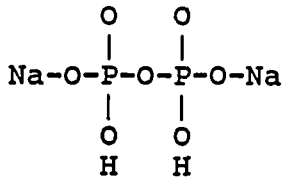


monosodium orthophosphate

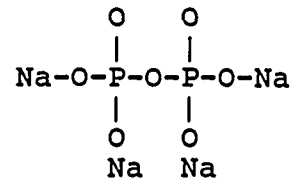


disodium orthophosphate

PYROPHOSPHATES

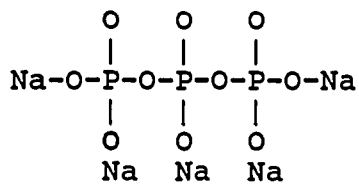


sodium acid pyrophosphate



tetrasodium pyrophosphate

POLYPHOSPHATES



sodium tripolyphosphate

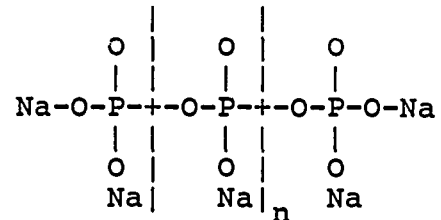
sodium polyphosphate, glassy
(n=8-13, Ellinger, 1972)

Figure 1. Structure and classification of some common sodium phosphates

Water holding capacity (WHC)

Increased water holding capacity is probably the most important aspect of phosphate addition to meats, both from an economic and a product quality standpoint. Prior to the use of phosphates in meat products, sodium chloride (salt) had been recognized for its ability to influence the water holding capacity and binding quality of meat products (Hamm, 1960). First, addition of salt to meat shifts the isoelectric point of the muscle to a lower pH. This effect is attributed to the chloride anion instead of the more hydrated sodium cation (Terrell and Olson, 1981). The shift of the isoelectric point is important since the isoelectric point is considered to be the point of minimum water holding capacity. The explanation of this phenomenon is that at the isoelectric point, the net charge of the proteins is zero and therefore, least repulsion of like charges takes place. That results in the fibers being closer together and in less space for bound water. As the pH of the meat shifts away from that at the isoelectric point, the net charge of the proteins increases and results in increased interstitial space and in more room for water to enter and be bound.

Another effect of salt which has an impact on water holding capacity, as well as other functional properties in meat, is the ability to solubilize the myofibrillar proteins (Paul, 1972). In this case, ionic strength is most

important because it is a determining factor in the solubility of the salt-soluble myofibrillar proteins. Protein solubility increases with increasing ionic strength (Ishioroshi et al., 1979) It is the myofibrillar proteins in solution which stabilize a meat emulsion and hold the water and fat in a protein matrix for emulsion products such as frankfurters or bologna.

Bendall (1954) was among the first to study the effect of inorganic alkaline phosphates on muscle. He studied the swelling of lean rabbit muscle treated with orthophosphate, pyrophosphate, SPG of 12-14 PO_4 residues, SPG of 20-30 PO_4 residues, and potassium metaphosphate of at least 10,000 PO_4 residues per molecule. He concluded that the alkaline polyphosphates increased muscle fiber swelling due to a non-specific ionic strength effect and that TSPP had an effect on WHC greater than could be attributed to an increase in ionic strength alone. The specific pyrophosphate effect was thought to be caused by the ability of TSPP to dissociate actomyosin to actin and myosin. Swift and Ellis (1956) found no real differences between orthophosphate and pyrophosphate in terms of rabbit muscle volume. Their results disagreed with the concept of a specific effect of pyrophosphate. The authors concluded that only pH and ionic strength influenced the extent to which pyrophosphates increased the WHC of ground meat.

Shults and Wierbicki (1973) stated that the maximum effect on WHC would be achieved by addition of 0.2-0.4% phosphate. Hamm (1971) summarized the manner in which alkaline phosphates affect water holding capacity. He stated that WHC is affected by an increase in pH, an increase in ionic strength, the ability of phosphates to bind to meat proteins, and the ability of phosphates to dissociate actomyosin to actin and myosin.

Phosphates can have a pronounced effect on the pH of raw or cooked meat. The order of increasing effectiveness for the alkaline phosphates in raising the pH of raw beef was reported to be SPG < STPP < TSPP (Shults et al., 1972). In contrast, Tims and Watts (1958) had observed that for effect on WHC, SPG < TSPP < ortho-P < STPP. Hamm (1960) found that WHC was affected more in the order: ortho-P < TSPP < SPG < STPP. Shults et al. (1972) and Wierbicki et al. (1976) rated the alkaline phosphates in order of increasing effect on the WHC of meats as SPG < STPP < TSPP, which corresponded to the manner in which those phosphates increase pH. Knipe (1982) cited Jauregui as proposing that nearly 80% of the effect of STPP and TSPP on WHC was due to a pH effect. Hamm (1971) later postulated that STPP was most effective because it hydrolyzed to TSPP plus ortho-P. Sutton (1973) also considered phosphate breakdown when he

saw conformational changes in cod muscle treated with STPP, and stated that possibly the effect was caused by TSPP resulting from STPP hydrolysis.

All of the phosphates mentioned so far as affecting positively the WHC of meat are alkaline phosphates. Since these phosphates raise the pH of meat away from the isoelectric point, the integral role of pH in determining the effect of a particular phosphate on the WHC of meat is clearly demonstrated. Sodium acid pyrophosphate (SAPP) however, is an acid phosphate which reduces the pH of meat. As would be expected, SAPP has been shown to increase processing shrinkage (Terrell et al., 1982), decrease cooked yields (Hargett et al., 1980), and diminish the swelling of raw meat (Hellendoorn, 1962). Miller et al. (1986) used STPP, SAPP, and SPG in restructured steaks and found that while the control steaks had significantly less moisture than steaks with any phosphate, taste panelists rated the control steaks significantly more juicy than those treated with STPP and SAPP. The control steaks had higher mean juiciness scores than any phosphate treatment.

The ability to dissociate actomyosin is well documented for TSPP (Mommaerts, 1948; Fukazawa et al., 1961; Yasui et al. 1964a; and Kiely and Martonosi, 1968), and is based upon comparisons with adenosine triphosphate (ATP). Mommaerts

(1948) had reported that ATP decreased the viscosity of a solution of actomyosin, but when ATP was hydrolyzed to adenosine diphosphate (ADP) plus orthophosphate, the viscosity increased to almost the original level. Yasui et al. (1964a) observed the same changes with ATP. When pyrophosphate was added to an actomyosin solution, the viscosity was lowered (Mommaerts, 1948; Yasui et al., 1964a). Mommaerts (1948) reported that once the viscosity had been decreased by phosphate addition, no subsequent increases in viscosity were observed. In contrast, Yasui et al. (1964a) stated that as the TSPP was hydrolyzed to orthophosphate, the viscosity increased much in the same way as observed for ATP and ADP. Kiely and Martonosi (1968) observed that pyrophosphate and actin competed for the same binding sites on myosin.

The reduction in viscosity by phosphates has been reported in meat batters other than protein solutions. Swift and Ellis (1957) reported that the increase in emulsion temperature normally seen with increases in the length of chopping time, was much lower when phosphate was added. Kena, a commercial phosphate blend, caused a reduction in viscosity of mechanically deboned poultry (Schnell et al., 1973) and SAPP was also reported to lower emulsion viscosity and reduce frictional heat buildup (Hargett et al., 1980).

The specific pyrophosphate effect of dissociating actomyosin is important to water holding capacity if protein extraction is less than maximum, because it can increase the amount of proteins in solution. The addition of pyrophosphate caused considerably greater extraction of soluble protein from native myofibrils than did STPP or SPG (Fukazawa et al., 1961). Sherman (1961a, b, 1962) believed that the manner in which phosphates affected WHC was by increased protein solubilization. The protein forms a gel upon heating, retaining fat and water in a protein matrix.

Bendall (1954) found that the extracted fluid from rabbit muscle was viscous when 1.0% NaCl and 0.5% TSPP were used. Increased viscosity indicated a substantial increase in the concentration of soluble protein in the extracted fluid. When NaCl was used alone at the same ionic strength as the salt-phosphate combination, Bendall (1954) reported no increase in viscosity of the extracted fluid. That supported his theory of the specific effect of pyrophosphate. Yasui et al. (1964b) stated that increased protein solubilization by phosphates was due to ionic strength increases, and that it may be a non-specific ionic strength effect.

Knipe et al. (1985) found that tetrapotassium pyrophosphate (TKPP) was better at solubilizing protein than potassium tripolyphosphate (KTPP), TSPP or STPP. While the

pyrophosphate anion was also better at solubilizing protein than the tripolyphosphate anion, there was no difference between the sodium or potassium cations.

Prusa and Bowers (1984) studied protein extraction from frozen, thawed turkey muscle. They found that STPP increased the concentration of extracted protein, increased the number of electrophoretically separated higher molecular weight protein bands and increased high performance liquid chromatography peak areas of extracts. Prusa et al. (1984) reported that the viscosity of turkey breast muscle extracts was highest when salt and STPP were combined.

Prusa et al. (1985) related increased protein solubility and WHC in an attempt to explain a larger expansion maximum of turkey batter during heating caused by salt and STPP. The batters containing only salt expanded to a maximum when heated in an electrical resistance oven by a current of 40 volts and then contracted. However, the batters containing a combination of STPP and salt began to expand at the lowest voltage tested (23 volts), expanded more, and later contracted less than any other batter. Those results were attributed to myofibrillar protein solubilization and consequent increased WHC and emulsion stability brought about by STPP.

Texture

Texture, another major functional property, also is affected by increased soluble protein and, thus, by phosphates (Trout and Schmidt, 1986). Other workers have studied the effect of phosphate addition on texture. Baldwin and deMan (1968) reported increased tenderness of meat, as measured by use of the Kramer Shear Press, with addition of ortho-P, STPP, TSPP, and SPG, possibly due to increased water holding. McMahon and Dawson (1976), by working with mechanically deboned meat (MDM), found that 0.5% Kena, a commercial phosphate blend, increased total shear values to a constant degree as % MDM increased. Addition of STPP was important to firmness, texture preference, and breaking force in fish patties prepared from minced sheepshead (Ahmed et al., 1983).

Puolanne and Terrell (1983) found different phosphate effects on tenderness depending on the rigor-state of the meat. STPP, at a level of 0.375%, increased Instron force of rupture values in pre-rigor meat, while the force of rupture value was decreased by the phosphate in post-rigor meat.

Keeton et al. (1984) reported that while 0.5% STPP did not affect yields, it did increase sensory firmness. In the same study, by using textural profile analysis (TPA),

fractionability and hardness were also increased with STPP addition.

STPP used in the manufacture of restructured steaks resulted in an increase in the maximum force required to shear the sample (Miller et al., 1986). However, taste panelists in the same study gave phosphate treatments lower bite scores, indicating less resistance when compared to control steaks. Lamkey et al. (1986) reported increased bind in restructured beef steaks caused by the addition of Brifisol 414, a commercial blend of SAPP, TSPP, and SPG.

Synergism with salt

Although phosphates can modify meat proteins and their functional properties of texture and water and fat binding (yield), several studies have shown that salt interacts with the effect of phosphates and may enhance their effect. Mahon (1961) stated that 4.0% NaCl was the optimum level to combine with phosphates to achieve maximum WHC. Theno et al. (1978a, b) found that salt, in conjunction with phosphates, was responsible for the solubilization of protein during massaging of pork muscle, and that 1.0% salt, without phosphate, was insufficient to produce adequate meat binding, even with 24 hours of massaging. Lamkey et al. (1986) reported lower cooking losses in restructured beef steaks treated with phosphates, and lowest when salt and

phosphate were combined, resulting in a 4.12% decrease in cooking loss compared to salt alone. The authors went on to state that the combination of salt and phosphate would decrease cooking loss to a greater extent than either ingredient added separately. The alkaline phosphates have little effect when the salt level is below 0.8% (Bendall, 1954; Hellendoorn, 1962), greater than 2.0% (Trout and Schmidt, 1984), and between 1.25 and 1.5% if the pH is greater than 6.0 to 6.3 (Trout and Schmidt, 1984).

Trout and Schmidt (1984) also stated that the difference in effectiveness among alkaline phosphates, in terms of increased meat cook yield and tensile strength, appeared to be directly related to the increase in both pH and ionic strength brought about by the different phosphates. In later work, Trout and Schmidt (1986) concluded that phosphates increase the functional properties of restructured beef rolls in a way separate from increasing pH and ionic strength. Their results showed that phosphates increased tensile strength at high ionic strength and pH; a situation which could not be duplicated by increasing pH or by increasing the ionic strength with NaCl. Trout and Schmidt (1986) postulated that the mode of action of alkaline phosphates was to increase muscle protein functionality mainly by altering hydrophobic interactions. They observed that there was a linear decrease in

effectiveness of the phosphates with increasing chain length, due in part to hydrophobic interactions. Also, that pH changes had no effect on the activity of the phosphates, indicating a lack of electrostatic effect.

Antioxidant action

Watts (1950) attributed antioxidant effects of phosphates to their ability to chelate free metallic cations from the meat system. Watts (1950) also stated that the order of increasing effectiveness in inhibiting rancidity in lard was ortho-P < TSPP < SPG. The addition of 0.5% polyphosphate to deboned poultry meat was effective in maintaining lower TBA values and also resulted in lower total bacterial plate counts for a limited period (McNeil et al., 1973). Thomson et al. (1979) found that addition of 6.0% commercial polyphosphate to water at 70-90°C delayed oxidative deterioration of broiler chickens for up to 26 days at 2°C.

In more recent studies, Smith et al. (1984) found that phosphates in pork and beef roasts allowed the roasts to be reheated after 1 and 3 days of refrigeration with reduced warmed-over-flavor in pork, but was not successful in decreasing warmed-over-flavor in beef. In restructured steaks, salt dramatically increased the rate of rancidity development, whereas the addition of phosphate reduced it

(Lamkey et al., 1986). In that study, when salt and phosphate were combined, the rate of rancidity development was below that of the control steaks, but higher than in steaks treated with phosphate alone. In another study involving restructured steaks, STPP slowed lipid oxidation, as indicated by lower thiobarbituric acid (TBA) values, better than did SAPP and SPG (Miller et al., 1986). SAPP increased TBA values, probably by decreasing the pH. TBA values were also higher with SPG, presumably due to increased free radical formation as a result of increased numbers of phosphate groups (Miller et al., 1986).

Color

The unique property of SAPP, as compared to other phosphates, is the ability to lower the pH of a meat mixture. Addition of 0.5% SAPP reduces the pH approximately 0.5 pH units (Haymon, 1981). For that reason, SAPP was approved for use in cooked, cured sausage as a cure accelerator in 1972 (USDA, 1972). The reduction in pH effectively increases the speed of cured color development (Haymon, 1981).

Knipe (1982) cited Watts as reporting that STPP and SPG stabilized the cured color of hams from fading. Vollmar and Melton (1981) found that panelists assigned higher color scores to hams containing phosphates.

Antimicrobial Properties of Phosphates

The literature concerning the antimicrobial properties of phosphates can be divided into that work done by use of laboratory media and the work conducted with food systems. Each type of work has its own purposes and merits. However, it is not always possible to carry conclusions from one type of work to the other. Reviews of this topic have been written by Hargreaves et al. (1972) and Tompkin (1983).

Antimicrobial properties demonstrated in laboratory media

There have been several researchers who have demonstrated that culture age plays a factor in the antimicrobial effect of polyphosphates. Field and Liechstein (1957) reported that if cultures of Propionibacteria were preincubated, they grew faster in a medium containing 0.5% orthophosphate. The increased sensitivity to phosphates of younger bacterial cultures was confirmed by Firstenberg-Eden et al. (1981) and Molins et al. (1984). Firstenberg-Eden et al. (1981) reported that cultures of Moraxella-Acinetobacter which were 48 hours old were more resistant to phosphates than cultures that were only 24 hours old. Cultures that were 3 hours old were more affected by phosphates than 24 hour old cultures in laboratory media (Molins et al., 1984).

Another factor which might be important in assessing results from work conducted with phosphates in laboratory media, is the possibility of hydrogen peroxide formation. When a medium containing both phosphate and glucose is heated and later exposed to atmospheric oxygen, formation of superoxide radicals can occur (Carlsson et al., 1978). Hydrogen peroxide can be extremely lethal to bacteria (Miller, 1969) and may explain some of the results seen with phosphate.

Post et al. (1963) attributed inhibition of "wild" populations of bacteria to the SPG used to dissolve calcium alginate swabs. These workers found that the bacteria were inhibited proportionately to the concentration of SPG in nutrient agar, from 0.01 to 2.00%. The Gram-negative bacteria also were more resistant than the Gram-positive organisms. Lysis and inhibition could be prevented by addition of NaCl or $MgSO_4 \cdot 7H_2O$. That result suggested that the inhibition resulted from chelation of essential cations necessary for bacterial growth and development.

Irani and Callis (1962) studied the sequestration of calcium and magnesium by sodium and potassium polyphosphates. These workers found that the sensitivity of calcium sequestration to pH changes decreased with increased polyphosphate chain length. TSPP was also shown to sequester Ca^{++} above pH 7.0. The sequestration of magnesium

was also studied at pH 9.0 and 10.5 (Irani and Callis, 1962). TSPP and STPP were the best sequestrants on a weight basis. In another study, TSPP had maximum ferric iron sequesterant capabilities at a pH slightly below 7.5, and the ability to complex with ferric iron decreased with increasing polyphosphate chain length (Irani and Morgenthaler, 1963).

Another characteristic of microbial inhibition caused by chelation is the formation of small or "petite" colonies. Schulman and Dwyer (1964) found that Staphylococcus pyogenes formed small colonies when exposed to metal chelating agents. Impaired function of the cytochrome system occurred with subsequent decreases in the synthesis of ATP. Nagai et al. (1961) reported that small colonies of Saccharomyces lacked the absorption bands of cytochrome a and b of normal strains. Small colony formation was also reported by Firstenberg-Eden et al. (1981) in Moraxella-Acinetobacter exposed to phosphates.

Chelation was reported by Elliott et al. as the cause for the inhibition of pseudomonads grown in lab media containing 1.0% Kena (75% STPP, 25% TSPP). Growth of non-fluorescent pseudomonads was completely inhibited by 1.0% Kena, while fluorescent strains grew after an extended lag phase. Phosphate inhibition was reportedly reversed by addition of pyoverdine, bacteriological peptone, and

magnesium ions (Elliott et al., 1964). Gray and Wilkinson (1965) observed that SPG had effects similar to that of EDTA and concluded that the bacterial inhibition was due to chelation of metal ions on the cell wall. This chelation was responsible for leakage of cell solutes, loss of viability, and rapid lysis of the cell.

Inorganic phosphates have recently been shown to affect the antibiotic resistance of the lactic streptococci (Sinha, 1984, 1985). Inorganic ortho-P and organic beta-glycerophosphate increased the resistance of strains of lactic streptococci to streptomycin, neomycin and kanamycin (aminoglycoside antibiotics) (Sinha, 1984). However, those phosphates also decreased resistance of these same organisms to penicillin, erythromycin, and tetracycline. The organic phosphate was not as effective as the inorganic orthophosphate. Later, Sinha (1985) found that the difference in resistance to tetracycline was caused by steadily increased uptake of the antibiotic when dipotassium orthophosphate was in the medium, while no increase in antibiotic uptake occurred if the orthophosphate was omitted from the medium. The author suggested that resistance to tetracycline was due to a reduction in the normal uptake of the antibiotic, which could be reversed with phosphate addition.

Some of the earliest work with phosphates centered on the use of phosphate buffers and phosphates in sporulation media in the determination of the heat resistance of bacterial spores. Williams and Hennessee (1956) reported that the heat resistance of Bacillus stearothermophilus was decreased by increasing disodium orthophosphate in the heating menstruum from 8.3 mM to 66.7 mM. El-Bisi and Ordal (1956) found that spores of Bacillus coagulans var. thermoacidurans produced on media with a high concentration of phosphate were more sensitive to heat because the excess phosphates chelated calcium and other cations necessary for sporulating cells. That result was later confirmed and it was reported that sporulation medium fortified with Ca^{++} and Mn^{++} yielded spore crops with increased heat resistance (Amaha and Ordal, 1957). According to Ordal and Lechowich (1958), the death rate of Bacillus coagulans var. thermoacidurans spores was increased with high levels (125-250 mM) of phosphate in the heating menstruum. The death rate was also increased with low levels (2.5-10 mM) of phosphate. The highest heat resistance was found in heating menstruum containing 25 mM of phosphate. The researchers postulated that a) increased death rate with high phosphate levels was due to increased spore release of Ca^{++} and Mn^{++} caused by phosphate anions, and b) increased death rate at low phosphate levels was caused by osmotic and ionic

concentration differences between the spores and the heating menstruum. Walker (1964) also reported that different phosphate concentrations in the buffer used as a heating menstruum had an effect on the destruction of spores of Bacillus megaterium. The author observed the greatest rate of kill in distilled water, followed by 200 mM phosphate buffer. The maximum survival was found in phosphate buffers between 5 and 50 mM.

Finley and Fields (1962) observed a heat-induced dormancy when Bacillus stearothermophilus spores were heated between 80 and 100°C, with heat activation occurring above 100°C. If spores were heated in 8.33 mM phosphate buffer at 110-115°C, germination and growth were reduced. The same authors cited work of Brachfeld who observed that 50 mM phosphate buffer decreased the number of spores surviving, and that 0.5-1.25 mM phosphate buffer did not influence spore germination or multiplication of vegetative cells of Bacillus stearothermophilus.

Phosphates have also been reported to affect the heat resistance of vegetative cells. Garibaldi et al. (1969) reported a 6 log cycle reduction in the number of salmonellae in egg white after 60 hours at 28°C with the addition of EDTA or 4.0% Kena. D values for Salmonella typhimurium were reduced by a factor of 2 and 3.4 with Kena and EDTA respectively. D values for a heat resistant

Salmonella senftenberg were reduced by a factor of 1.7 and 9 with Kena at pH 5.3 and 9 respectively. This work was followed by a patent for pasteurizing egg whites at pH 9 in 3.5 minutes at 134°F (Kohl, 1971). In much later work, Seward et al. (1986) reported that STPP reduced heat resistance of salmonellae, and that heat sensitization was enhanced by increasing the heating time or reducing the inoculum size. However, when the inoculum was less than 10^3 cells/ml, 50°C was so bactericidal that the effect of the phosphate could not be detected. Those workers also studied chelation effects on heat resistance and found that EDTA reduced heat resistance of salmonellae. Addition of 0.05% $MnSO_4$, $MnCl_2 \cdot 4H_2O$, and $CaCl_2$ reduced the effect by a factor of 4 and also reduced subsequent antibacterial effects of polyphosphates.

Other workers have studied the effect of phosphate buffers on the germination of spores. Riemann and Ordal (1961) found that STPP in the phosphate buffer inhibited the germination of Clostridium sporogenes PA3679 and that calcium addition reversed the inhibition. This result was confirmed by Holdom and Foster (1967) who observed that phosphate in concentrations greater than 20 mM caused Bacillus megaterium spores to germinate and lyse. Addition of Ca^{++} , Mn^{++} , or Mg^{++} prevented lysis. Gould (1964) reported that morphologically defective cells emerged from

bacillus spores, but did not multiply in the presence of 0.2% SPG. At concentrations approaching 1.0% SPG, the spores germinated but were inhibited before lysis of the spore wall, whereas at SPG levels greater than 1.0%, the rate of spore germination was reduced.

Chen et al. (1973) studied the tolerance to Kena (STPP, TSPP, SAPP) of seventeen strains of bacteria isolated from spoiled poultry. Among seven Gram-positive coccoid isolates, one, Streptococcus lactis, could tolerate the maximum level of 6.0% tested; one could tolerate 1.0%; one isolate could tolerate 0.5%; and the remaining four could not grow in the presence of even 0.5% phosphate. The Gram-positive rods (Bacillus spp.) tested could tolerate 1.5 and 3.0% phosphate. Eight Gram-negative rods were also tested but only one, Alcaligenes faecalis (maximum tolerance at 1.0%), did not grow in the presence of 6.0% Kena. Molins et al. (1984) also reported increased resistance to phosphates by Gram-negative organisms.

Much of the current work on phosphates has been directed toward inhibition of Clostridium botulinum due to the questionable safety of sodium nitrite as a preservative in cured meats. The activity of SAPP against Clostridium botulinum is affected by the pH of the medium. Wagner and Busta (1985a) observed that growth from spores of Clostridium botulinum was greatly affected by a decrease in

pH from 5.85 to 5.55. Addition of SAPP at pH 5.85 and pH 5.55, however, did not affect botulinal growth from spores, while vegetative cells were definitely inhibited by addition of SAPP at pH 5.55 but not at pH 5.85. The same authors (Wagner and Busta, 1985b) found that addition of 0.4% SAPP alone allowed normal logarithmic growth of Clostridium botulinum, followed by lysis at pH 5.55 and 5.85. However, either no toxicity or delayed toxicity (about 24 hours after the control was affected) was observed. Cells were also longer and wider than they would normally be at pH 7.0 and 5.5.

Seward et al. (1982) reported that 0.5% STPP, added to medium containing 1.5% sorbate, prevented normal cell growth of Clostridium botulinum type E to an extent greater than sorbate alone. Wagner and Busta (1984) found that increasing levels of SAPP, from 0.0 to 0.4%, caused concurrent increases in inhibition of C. botulinum. There was no SAPP/NaCl interaction reported in that study. In another report, Wagner and Busta (1985c) stated that orthophosphate at 0.4% did not affect Clostridium botulinum vegetative cell growth, development or toxicity. However, while SAPP did not inhibit vegetative cell growth and development, botulinal cells were longer and wider than normal, with no toxicity or delayed toxicity. Also, addition of SAPP to known toxic Clostridium botulinum

supernatant delayed (4-8 hours) the death of mice, while addition of trypsin to known non-toxic Clostridium botulinum supernatant caused the supernatant to become toxic. Those results led to the hypothesis that SAPP affected the protease needed to activate the protoxin. Later, Wagner and Busta (1986) reported that SAPP, labeled with [³²P], appeared to bind cells and protein fragments from cells. An increase in [³²P] after the onset of the stationary phase, indicated the ability of SAPP to bind anion sites of proteins and other sources more readily than orthophosphate. Also, RNA had a significantly larger proportion of the radioactive label than DNA. Wagner and Busta (1986) cited Stahl and Ebel as reporting that polyphosphate-RNA complex formation is apparently due to an ionic interaction between phosphate groups and nucleic acid bases with Mg⁺⁺ possibly serving as a bridge. Wagner and Busta (1986) suggested that synthesis of proteases produced from RNA may be affected by the association of SAPP with the RNA of Clostridium botulinum cells.

Antimicrobial properties in meats

The inhibition of clostridia by phosphates has also been studied in meat systems. Much of that work was done with phosphates in combination with sodium nitrite, sorbic acid, and/or potassium sorbate in an attempt to find

suitable means of reducing nitrite levels in meat products. The work at this point is not definitive, but it is encouraging that there are some phosphates, especially SAPP, which may prove beneficial in the future.

SAPP had no effect or interaction with sodium nitrite in the inhibition of Clostridium botulinum in canned, comminuted pork (Ivey and Robach, 1978). However, SPG became increasingly inhibitory as the level of nitrite increased, so that botulinum outgrowth could be prevented by 156 ppm of sodium nitrite and 0.5% SPG. Molins et al. (1985b), working with cooked bratwurst inoculated with Clostridium sporogenes PA3679 and containing 0.5% SAPP, STPP, TSPP, or SPG, reported no microbiological differences during 7 days of refrigerated storage. However, during subsequent storage at room temperature for 48 hours, SAPP inhibited aerobic and anaerobic bacteria, including Clostridium sporogenes, followed in effect by TSPP and STPP, with no effect by SPG. In a later study, inhibition of Clostridium sporogenes by SAPP was enhanced by at least 100 ppm sodium nitrite (Molins et al., 1986).

Tompkin (1983) reported that 0.4% STPP, without nitrite, resulted in slightly delayed botulinal growth. Phosphate addition, when combined with 156 ppm of sodium nitrite, increased botulinal inhibition. Also, the rate of nitrite depletion decreased with increasing phosphate

concentration. Roberts et al. (1981a) stated that 0.3% of a commercial polyphosphate (Curafos 700) increased botulinal toxin production in pork slurries of low pH (5.5-6.3). However, high pH (6.3-6.8) slurries had decreased toxin production with similar addition of 0.3% polyphosphate (Roberts et al., 1981b). As a result of that work, regression equations for predicting botulinal toxin production were later published by Robinson et al. (1982).

Working with beef/pork frankfurters, Wagner and Busta (1983) observed that 0.4% SAPP did not affect aerobic mesophilic bacterial counts, but delayed toxicity when combined with sodium nitrite (40 ppm) and potassium sorbate (0.26%) or with sodium nitrite (120 ppm) alone. Samples containing SAPP first became toxic between 12 and 18 days at 27°C, while samples without SAPP first became toxic between 6 and 12 days. Jarvis et al. (1977) had also found that the pyrophosphates were more inhibitory to Clostridium botulinum than STPP or longer chain phosphates, and that spoilage was less affected by phosphates than toxicity. Nelson et al. (1983) observed that SPG and STPP, in combination with nitrite (40 ppm) and sorbic acid (0.20%), were less effective than SAPP in delaying botulinal toxin production in the pH range 5.87 - 6.19 in chicken frankfurter emulsions. The use of SPG and STPP resulted in toxin production between days 4 and 14 at 27°C, while SAPP

treatments were first toxic between 8 and 24 days. In total percentage of toxic samples, SPG was less effective (40-60% toxic samples) when compared to SAPP (9-52% toxic samples). Barbut et al. (1986) found that decreasing salt content from 3.0 to 1.0% in turkey frankfurters increased the speed of toxin production by an average of 3 days. Toxin was detected earlier when 0.4% STPP was added, SPG had no effect, and SAPP delayed toxin production by one day at 27°C, possibly due to lower pH.

Madril and Sofos (1986) observed that 0.5% SAPP and 1.3% NaCl (2.3% brine) reduced the rate of anaerobic, mesophilic bacterial growth, delayed initial formation of gas in cans of comminuted meat, reduced the rate of gas production, and at pH<6.3, also inhibited microbial growth from increasing above 10^5 CFU/g for up to 2 weeks at 27°C. However, at pH 5.7, the 2.3% brine with 0.4% SAPP was less effective than 4.1% brine (2.5% NaCl) without 0.4% SAPP in delaying gas production, which the authors stated indicated that SAPP might be more beneficial above pH 5.7. SAPP also showed antimicrobial activity at pH 6.3, indicating inhibitory properties distinct from reduced pH. Madril and Sofos (1986) concluded that SAPP may be more inhibitory at pH 6.0 than at 5.7, but additional work was needed for verification of their conclusion.

Shelf life extension

The preservative capabilities of phosphates were probably first tested in an industrial application to increase the shelf life of poultry. Spencer and Smith (1962) reported a 1-2 day extension of shelf life of fryers chilled in water containing 7.0% Kena. Kena, at 8.0%, or STPP, at 6.0%, resulted in lower bacterial counts for 20 days at 5°C compared with chilling water with no phosphate (Steinhauer and Banwart, 1963). Elliott et al. (1964) observed 17 and 25% increases in shelf life of chickens chilled with water containing 3.0 and 8.0% Kena, respectively. The same researchers found 17 and 67% increases in shelf life if the chickens were kept in continuous contact with solutions of 3.0 and 8.0% Kena, respectively. Also, if the ice used for the chilling tanks was made with water containing 8.0% Kena, there was a 60% increase in shelf life. Chen et al. (1973) reported that 3.0% Kena solution, applied either as precooking or presoaking treatments, eliminated most of the surface organisms of chickens. However, the phosphate also digested most of the skin of the poultry when cooked.

In contrast to reports of work conducted with other systems, Thomson et al. (1979) observed that addition of 6.0% polyphosphate (Kena FP-28, STPP and SPG) did not

influence the effectiveness of hot water (65-90°C) in destroying Salmonella in chicken. The reason phosphates did not affect the heat resistance of Salmonella was thought to be the protection afforded by the rough surface of chicken skin.

Snyder and Maxcy (1979) used SPG to reduce the water activity (A_w) of ground meat and meat exudate to study inhibition of Moraxella-Acinetobacter. They reported that Moraxella-Acinetobacter was unable to grow in meat or meat exudate containing 0.5% SPG and 20% added water or in meat or meat exudate with 1.0% SPG and 30% added water. Sikes and Maxcy (1980) observed that the depth to which Serratia marcescens could invade ground beef or pork was reduced by 16% and 84%, respectively, with addition of 0.5% STPP.

Hargreaves et al. (1972) cited Kitchell, who studied the effect of polyphosphate addition to Wiltshire cured bacon. Average surface counts were 70% higher on normal bacon than on bacon injected with polyphosphate. Gram-variable organisms dominated the flora of the normal bacon, but were largely inhibited on phosphate-treated bacon. Those authors speculated that if the normal Gram-positive flora of bacon were inhibited by phosphate, then the safety of bacon would have to be reexamined since those organisms serve as competitors and antagonists for pathogenic organisms such as Staphylococcus aureus.

No statistical difference was observed in refrigerated uncooked bratwurst containing 0.5% SAPP, TSPP, STPP, or SPG, even though the treatments containing SAPP were consistently lower in bacterial counts (Molins et al., 1985a). At the same time, large differences occurred in the pH of the various phosphate treatments, indicating a lack of effect due to pH. A possible explanation of no difference between phosphate treatments was enzymatic hydrolysis of the phosphates to orthophosphates. In later work, Molins et al. (1987a) observed that 0.4% TSPP, STPP, and three commercial phosphate blends (Brifisol 414B, Brifisol 614P, and Brifisol 414P) did not reduce mesophiles, psychrotrophs, lactic acid bacteria, presumptive Staphylococcus aureus, or viable anaerobic spores when added to beef patties, frozen and held at -20°C for 90 days. Neither did phosphates prevent spoilage when the patties were subjected to 24 hours of temperature abuse at 24-25°C.

SAPP was able to inhibit psychrotrophic bacteria when added at 1.0% to ground pork (Molins et al., 1987b). An increase of 50% in shelf life was achieved with 1.0% SAPP compared to control treatments and to 0.5 and 1.0% orthophosphate. One percent orthophosphate was even less inhibitory than 0.5%. In contrast, Hoes et al. (1980) reported higher total counts in phosphate-injected than non-

injected pork loins. However, that result could also be the difference between injection and non-injection since no loins were injected with fluid without phosphate.

Mol et al. (1971) reported the presence of an unclassified streptobacterium in vacuum-packed, sliced, cooked meat products. The streptobacterium was similar to Lactobacillus, but the researchers were unable to classify it as any known Lactobacillus. They found that it quickly became the predominant organism in vacuum-packed, sliced, cooked meat because of its ability to grow at 8°C. The organism was not affected by omitting polyphosphate (Hamine OX, 0.45%) from the berliner sausage formulation, by increasing the concentration of sodium nitrite to 200 ppm, by adjusting the pH of the product with 0.3% glucono-delta-lactone, or by combinations of these factors. The atypical streptobacterium was later studied by Nielsen and Zeuthen (1983) along with Brochothrix thermosphacta and Serratia liquefaciens. A low pH mixture of sodium pyrophosphate, sodium tripolyphosphate, and sodium polyphosphate inhibited the growth of Brochothrix thermosphacta and Serratia liquefaciens with little effect on the growth of the atypical streptobacterium. However, lactic acid accumulated less rapidly in sausage containing phosphate.

Objective of this research

The literature reviewed indicates that the various pyro- and polyphosphates have different effects on the functional properties of a meat system and also differ in antimicrobial activity within a meat system. Functional properties dominate the decision-making process to use phosphates in meat products and also which phosphate to use. Therefore, the major part of this research examines alkaline and neutral pyrophosphates, added to meat products for functional value, and effects of blending with sodium acid pyrophosphate in order to attain increased antimicrobial properties.

Explanation of dissertation format

This dissertation is divided into three parts, each being a complete paper either submitted (Part I) or to be submitted (Parts II and III) to a professional scientific journal. Part I examines the microbiological effects of sodium acid pyrophosphate and tetrasodium pyrophosphate blends on uninoculated cooked pork sausage. Part II studies the effects of sodium acid pyrophosphate and trisodium pyrophosphate blends on bacteria in uninoculated cooked pork sausage. Part III is a study of the effects of addition of selected commercial phosphate blends on uninoculated cooked

pork sausage, as well as the effect of different levels of phosphate addition, including levels higher than the 0.5% phosphate presently permitted by law.

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PART I.

THE EFFECT OF ACID AND ALKALINE PYROPHOSPHATE BLENDS
ON THE NATURAL FLORA OF A COOKED MEAT SYSTEM

THE EFFECT OF ACID AND ALKALINE PYROPHOSPHATE BLENDS
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J. A. Marcy, A. A. Kraft, D. K. Hotchkiss, R. A. Molins,
D. G. Olson, H. W. Walker, and P. J. White
Departments of Food Technology, Statistics,
Animal Science, and Food and Nutrition
Iowa State University
Ames, Iowa 50011

Running title: phosphate effects on bacteria in
meat products

All authors are at Iowa State University, Ames, Iowa 50011.
Authors Marcy, Kraft, and Walker are with the Department of
Food Technology. Author Hotchkiss is with the Department of
Statistics. Authors Molins and Olson are with the
Department of Animal Science, and author White is with the
Department of Food and Nutrition.

ABSTRACT

Combined effects of acid and alkaline pyrophosphates on the natural flora of a cooked meat system. J. A. Marcy, A. A. Kraft, D. K. Hotchkiss, R. A. Molins, D. G. Olson, H. W. Walker, and P. J. White. Departments of Food Technology, Statistics, Animal Science, and Food and Nutrition, Iowa State University, Ames, Iowa 50011.

Cooked pork sausage was prepared by using tetrasodium pyrophosphate (TSPP) and sodium acid pyrophosphate (SAPP) at 0 and 0.4% of the meat weight either separately or in different combinations. Samples were vacuum packaged and held in a refrigerated display case at 5°C for 21 days. Samples were also stored at room temperature (20-22°C) for 24 and 48 hours. Neither phosphate had an effect on microbial counts during refrigerated storage up to 21 days, but SAPP caused significantly lower counts of mesophilic and facultative anaerobic organisms after 48 hours of temperature abuse. The organisms affected were streptococci or very similar coccobacilli.

INTRODUCTION

It is becoming increasingly evident that phosphates, under certain conditions, have the potential for enhancing the microbial safety and stability of foods (Tompkin, 1983). These specific conditions have yet to be determined and are the subject for much research. In the meantime, phosphates are being used more widely than ever before in meat and poultry products since the revision in USDA regulations in 1982 (USDA, 1982). This use, and the regulatory limit of 0.5%, is based upon functional properties of phosphates and not on any direct or indirect antimicrobial properties that may occur (Tompkin, 1983).

Much of the current research with polyphosphates in meat has dealt with inhibition of either clostridial growth or, in the case of C. botulinum, toxin production. Tompkin (1983) has reviewed much of this work. Nelson et al. (1983) reported that toxin production was affected by the presence of 0.4% sodium acid pyrophosphate (SAPP), sodium hexametaphosphate (SHMP), and sodium tripolyphosphate (STPP) in combination with sorbic acid and nitrite, with SAPP inhibiting toxin production to a greater degree in poultry frankfurter formulations. Wagner and Busta (1983) reported similar results in beef/pork frankfurter formulations. These results are in agreement with a report by Jarvis et

al. (1977) that, in a cured meat system, the diphosphates or pyrophosphates are more inhibitory than the triphosphates or longer polyphosphates, with spoilage being less affected than toxicity. Wagner and Busta (1985) reported that a mechanism for toxicity inhibition was inhibition by SAPP of the production or function of the protease responsible for botulinal toxin activation.

Growth of clostridial species can also be inhibited by pyrophosphate addition. SAPP, alone or in combination with sodium nitrite, was able to inhibit C. sporogenes PA3679 during storage at 24°C for 48 hours (Molins et al., 1985a, 1986).

The objective of this work was to demonstrate that differences in the antimicrobial action of two pyrophosphates, tetrasodium pyrophosphate (TSPP) and sodium acid pyrophosphate (SAPP), in cooked meat were independent of the number of phosphorous atoms present and, if the two phosphates exhibited a synergistic effect when used in combination, to determine their optimum ratio. Both phosphates contained two phosphorous atoms per molecule.

MATERIALS AND METHODS

Pork trimmings with approximately 20% fat were obtained from the Iowa State University Meat Laboratory. The trimmings were ground through a 1/8" plate and mixed in a ribbon blender for 5 minutes. The meat was packaged in polyethylene bags containing 6 kilograms of meat and frozen to -28°C in an air blast freezer. One bag of meat was removed from the freezer and allowed to thaw at 5°C for 2 days prior to making sausage.

Sausage was prepared with 2.0% salt, 0.5% pepper, and 10% added moisture (phosphate solutions and/or water). All expressed percentages are relative to the weight of the meat used in the formulation. Food grade tetrasodium pyrophosphate (TSPP) and food grade sodium acid pyrophosphate (SAPP) were added as 5% w/v solutions to a total phosphate concentration of 0.4% of meat weight except for the control treatment. Six treatments were used as shown in Table 1. Each batch was mixed, stuffed into 30mm collagen casings (Teepak, Chicago, IL), and cooked in an 80°C water bath to an internal temperature of $65.5 \pm 1^\circ\text{C}$. The total time from start of mixing to the end of the cook was less than 20 minutes to inactivate the phosphatases present in the raw meat as quickly as possible (Awad, 1968, Sutton, 1973, and Molins *et al.*, 1985a, b). After cooking,

the sausages were cooled for 2 hours at 5°C and placed in individual 6 oz Whirl-pak bags (NASCO, Ft. Atkinson, WI). One sausage from every treatment, for a total of six sausages per bag, was vacuum packaged in a Curlon 892 (Curwood, Inc., New London, WI) bag (O_2 permeability < 1 ml/645 cm²/24 hr at 22.8°C and 0% RH) , and placed in a refrigerated display case at approximately 5°C until sampled.

Samples were evaluated for pH, mesophiles, psychrotrophs, total facultative anaerobes, and anaerobic spores after 0, 1, 4, 7, 14, and 21 days of refrigerated storage. Samples were also subjected to 24 and 48 hours of room temperature (20-22°C) storage after either 3 or 7 days of refrigerated storage. Mesophilic and psychrotrophic counts were made using Trypticase Soy Agar (BBL) and incubating at 30°C and 7°C respectively. Total facultative anaerobic and anaerobic spore counts were made with the anaerobic pouch method of Bladel and Greenberg (1965) using Brewer's Anaerobic Agar (Difco). Five replications were made for refrigerated storage and four replications for room temperature storage. Results were evaluated by using release 5.08 of the Statistical Analysis System (SAS Institute Inc., 1985).

Cultures were isolated from representative plates through 3 replications of the refrigerated storage and all 4

replications of the temperature abuse storage. Cultures were identified to group or genus by the scheme of Harrison et al. (1981). Subsequent tests were done on streptococci according to Sherman's criteria for enterococci, and included growth at 10°C, 45°C, 6.5% NaCl, pH 9.6, and reduction in 0.1% methylene blue milk (Wilkerson et al., 1961; and Pratt, 1969).

RESULTS AND DISCUSSION

TSPP and SAPP had few significant effects on the natural flora of cooked vacuum packaged pork sausage refrigerated for 21 days. The data from the mesophilic counts are represented in Figure 1. Mesophilic organisms did not increase or decrease consistently during the 21-day storage, but remained relatively constant. Numbers of mesophiles fluctuated more after day 7, at which time the variance in the data increased approximately eightfold. Significant differences were observed between the control treatment and all treatments containing phosphates on days zero ($P=.0280$) and one ($P=.0023$) of storage, in that the control treatment had significantly fewer cfu/gram of mesophilic organisms than the treatments containing phosphates. Hoes et al. (1980) reported that injection with phosphate also increased mesophilic organisms; however, the control in that study was not injected, and the difference could have been related to the injection operation. In our work, there was very sporadic growth of psychrotrophic organisms. Anaerobic spores were, for all practical purposes, nondetectable. The level of facultative anaerobic organisms remained relatively constant over the 21 days, on the order of 100 cfu/gram or less, with no meaningful or statistical differences.

The pH of all treatments stayed relatively constant over the 21-day period and the means are given in Table 2. The pH values of the treatments also remained stable during temperature abuse at 20-22°C and were directly proportional to the amount of each phosphate added. Nielsen and Zeuthen (1983) reported that addition of a low pH mixture of sodium pyrophosphate, sodium tripolyphosphate, and sodium polyphosphate had a stabilizing effect upon pH in sausage during storage at 8°C.

Sausages were also examined for changes during a short (48 hours) period of temperature abuse. All treatments had approximately the same level of mesophilic and facultative anaerobic organisms when removed from refrigerated storage. Upon subsequent temperature abuse at 20-22°C, large differences were noted between treatments with a significant linear effect after 48 hours of temperature abuse for both mesophilic (Figure 2) and facultative anaerobic bacteria (Figure 3). Mesophilic counts after 48 hours at 20-22°C decreased in a linear pattern from \log_{10} 7.681 for the control treatment to \log_{10} 5.704 for the treatment containing 0.4% SAPP ($P=.0141$). Facultative anaerobic counts after 48 hours at 20-22°C decreased in a linear pattern from \log_{10} 7.595 for the control treatment to \log_{10} 5.497 for the treatment containing 0.4% SAPP ($P=.0039$).

Culture identification from samples in refrigerated storage is given in Table 3. The total number of isolates identified was 811 (313 from refrigerated storage and 498 from temperature abuse storage). The results from refrigerated storage indicate a typical flora of cooked, vacuum-packaged meat containing pepper. Allen and Foster (1960) reported that the flora of vacuum-packed, sliced, processed meats changed from Gram-positive, catalase-positive to 100% Gram-positive, catalase-negative organisms between 10 and 20 days of refrigerated storage. The organisms were predominantly rods. This total change in flora was not seen in the present work with catalase-positive organisms still found on day 21. Later, Mol et al. (1971) found that unclassified streptobacteria grew fastest at 8°C and were able to outgrow other organisms in vacuum-packed, sliced, cooked meat products. These streptobacteria were not affected by 0.45% polyphosphate (Hamine Ox) or by 0.3% GDL (glucono-delta-lactone) alone or in combination with the polyphosphate even though the pH range was 6.30 to 6.10.

Table 4 represents the results of the identification of the 498 cultures taken during temperature abuse storage at 20-22°C. After 48 hours of temperature abuse, streptococci were the predominant organisms. These organisms did not

seem to always be typical streptococci; many exhibited coccobacilli morphology. They were also different from the unclassified streptobacterium described by Mol et al. (1971) in terms of growth temperatures. The unclassified streptobacterium of Mol et al. grew at 10°C but not at 45°C. Only 14 of the 213 Streptococcus tested did not grow at 45°C (6.6%), while only 128 of 213 grew at 10°C (60%). The streptococci isolated here were tested for Sherman's criteria (growth at 10°C, 45°C, pH 9.6, and 6.5% NaCl). To be classified as an enterococcus, the isolate must be positive for four tests, growth at 10 and 45°C, pH 9.6, and in the presence of 6.5% NaCl. Of the 213 isolates tested, 121 (56.8%) were positive for all four tests and 92 (43.2%) were negative for at least one test, the most common being no growth at 10°C.

In general, Gram-negative organisms have been found to be more resistant to phosphate addition than Gram-positive organisms. However, Chen et al. (1973) reported that Streptococcus lactis was very resistant to the commercial phosphate blend, Kena (STPP, TSPP, and SAPP). Nielsen and Zeuthen (1983) reported that Brochothrix thermosphacta and Serratia liquefaciens were strongly inhibited by addition of a low pH phosphate mixture whereas an atypical streptobacterium was only slightly inhibited by phosphate

addition. The streptococci isolated here were in fact inhibited by increasing levels of SAPP with little effect attributed to TSPP.

CONCLUSIONS

The acid and alkaline pyrophosphates were very different in their effect on bacterial populations when these populations were not controlled by refrigeration. There was no synergistic effect between the two types of pyrophosphates. Instead, there was a significant linear effect with the level of sodium acid pyrophosphate added. The decrease in counts as the level of SAPP was increased was a straight-line function from the control with no added phosphate to the treatment with 0.4% SAPP alone, and was approximately a difference of two log cycles for both the mesophilic and the facultative anaerobic bacteria present. SAPP produced significantly lower counts after 48 hours of temperature abuse.

The pH of the product, although directly proportional to the level of each phosphate added, was not the primary determining factor in the level of inhibition exhibited in that the control treatment had the same pH as the treatment containing 0.2% TSPP and 0.2% SAPP, but the mesophilic and the facultative anaerobic counts after 48 hours of temperature abuse were significantly different. However, the pH might have a secondary effect on the microorganisms by affecting the chelating ability of the phosphates present.

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Table 1. Phosphate treatments in percent of meat weight

<u>TREATMENT</u>	<u>TETRASODIUM PYROPHOSPHATE</u>	<u>SODIUM ACID PYROPHOSPHATE</u>
1	0.0%	0.0%
2	0.4%	0.0%
3	0.3%	0.1%
4	0.2%	0.2%
5	0.1%	0.3%
6	0.0%	0.4%

Table 2. Phosphate effect on pH of cooked sausage

<u>TRT</u>	<u>TSPP</u>	<u>SAPP</u>	<u>pH</u>
1	0.0%	0.0%	6.23
2	0.4%	0.0%	6.50
3	0.3%	0.1%	6.34
4	0.2%	0.2%	6.23
5	0.1%	0.3%	6.12
6	0.0%	0.4%	6.00

Table 3. Cultures identified during refrigerated storage^a

Bacillus	Micrococcus	Coryneform	Pseudomonas
39.94%	22.04%	16.61%	7.67%
Streptococcus	Coliform	Lactobacillus	Staphylococcus ^b
6.39%	3.83%	2.24%	1.28%

^a313 total organisms identified.

^bAll isolates were coagulase-negative.

Table 4. Culture identifications during temperature abuse.^a
Percent of total isolations for each treatment and time

HOURS ^b		TREATMENT					
		1	2	3	4	5	6
0	Streptococcus	19	26	21	14	8	-
	Bacillus	41	33	40	38	50	32
	Coryneform	7	-	11	3	-	4
	Micrococcus	22	38	21	28	38	39
	Staphylococcus ^c	4	3	7	10	4	11
	Lactobacillus	7	-	-	7	-	7
	Clostridia	-	-	-	-	-	7
24	Streptococcus	100	36	19	13	-	27
	Bacillus	-	-	23	43	45	50
	Micrococcus	-	54	50	22	41	14
	Staphylococcus ^c	-	10	8	22	14	9
48	Streptococcus	100	100	100	97	84	93
	Bacillus	-	-	-	-	10	7
	Micrococcus	-	-	-	3	6	-

^a498 total organisms identified.

^bHours of temperature abuse at 20-22°C.

^cAll isolates were coagulase-negative.

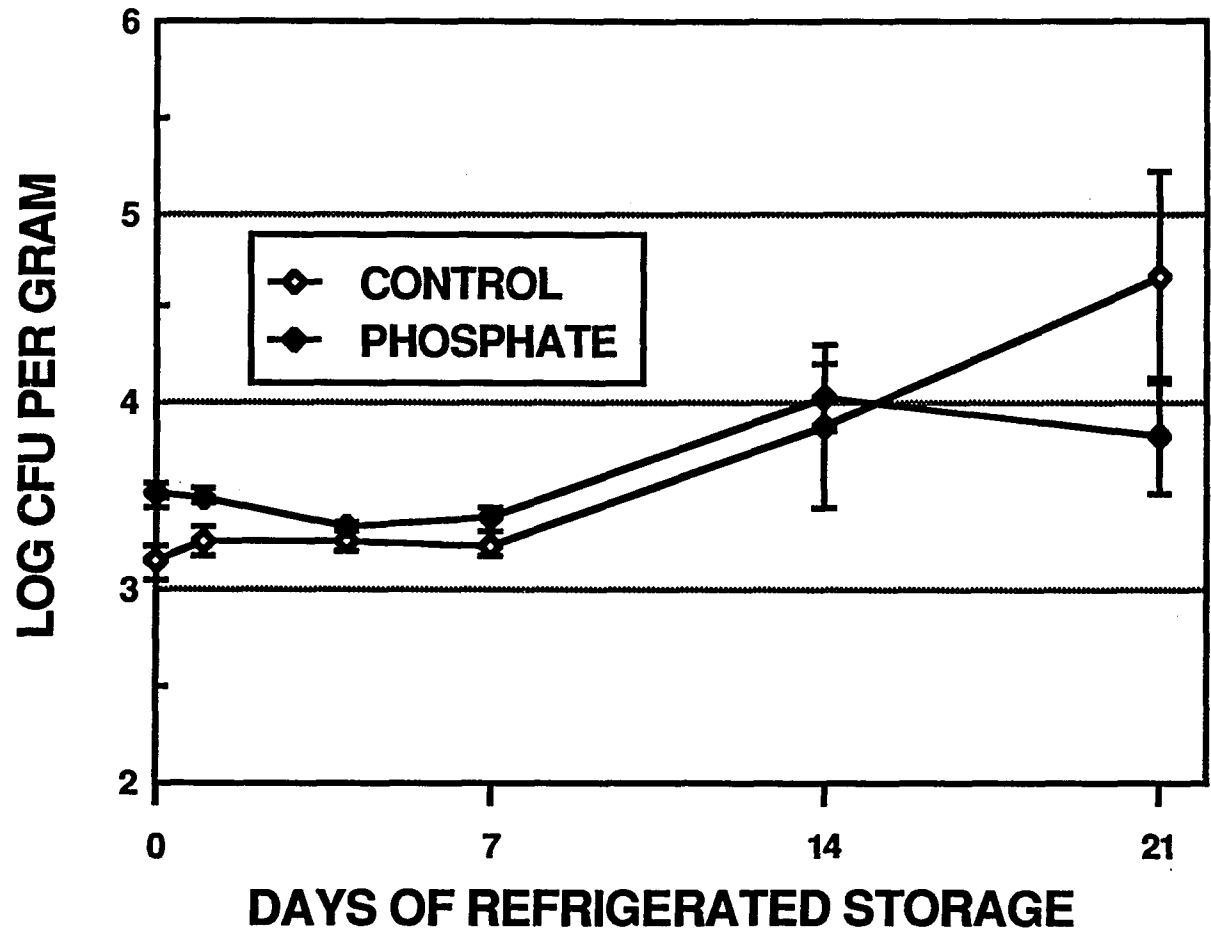


Figure 1. Mean responses of mesophilic bacteria during refrigerated storage at 5°C. Phosphate refers to the mean of all phosphate treatments. Overall S.E.M.=0.3643

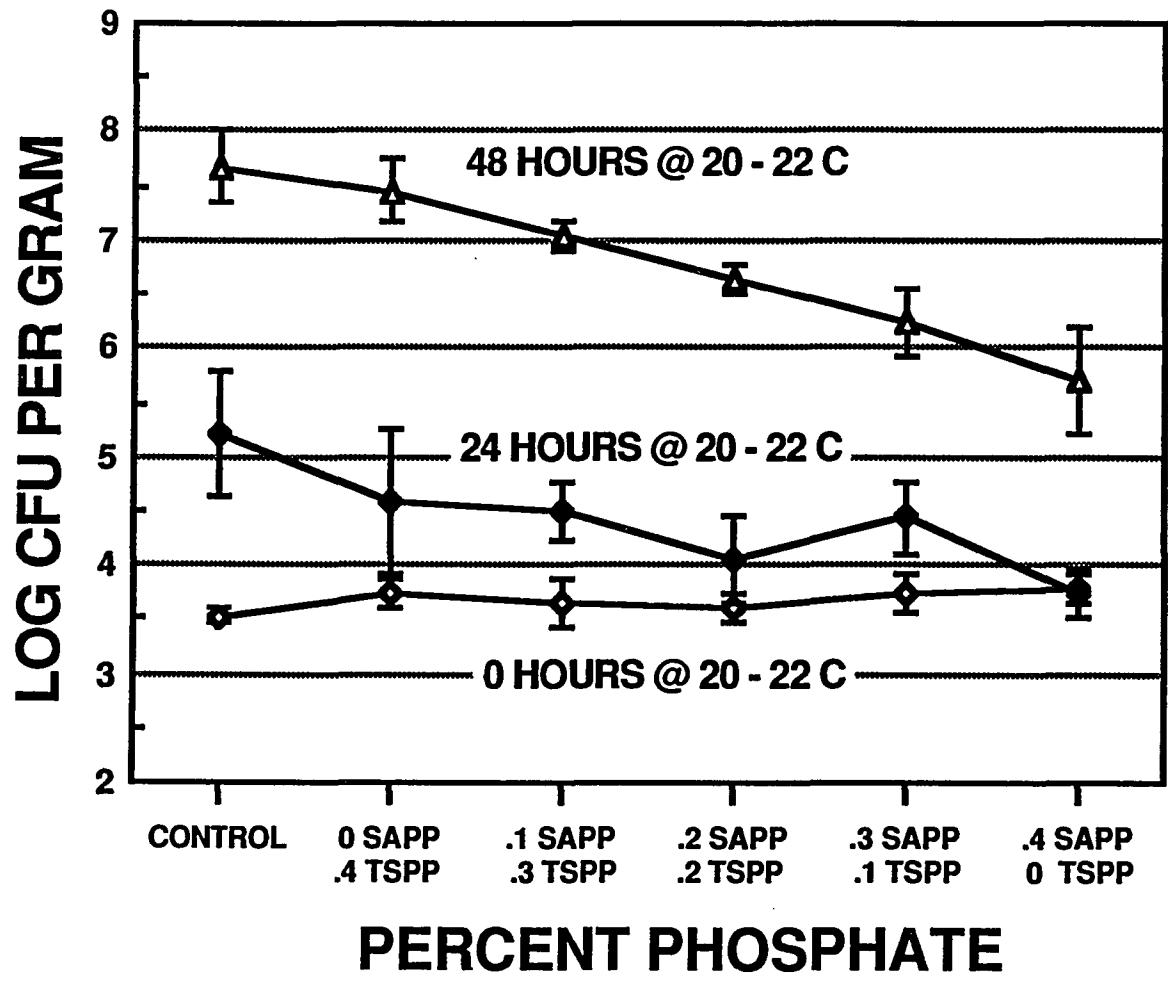


Figure 2. Mean responses of mesophilic bacteria after 0, 24, and 48 hr temperature abuse (20-22°C) storage. Overall S.E.M.=0.3027

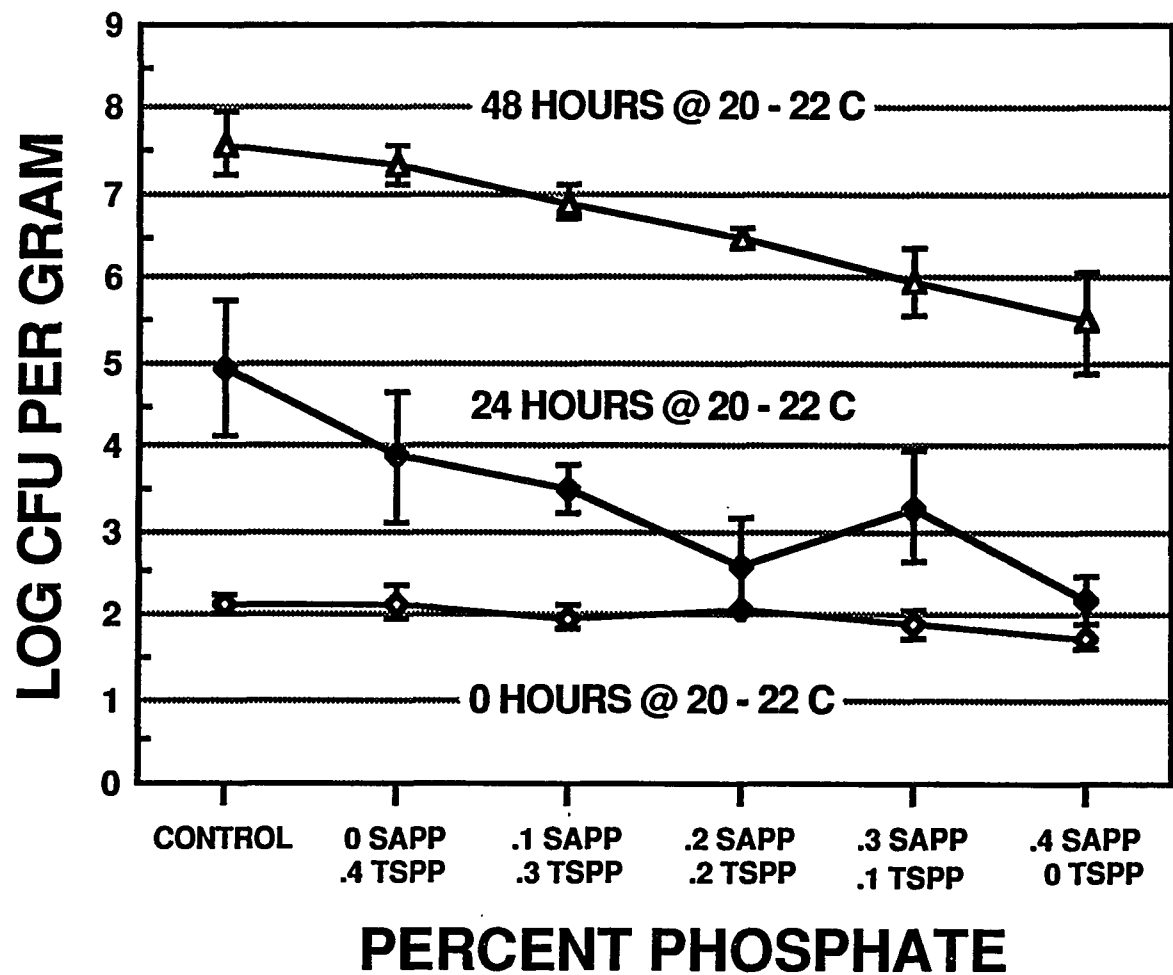


Figure 3. Mean responses of facultative anaerobic bacteria after 0, 24, and 48 hr temperature abuse (20-22°C) storage. Overall S.E.M.=0.3857

PART II.

EFFECT OF ACID AND NEUTRAL PYROPHOSPHATES
ON THE NATURAL BACTERIAL FLORA OF A COOKED MEAT SYSTEM

EFFECT OF ACID AND NEUTRAL PYROPHOSPHATES
ON THE NATURAL BACTERIAL FLORA OF A COOKED MEAT SYSTEM

J. A. Marcy, A. A. Kraft, D. K. Hotchkiss, R. A. Molins,
D. G. Olson, H. W. Walker, and P. J. White
Departments of Food Technology, Statistics,
Animal Science, and Food and Nutrition
Iowa State University
Ames, Iowa 50011

Running title: phosphate effects on bacteria in meat
products

All authors are at Iowa State University, Ames, Iowa 50011.
Authors Marcy, Kraft, and Walker are with the Department of
Food Technology. Author Hotchkiss is with the Department of
Statistics. Authors Molins and Olson are with the
Department of Animal Science, and author White is with the
Department of Food and Nutrition.

ABSTRACT

Effect of acid and neutral pyrophosphates on the natural bacterial flora of a cooked meat system. J. A. Marcy, A. A. Kraft, D. K. Hotchkiss, R. A. Molins, D. G. Olson, H. W. Walker, and P. J. White. Departments of Food Technology, Statistics, Animal Science, and Food and Nutrition, Iowa State University, Ames, Iowa 50011.

The microbiological effects of selected pyrophosphates were studied in cooked, vacuum-packaged pork sausage stored at 7°C for 21 days, or at 20-22°C for 24 and 48 hours. Neutral trisodium pyrophosphate (PYRO-3) and sodium acid pyrophosphate (SAPP) were tested at 0 and 0.4% of meat weight, separately or in combinations. Both phosphates had an effect after 21 days refrigerated storage (7°C), with lower mesophilic counts than controls with no phosphate. Both phosphates also resulted in significantly lower counts of mesophilic and facultative anaerobic microorganisms in sausage after 48 hours of elevated temperature (20-22°C) holding. The organisms affected were mainly streptococci.

INTRODUCTION

Recent research has shown that sodium acid pyrophosphate (SAPP) was more effective than other pyro- or polyphosphates as an inhibitor of Clostridium botulinum (Barbut et al., 1986; Madril and Sofos, 1986; Nelson et al., 1983; Wagner and Busta, 1984, 1985a, b, c), Clostridium sporogenes PA3679 (Molins et al., 1985b, 1986), and streptococci (Marcy et al., 1987) during storage of meat products at elevated temperatures (21-27°C). Toxicity of meat products containing Clostridium botulinum was affected more by SAPP than was the actual growth of the organisms (Jarvis et al., 1977; Wagner and Busta, 1985a, c, 1986), possibly due to inhibition of the protease responsible for activation of the protoxin (Wagner and Busta, 1985a, 1986).

Phosphates are used for their effect on the functional properties of meat, particularly for improving water holding capacity (Tompkin, 1983). However, compared to other phosphates, SAPP has also been reported to be relatively inefficient in improving the functional properties of meat proteins (Hargett et al., 1980; Hellendoorn, 1962). For that reason, it might be expected that SAPP would best be utilized in conjunction with other phosphates. That would insure desired formulation results, while gaining possible

product safety and/or extended shelf life from the incorporation of sodium acid pyrophosphate.

The objective of this work was to compare sodium acid pyrophosphate (SAPP), a compound of known antimicrobial activity and allowed for use in the U.S. meat industry, with trisodium pyrophosphate (PYRO-3), a neutral pyrophosphate used in meat products in Germany but not yet evaluated in terms of antimicrobial properties in meat.

MATERIALS AND METHODS

Pork trimmings (20% fat) were obtained from the Iowa State University Meat Laboratory. The trimmings were ground through a 1/8 in. (32 mm) diam. plate and mixed for 5 min. by using a ribbon blender. The ground meat was packaged in polyethylene bags containing 6 Kg of meat and frozen to -28°C in an air blast freezer.

One bag of meat was removed from the freezer and allowed to thaw at 5°C for 48 hr. Sausage was formulated with 2.0% salt, 0.5% pepper, and 10% added moisture (phosphate solutions and/or water). All expressed percentages are relative to the weight of the meat used in the formulation. Food-grade trisodium pyrophosphate (PYRO-3) and sodium acid pyrophosphate (SAPP) (BK Ladenburg Corp., Cresskill, NJ) were added to the meat as 5% w/v aqueous solutions to obtain a total phosphate concentration of 0.4% of meat weight. The control treatment was balanced for added water. The 6 treatments used are shown in Table 1. Each batch was mixed for 2 min. by using a Kitchen Aid Model 4 mixer (The Hobart Manufacturing Co., Troy OH), stuffed into 30mm collagen casings (Teepak, Chicago, IL) by means of an Oster Food and Meat Grinder (Oster, Milwaukee, WI), and cooked in a Magni Whirl water bath (Blue M Electric Co., Blue Island, IL) set at 80°C to an internal temperature of

65.5 ± 1°C. Cooking end point was determined potentiometrically by using a model 3476A Digital Multimeter (Hewlett-Packard, Loveland, CO). The total time from start of mixing to the end of the cook was less than 20 minutes in order to inactivate the phosphatases present in the raw meat as quickly as possible (Awad, 1968, Sutton, 1973, and Molins et al., 1985a, b). After cooking, the sausages were cooled for 2 hr at 5°C and placed in individual 6-oz Whirl-pak bags (NASCO, Ft. Atkinson, WI). One sausage from every treatment, for a total of six sausages per bag, was vacuum packaged in a Curlon 892 (Curwood, Inc., New London, WI) bag (O_2 permeability < 1 ml/645 cm²/24 hr at 22.8°C and 0% RH), and placed in a refrigerated incubator at approximately 7°C until sampled.

Samples were evaluated for pH, mesophiles, psychrotrophs, and total facultative anaerobes after 0, 1, 4, 7, 14, and 21 days of refrigerated storage. Samples were also subjected to 24 and 48 hours of room temperature (20-22°C) storage after either 3 or 7 days of refrigerated storage. On sampling days, 33 g samples of sausage were blended with 297 ml of 0.1% sterile peptone water by using a Stomacher Lab Blender 400 (Tekmar Co., Cincinnati, OH). Serial dilutions were prepared according to standard procedures. Mesophilic and psychrotrophic counts were determined by using Trypticase Soy Agar (BBL) and incubating

at 30°C for 48 hr and 7°C for 7 days, respectively. Total facultative anaerobic counts were determined by using the anaerobic pouch method of Bladel and Greenberg (1965) and Brewer's Anaerobic Agar (Difco) (30°C, 48 hr).

Cultures were isolated from representative plates throughout three replications of the refrigerated storage and the temperature abuse storage. Cultures were identified to group or genus by the scheme of Harrison et al. (1981).

Measurement of sausage pH was performed by using a Radiometer 28 meter (Radiometer, Copenhagen, Denmark) equipped with an Orion 9163 probe (Orion Research, Cambridge, MA).

Five replications were made for refrigerated storage and room temperature storage. Results were evaluated using release 5.08 of the Statistical Analysis System (SAS Institute Inc., 1985).

RESULTS AND DISCUSSION

Phosphate in general had several significant effects on the natural flora of cooked, vacuum-packaged pork sausage held at 7°C for 21 days. Significant differences ($P < 0.01$) were observed between the control treatment and all treatments containing phosphates on day 1 of storage at 7°C; the control treatment had significantly fewer cfu/gram of mesophilic organisms than the treatments containing phosphates. That was in agreement with previous experience in this lab (Marcy *et al.*, 1987). One possible explanation of this occurrence is that small amounts of phosphate carryover in the diluent helped recovery of injured organisms early in the storage (R. A. Molins, Animal Science Department, Iowa State University, Ames, Iowa, personal communication). Data from the mesophilic bacterial counts are presented in Fig. 1. It was noted that mesophilic counts fluctuated after day 7, at which time the variance in the data increased approximately twentyfold. There was no difference ($P > 0.05$) in mesophilic numbers between the control and phosphate-treated sausage for the remainder of the 7°C storage period until after 21 days, when the control treatment had higher ($P < 0.05$) mesophilic counts than the sausages containing phosphate. However, there was no significant difference between the phosphates used ($P > 0.05$).

Psychrotrophic microorganisms could only be detected occasionally in the sausage throughout the low temperature experimental period and did not follow any regular growth pattern. The level of facultative anaerobic organisms remained relatively constant over the same period of time, at about 100 cfu/g or less in all sausages.

The pH of all treatments remained constant over the 21 day period at 7°C and also during temperature abuse of the sausage at 20-22°C (Table 2). The pH was directly related to the amount of each phosphate added and decreased as the amount of SAPP in the formulation increased. Maximum pH differences, however, were only 0.2 pH units.

Sausages were also examined for microbiological changes during a 48 hour period of temperature abuse at 20-22°C following 7 days storage at 7°C. Although all treatments had approximately the same level of mesophilic and facultative anaerobic bacteria when removed from refrigerated storage, differences were noted between treatments upon exposure to elevated temperature (20-22°C). A significant linear treatment effect was observed after 48 hours on both mesophilic (Fig. 2) and facultative anaerobic bacteria (Fig. 3). Mesophilic counts after 48 hours at 20-22°C decreased in a linear pattern from \log_{10} 6.961 for the control treatment (no phosphate) to \log_{10} 6.337 for the treatment containing 0.4% SAPP ($P < 0.05$). Facultative

anaerobic counts differed earlier. After 24 hours at 20-22°C, the control treatment contained \log_{10} 5.1801 cfu/g while phosphate-treated sausage had an average of \log_{10} 4.0646 cfu/g ($P < 0.01$). After 48 hours at 20-22°C, the facultative anaerobic counts in control samples remained significantly higher ($P < 0.01$) than in samples containing phosphate, but there was no difference between the various phosphate treatments.

The results of the identification of 334 cultures isolated from sausage held at 7°C are shown in Table 3. In general, the results agreed with the typical flora expected in cooked, vacuum-packaged meat containing pepper. However, the change from predominantly Gram-positive, catalase-positive bacteria to Gram-positive, catalase-negative organisms between 10 and 20 days of refrigerated storage reported by Allen and Foster (1960) was absent in this work. Table 4 shows the results of the identification of 258 cultures taken from sausage immediately after 7 days storage at 7°C and after 24 and 48 hours at 20-22°C. After 24 hr, no streptococci were recovered from phosphate-treated sausage. Streptococcus was the only bacterial genus found in control sausage after 48 hours at 20-22°C, but such predominance decreased as the concentration of SAPP increased. The 0.4% SAPP treatment had only 15% streptococci after 48 hr. The streptococci isolated were

also inhibited by PYRO-3 in sausage held at 20-22°C but to a lesser extent than by SAPP.

Chen et al. (1973) reported that, in general, Gram-positive bacteria were more susceptible to phosphate inhibition than were Gram-negative microorganisms. The exception was Streptococcus lactis. Although those authors used a phosphate blend (Kena) that contained SAPP along with STPP and TSPP, the total level of SAPP in that blend did not likely amount to 0.4%, which proved to be inhibitory to streptococci in our study. A similar explanation may apply to the findings of Nielsen and Zeuthen (1983), who reported that a low-pH phosphate mixture was only slightly inhibitory to an atypical streptobacterium, while strongly inhibitory to Brochothrix thermosphacta and Serratia liquefaciens.

CONCLUSIONS

Sodium acid pyrophosphate and trisodium pyrophosphate inhibited mesophilic bacteria in cooked, vacuum-packaged pork sausage during refrigerated storage (7°C) and subsequent temperature abuse (20-22°C). Significant differences during refrigerated storage were only observed one day after cooking and after 21 days of storage.

The main organisms inhibited during elevated temperature holding of sausage were streptococci. SAPP alone inhibited the streptococci more effectively than did PYRO-3 alone or in combination, although the total numbers of microorganisms were not significantly different within the phosphate-containing treatments.

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Mention of any company or product name does not constitute endorsement.

Table 1. Phosphate treatments in percent of meat weight

<u>TREATMENT</u>	<u>TRISODIUM PYROPHOSPHATE</u>	<u>SODIUM ACID PYROPHOSPHATE</u>
1	0.0%	0.0%
2	0.4%	0.0%
3	0.3%	0.1%
4	0.2%	0.2%
5	0.1%	0.3%
6	0.0%	0.4%

Table 2. Phosphate effect on pH of cooked sausage

<u>TRT</u>	<u>PYRO-3</u>	<u>SAPP</u>	<u>pH</u>
1	0.0%	0.0%	6.22
2	0.4%	0.0%	6.21
3	0.3%	0.1%	6.16
4	0.2%	0.2%	6.11
5	0.1%	0.3%	6.06
6	0.0%	0.4%	6.00

Table 3. Cultures identified during refrigerated storage^a

Bacillus	Pseudomonas	Coryneform
57.78%	12.28%	11.38%
Lactobacillus	Micrococcus	Streptococcus
8.98%	6.59%	2.99%

^a334 total organisms identified.

Table 4. Culture identifications during temperature abuse.^a
Percent of total isolations for each treatment and time

HOURS ^b		TREATMENT					
		1	2	3	4	5	6
0	Streptococcus	-	-	-	7	-	-
	Bacillus	57	58	100	53	79	93
	Coryneform	14	21	-	20	21	7
	Micrococcus	29	21	-	20	-	-
24	Streptococcus	87	-	-	-	-	-
	Bacillus	-	53	39	80	53	80
	Coryneform	13	20	23	-	27	20
	Micrococcus	-	20	23	20	20	-
	Coliform	-	7	15	-	-	-
48	Streptococcus	100	80	79	73	82	15
	Bacillus	-	7	-	20	18	31
	Coryneform	-	-	-	-	-	31
	Micrococcus	-	-	7	-	-	8
	Coliform	-	13	14	7	-	-
	Lactobacillus	-	-	-	-	-	15

^a258 total organisms identified.

^bHours of temperature abuse at 20-22°C.

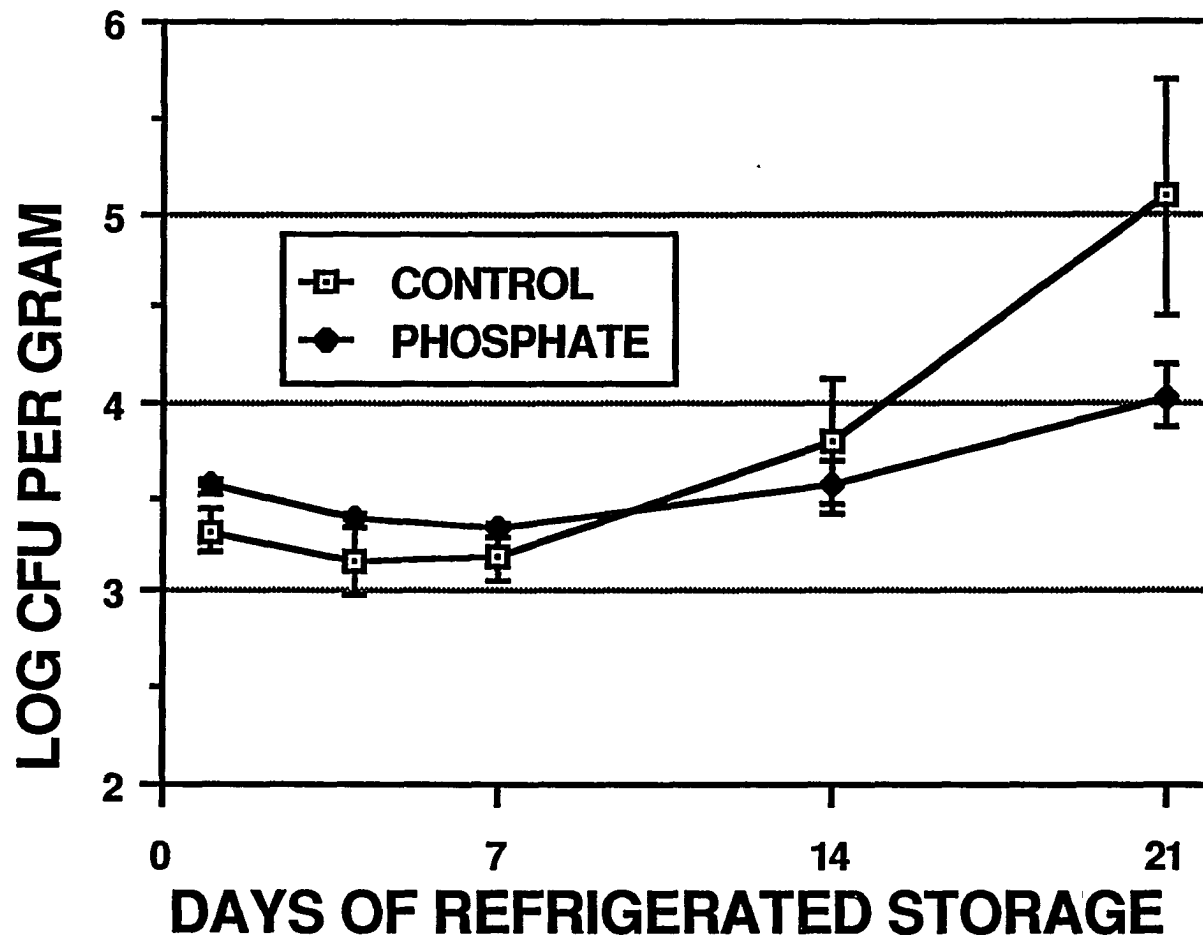


Figure 1. Mean responses of mesophilic bacteria during refrigerated storage at 7°C. Phosphate refers to the mean of all phosphate treatments. Overall S.E.M.=0.2408

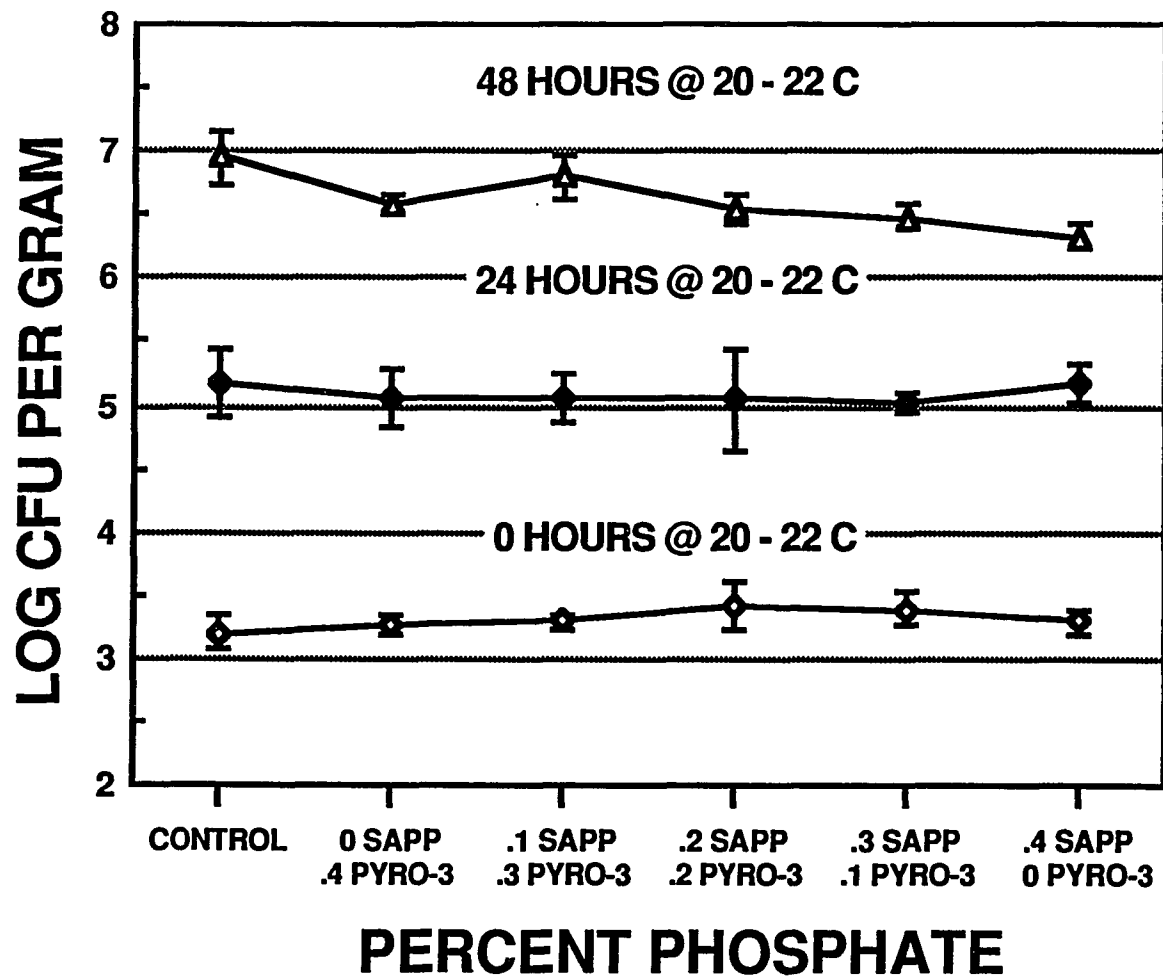


Figure 2. Mean responses of mesophilic bacteria after 0, 24, and 48 hr temperature abuse (20-22°C) storage. Overall S.E.M.=0.1774

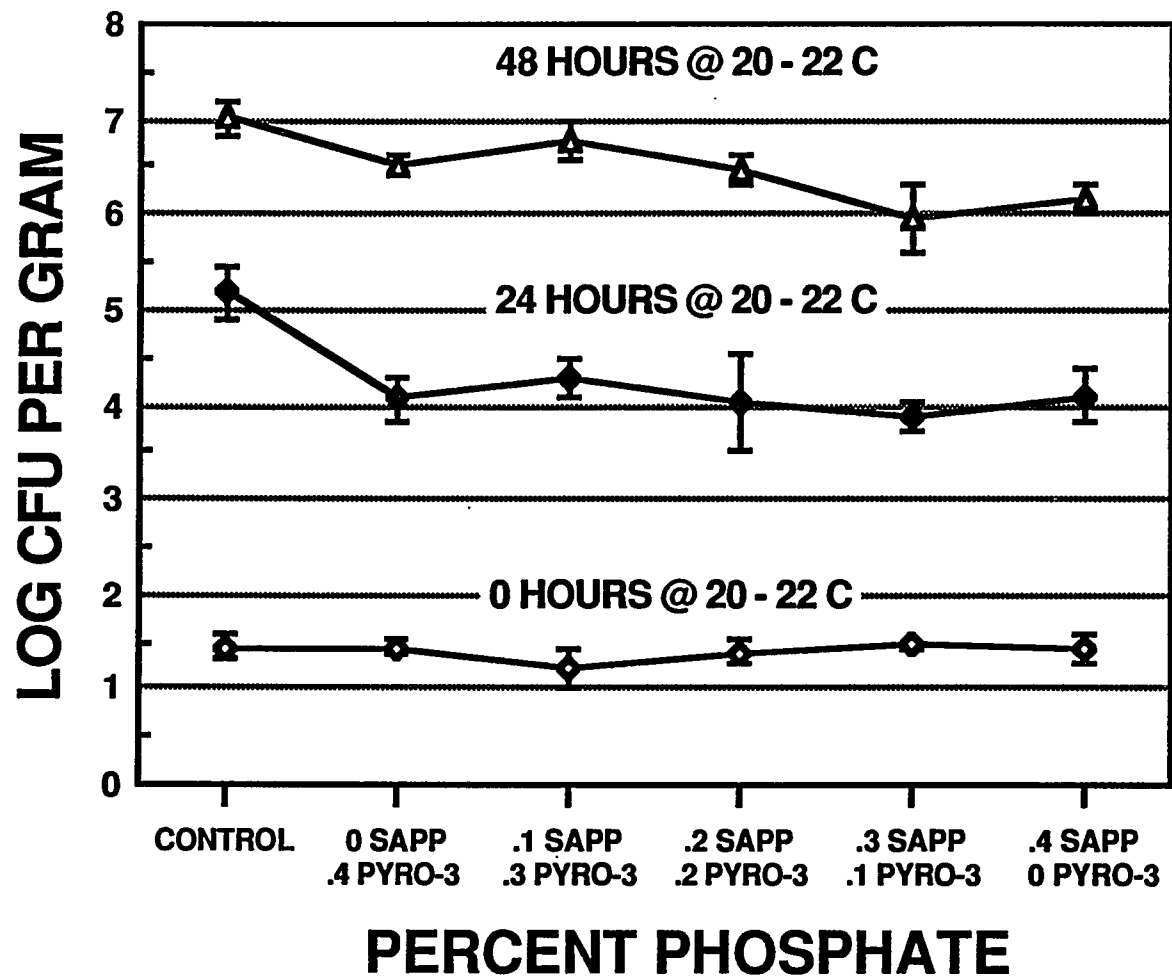


Figure 3. Mean responses of facultative anaerobic bacteria after 0, 24, and 48 hr temperature abuse (20-22°C) storage. Overall S.E.M.=0.1788

PART III.

EFFECTS OF SELECTED COMMERCIAL PHOSPHATE PRODUCTS
ON THE NATURAL BACTERIAL FLORA OF A COOKED MEAT SYSTEM

EFFECTS OF SELECTED COMMERCIAL PHOSPHATE PRODUCTS
ON THE NATURAL BACTERIAL FLORA OF A COOKED MEAT SYSTEM

J. A. Marcy, A. A. Kraft, D. K. Hotchkiss, R. A. Molins,

D. G. Olson, H. W. Walker, and K. Merkenich

Departments of Food Technology, Statistics,

and Animal Science

Iowa State University

Ames, Iowa 50011

and

BK-Ladenburg Corp.

Cresskill, New Jersey 07626

Running title: commercial phosphate effects on
bacteria in meat products

Author Merkenich is with BK-Ladenburg Corp., 50 Spring St.,
Cresskill, NJ. All other authors are at Iowa State
University, Ames, Iowa 50011. Authors Marcy, Kraft, and
Walker are with the Department of Food Technology. Author
Hotchkiss is with the Department of Statistics. Authors
Molins and Olson are with the Department of Animal Science.

ABSTRACT

Effects of selected commercial phosphate products on the natural bacterial flora of a cooked meat system. J. A. Marcy, A. A. Kraft, D. K. Hotchkiss, R. A. Molins, D. G. Olson, H. W. Walker, and K. Merkenich. Departments of Food Technology, Statistics, and Animal Science, Iowa State University, Ames, Iowa 50011, and BK-Ladenburg Corp., Cresskill, NJ.

Four commercial phosphate blends and a neutral pyrophosphate were used at three levels (0.30-0.65%) in the preparation of cooked sausage. Control treatments contained no phosphate. Vacuum-packaged sausage was stored at 5°C for 21 days or held at room temperature (20-22°C) for up to 48 hours after 7 days of refrigerated storage. Mesophilic and facultative anaerobic bacterial counts were highest in control treatments after 14 and 21 days at 5°C and after 24 and 48 hours at 20-22°C. For each phosphate, the level of phosphate addition was the determining factor in bacterial inhibition, since the highest phosphate level resulted in the lowest bacterial counts in sausage held at 5°C and 20-22°C. Phosphate type was not a determinant nor did it interact with phosphate level.

INTRODUCTION

Inorganic pyro- and polyphosphates have found much use in the meat industry for economic and product quality considerations. The blending of different pyro- and polyphosphates is also common to achieve particular properties, such as solubility and pH. Tompkin (1983) stated that it is the effect of phosphates on the functional properties of meat proteins, and not any antimicrobial activity which may occur, that determines the use and regulation of pyro- and polyphosphates.

Commercial phosphate blends were reported as being effective in increasing moisture retention in poultry (Spencer and Smith, 1962; May et al., 1963; Monk et al., 1963; Farr and May, 1970; Chen et al., 1973); in pork loins (Hoes et al., 1980); and processed meats (Hargett et al., 1980). Recently, Lamkey et al. (1986) reported that Brifisol 414¹ addition at 0.2 and 0.4% resulted in lower cooking losses and increased binding quality in restructured beef steaks. A combination of 0.2% Brifisol 414 and 0.2% NaCl decreased the cooking loss more than either ingredient added separately. The rate of oxidative rancidity development was significantly decreased with phosphate

¹Trademark of the BK-Ladenburg Corp. Cresskill, NJ.

addition to the restructured steak formulation. Molins et al. (1987b) reported that frozen, ground beef patties had higher cook yields when treated with Brifisol blends than did the control patties. Thaw drip and cook drip were significantly decreased when cod fillets were treated with Freez-gard² FP-19 and FP-65, commercial phosphate products (Woyewoda and Bligh, 1986).

Some commercial phosphate products have also been evaluated for antimicrobial capabilities. Spencer and Smith (1962) reported that 7% Kena³ in the chilling water, increased the shelf life of poultry by 1-2 days. Lower bacterial counts for 20 days at 5°C resulted from the use of 8% Kena in broiler chilling water (Steinhauer and Banwart, 1963).

The objective of this work was to compare selected phosphate blends and a neutral pyrophosphate to determine if certain phosphate products might be more capable of inhibiting microorganisms in sausage than other phosphate products. In addition, higher-than-legal levels of phosphate were studied to determine if potential bacterial inhibition by phosphates is presently being limited by the 0.5% maximum level imposed in meat products (USDA, 1982).

²Trademark of ERCO Industries Ltd., Islington, Ontario.

³Trademark of Stauffer Chemical Co., Washington, PA.

MATERIALS AND METHODS

All phosphates used in this study were furnished by BK-Ladenburg (Cresskill, NJ). The four commercial phosphate blends used were Brifisol 414, Brifisol 414-B, Brifisol 414-K, and Brifisol 512. The neutral pyrophosphate used was trisodium pyrophosphate (Pyro-3). The level of phosphate addition for each phosphate product was balanced by the amount of P_2O_5 present in each product (K. Merkenich, BK-Ladenburg Corp., Cresskill, NJ, personal communication) and are given in Table 1. In addition to sausage made with different levels of phosphate, four treatments were made with no phosphate and the geometric means of these quadruplicates were the responses for the control level of each phosphate. A separate analysis of variance was performed to determine no significant differences among the quadruplicates.

The order of preparation for the sausages was assigned at random. Fresh, 80% lean pork trim was obtained from a local purveyor and ground through a 1/8 in (32 mm) plate, followed by 5 minutes of mixing in a ribbon blender at the Iowa State University Meat Laboratory. The ground meat was held at 2°C for 16-18 hr prior to making sausage. The sausage formulation consisted of 500 g of pork, 2% salt, 0.5% pepper, 10% water, and phosphate if called for. All

phosphates were added in dry form and all percentages were relative to the weight of the meat. The sausage was mixed in a Kitchen Aid Model "4" (Hobart Mfg. Co., Troy, OH). After mixing, the sausage was stuffed into a 32mm cellulose casing (Teepak, Chicago, IL) by means of an Oster Food and Meat Grinder (Oster, Milwaukee, WI), and cooked to an internal temperature of $65.5 \pm 1^\circ\text{C}$ in a Magni Whirl water bath (Blue M Electric Co., Blue Island, IL) set at 80°C . Cooking end point was determined potentiometrically by using a model 3476A Digital Multimeter (Hewlett-Packard, Loveland, CO). The total time from start of mixing to the end of the cook was less than 20 minutes in order to inactivate the phosphatases present in the raw meat as quickly as possible (Awad, 1968, Sutton, 1973, and Molins *et al.*, 1985a, b). After cooking, the sausages were cooled for 2 hr at 5°C . Upon cooling, the sausages were removed, aseptically separated into 30 g samples, and each sample placed in a sterile Stomacher bag (Tekmar Company, Cincinnati, OH). The Stomacher bags containing the 30 g samples were vacuum packaged in Curlon 892 (Curwood, Inc., New London, WI) bags (O_2 permeability $< 1 \text{ ml}/645 \text{ cm}^2/24 \text{ hr}$ at 22.8°C and 0% RH), and placed in a refrigerated display case at approximately 5°C until sampled.

Samples were evaluated for pH at the time of manufacture. Mesophilic, psychrotrophic, and facultative

anaerobic organisms were enumerated after 1, 4, 7, 14, and 21 days of refrigerated storage. Other sets of samples were examined after 24 and 48 hr holding at room temperature (20-22°C) following 7 days refrigerated storage at 5°C. On sampling days, 30 g samples of sausage were blended with 270 ml of 0.1% sterile peptone water by using a Stomacher Lab Blender 400 (Tekmar Co., Cincinnati, OH). Serial dilutions were prepared according to standard procedures. Mesophilic and psychrotrophic counts were determined in all samples by using Trypticase Soy Agar (BBL) and incubating at 30°C for 48 hr and 7°C for 7 days, respectively. In addition, total facultative anaerobic counts were determined by using the anaerobic pouch method of Bladel and Greenberg (1965) and Brewer's Anaerobic Agar (Difco) (30°C, 48 hr).

Measurement of sausage pH was performed by using a Radiometer 28 meter (Radiometer, Copenhagen, Denmark) equipped with an Orion 9163 probe (Orion Research, Cambridge, MA).

Each sampling unit consisted of three vacuum packages containing one 30-g sample from each treatment. Package 1 contained two control treatments and the three samples for Brifisol 414. Package 2 contained one control treatment and the six samples for Brifisol 414-K and Brifisol 512. Package 3 contained one control treatment and the six samples for Brifisol 414-B and Pyro-3. Samples were

prepared for sampling at 1, 4, 7, 14, and 21 days of refrigerated storage, as well as 24 and 48 hours of room temperature storage, totaling 21 packages containing 133 samples per replication. Three replications were made for refrigerated and room temperature storage. Results were evaluated by using release 5.08 of the Statistical Analysis System (SAS Institute Inc., 1985). The design of the experiment was a split-plot design, with a five-by-four factorial in the whole plot. The degrees of freedom for level, phosphate-by-level, and the whole plot error were corrected for duplication of the control levels for each phosphate type. The subplot consisted of sampling days, during refrigerated storage, or sampling times during temperature abuse. The degrees of freedom for day/time-by-level, day-by-phosphate-by-level, and the subplot error were corrected for duplication of the control levels for each phosphate type.

RESULTS AND DISCUSSION

The results for effect of phosphate addition on pH are given in Table 2. The addition of Brifisol 414K and Brifisol 512 increased the pH of the sausage compared to that of the control. Addition of Brifisol 414, Brifisol 414B, and Pyro-3 had negligible effect on the pH of the sausage. There was a significant ($P < 0.001$) difference in pH due to replications, which shifted the mean pH higher than at first expected.

An overall view of the manner in which the different phosphate blends affected mesophilic bacterial counts during refrigerated storage at 5°C is shown in Fig. 1. At day 14, there appeared to be a considerable difference between Brifisol 414 and Pyro-3. However, the analysis of variance indicated that the effect was due to random error ($P > 0.25$). Figs. 2 and 3 show the same information plus error bars indicating the standard error associated with each mean. Fig. 2 presents information concerning the three Brifisol 414-derived blends and Fig. 3 presents Brifisol 512 and Pyro-3. The errors associated with the phosphate treatments became quite large after 7 days of storage. The increase in experimental error was likely caused by inconsistent growth of psychrotrophic microorganisms, which only grew to detectable levels sporadically in sausage after

7 days of refrigerated storage. These bacteria were present in different treatments at different times without replication. Considering that psychrotrophic bacteria may grow at temperatures in the mesophilic range, their presence and growth in some sausage but not in others within the same treatment would have caused the experimental error of the means of mesophilic counts to increase. For the most part, psychrotrophic bacterial numbers were less than 10 per gram of sample throughout the work.

Fig. 4 presents the manner in which the different phosphate levels affected mesophilic and facultative anaerobic bacterial growth in sausage after 14 and 21 days of refrigerated storage at 5°C. The counts in the control treatment (no phosphate) were consistently highest ($P < 0.001$), and those in sausage containing the highest level of phosphate (0.60 - 0.65%) were consistently lowest ($P < 0.001$). There was no difference between the counts in the two intermediate levels ($P > 0.05$). The results, averaged over all phosphates (Fig. 4), indicated that the level of phosphate addition was more important in terms of inhibition of mesophilic and facultative anaerobic bacteria than was the type of phosphate tested in this study. Effect of increased levels of phosphate had been observed by Elliott *et al.* (1964) who reported that broilers, chilled in ice water containing 3 and 8% Kena, had an increase in shelf

life of 17 and 25%, respectively. An increase in shelf life of 17 and 67% was achieved if the broilers were stored in continuous contact with 3 and 8% Kena solutions, respectively.

The phosphate-by-level interaction on mesophilic bacterial counts is shown in Fig. 5, with the mean for all phosphates displayed on the left side of the figure. Apparent differences between phosphate blends at the same levels can be seen (i.e., Brifisol 414-K at 0.32% and Brifisol 414-B at 0.30%). However, the data only suggested possible differences ($0.1 > P > 0.05$) that might be ascertained with further research. Fig. 5 also emphasizes the difference due to phosphate level in comparison to the type of phosphate used.

During the temperature abuse (20-22°C) phase of the experiment, the level of phosphate added to the sausage was very important in determining differences between observations. Mesophilic and facultative anaerobic counts for each time period and each phosphate level, averaged over all phosphate types, are presented in Fig. 6. Although differences between phosphates were insignificant ($P > 0.5$), the differences between levels were highly significant ($P < 0.0001$). At zero hours (i.e., the time when sausage was withdrawn from refrigeration after 7 days at 5°C), there was no difference in mesophilic or facultative anaerobic counts

due to phosphate level. After 24 and 48 hours at 20-22°C, mesophilic and facultative anaerobic counts in the control samples were significantly higher than the counts in sausage containing the two intermediate phosphate levels which, in turn, were significantly higher than the counts in product formulated with the highest level. Mesophilic bacterial populations differed by almost 2.5 log cycles in sausage with 0.60-0.65% phosphate compared with controls containing no added phosphate at 24 hours while facultative anaerobic counts differed by over 3 log cycles. Aerobic and facultative anaerobic counts were nearly identical after 48 hours and the bacterial counts in control sausages differed by almost 2 log cycles from the bacterial counts in sausages which contained 0.60-0.65% phosphate. This was in agreement with Molins et al. (1987a) who found that Brifisol 414B significantly inhibited mesophilic, psychrotrophic, and lactic acid microorganisms in ground beef patties which were temperature abused (24-25°C) after 90 days of frozen storage.

Results from the work on temperature abuse storage are encouraging in that a phosphate level higher than the 0.5% legal maximum had a positive effect in inhibiting bacterial growth in cooked, vacuum-packaged sausage. Such products are already in the market and might easily be temperature abused for short periods of time (24 hours or less).

Because of the close agreement between the aerobic mesophilic counts and the facultative anaerobic, mesophilic counts, and because of past experiences (Marcy et al., 1987a, b), it is believed that the organisms affected in this study by the higher levels of the phosphate blends were streptococci. This is in contrast with the report of Chen et al. (1973) that Kena was very inhibitory to several Gram-positive organisms, but Streptococcus lactis was resistant to 6% phosphate in laboratory medium. However, those authors found that chicken parts soaked in 3% Kena for 16 hours had greatly reduced numbers of surface microorganisms. Mol et al. (1971) observed that a commercial phosphate blend (0.45% by weight) did not affect the growth of an unclassified streptobacterium in berliner sausage.

Certain phosphates have been shown to inhibit microorganisms during both refrigerated storage as well as subsequent temperature abuse at room temperature (Molins et al., 1985a, b, 1986; Marcy et al., 1987a, b). In this work, the level of phosphate addition was more important than the type of phosphate added, especially during temperature abuse conditions.

CONCLUSIONS

The level of phosphate added to cooked, vacuum-packaged pork sausage was more important than the type of phosphate added in terms of bacterial inhibition, both during storage at 5°C and 20-22°C.

The addition of phosphate, at all levels tested, resulted in lower bacterial counts compared to the treatments without phosphate added, both during storage at 5°C and 20-22°C.

Addition of phosphate to sausage at 0.60 - 0.65% of meat weight resulted in lower bacterial counts compared to two other levels of phosphate that were within the legal restriction for phosphate addition to meat products.

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Mention of any company or product name does not constitute endorsement.

Table 1. Types and levels of phosphates used

PHOSPHATE	LEVELS (in percent of meat weight)			
Brifisol 414	0.0	0.30	0.45	0.60
Brifisol 414-B	0.0	0.30	0.45	0.60
Brifisol 414-K	0.0	0.32	0.48	0.62
Brifisol 512	0.0	0.30	0.45	0.60
Pyro-3	0.0	0.32	0.48	0.65

Table 2. pH values for each phosphate and level of phosphate addition

<u>PHOSPHATE</u>	<u>LEVEL OF PHOSPHATE ADDITION</u> <u>(IN PERCENT OF MEAT WEIGHT)</u>			
	<u>CONTROL</u>	<u>0.30-0.32%</u>	<u>0.45-0.48%</u>	<u>0.60-0.65%</u>
Brifisol 414	6.33 ± 0.07	6.29 ± 0.04	6.34 ± 0.03	6.34 ± 0.03
Brifisol 414-B	6.33 ± 0.07	6.32 ± 0.06	6.34 ± 0.06	6.37 ± 0.03
Brifisol 414-K	6.33 ± 0.07	6.45 ± 0.03	6.58 ± 0.02	6.71 ± 0.01
Brifisol 512	6.33 ± 0.07	6.38 ± 0.04	6.46 ± 0.03	6.50 ± 0.01
Pyro-3	6.33 ± 0.07	6.32 ± 0.05	6.35 ± 0.03	6.37 ± 0.03

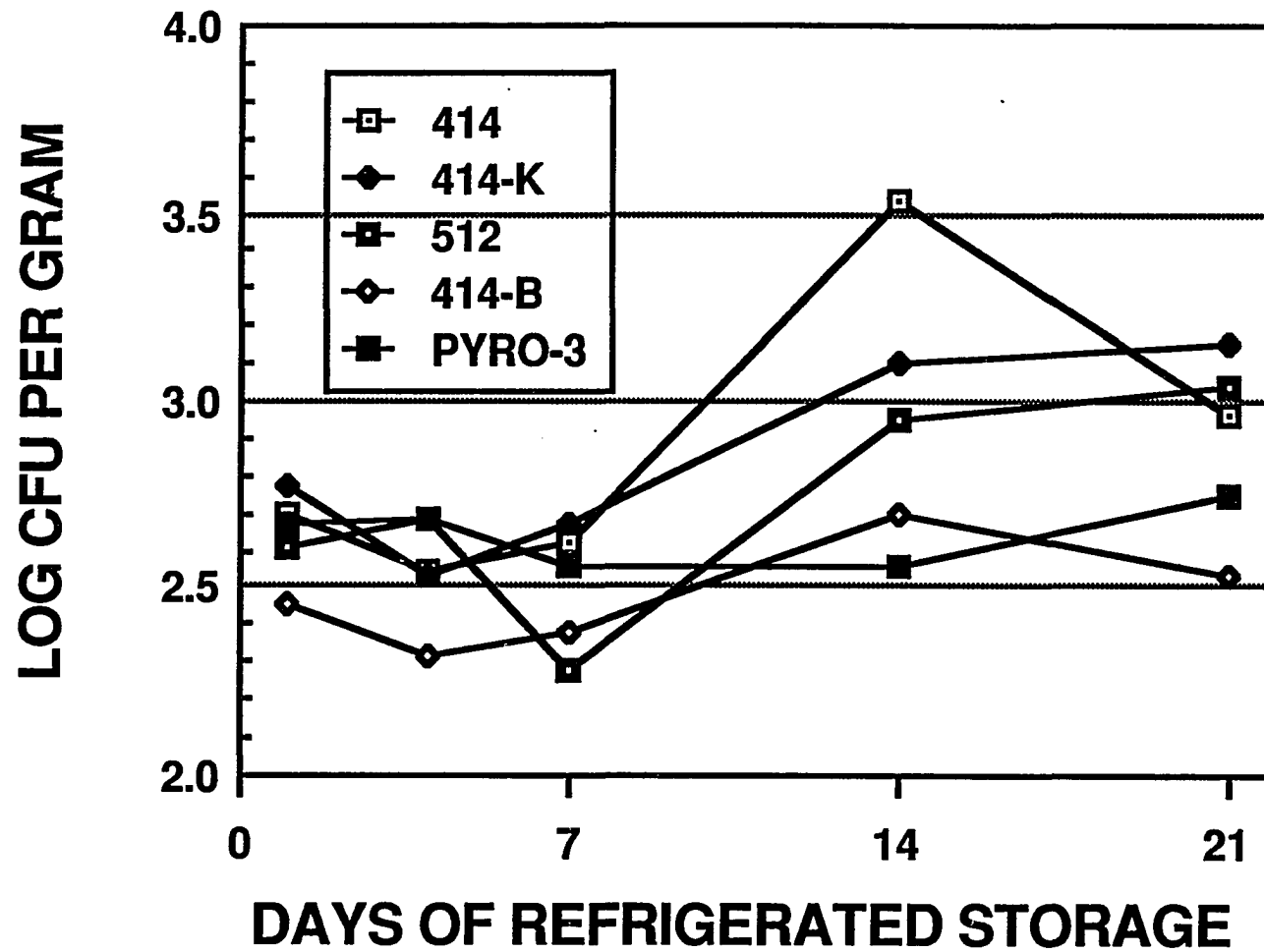


Figure 1. Mean responses of mesophilic bacteria for each phosphate during refrigerated storage at 5°C. Overall S.E.M.=0.2514

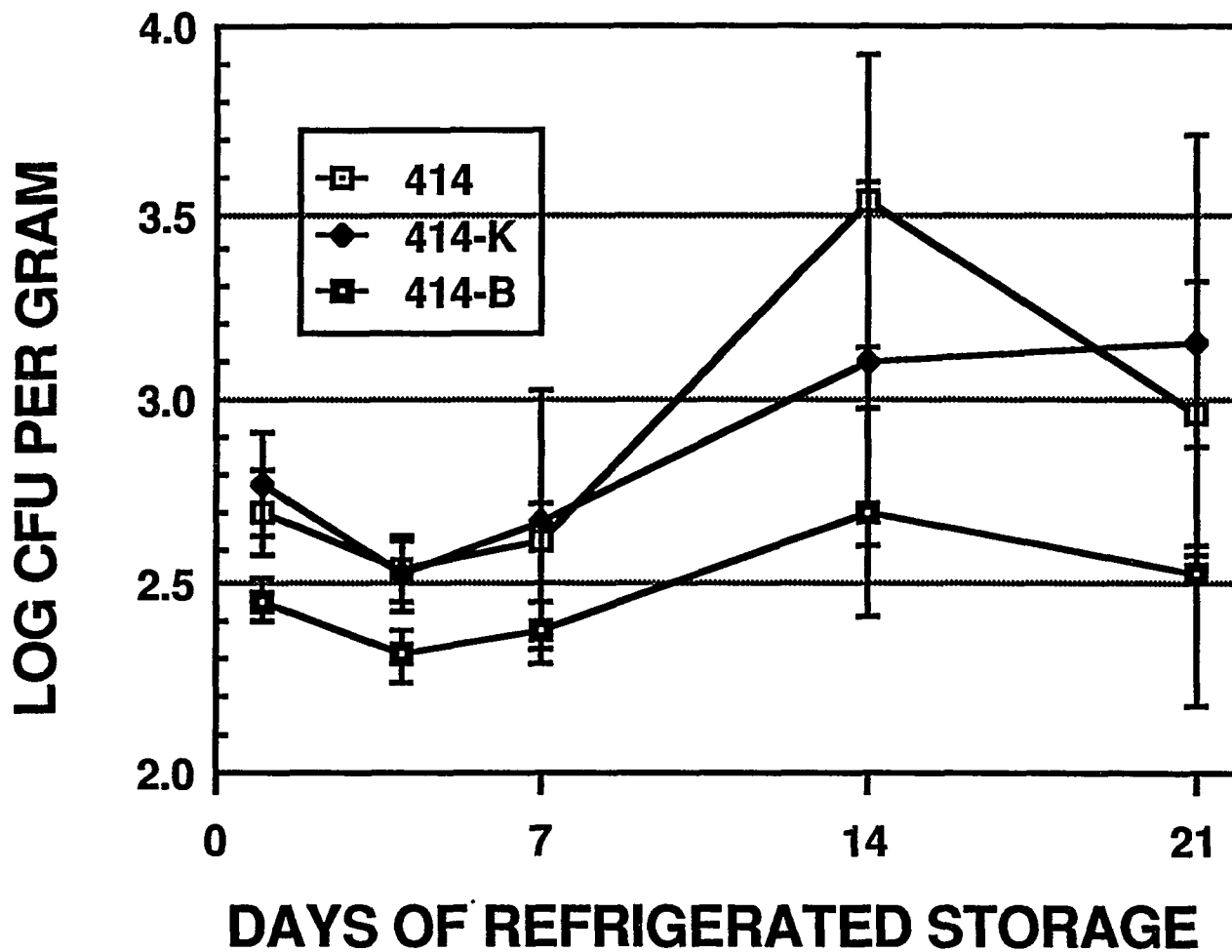


Figure 2. Mean responses of mesophilic bacteria for Brifisol 414, 414-B and 414-K during refrigerated storage at 5°C. Overall S.E.M.=0.2514

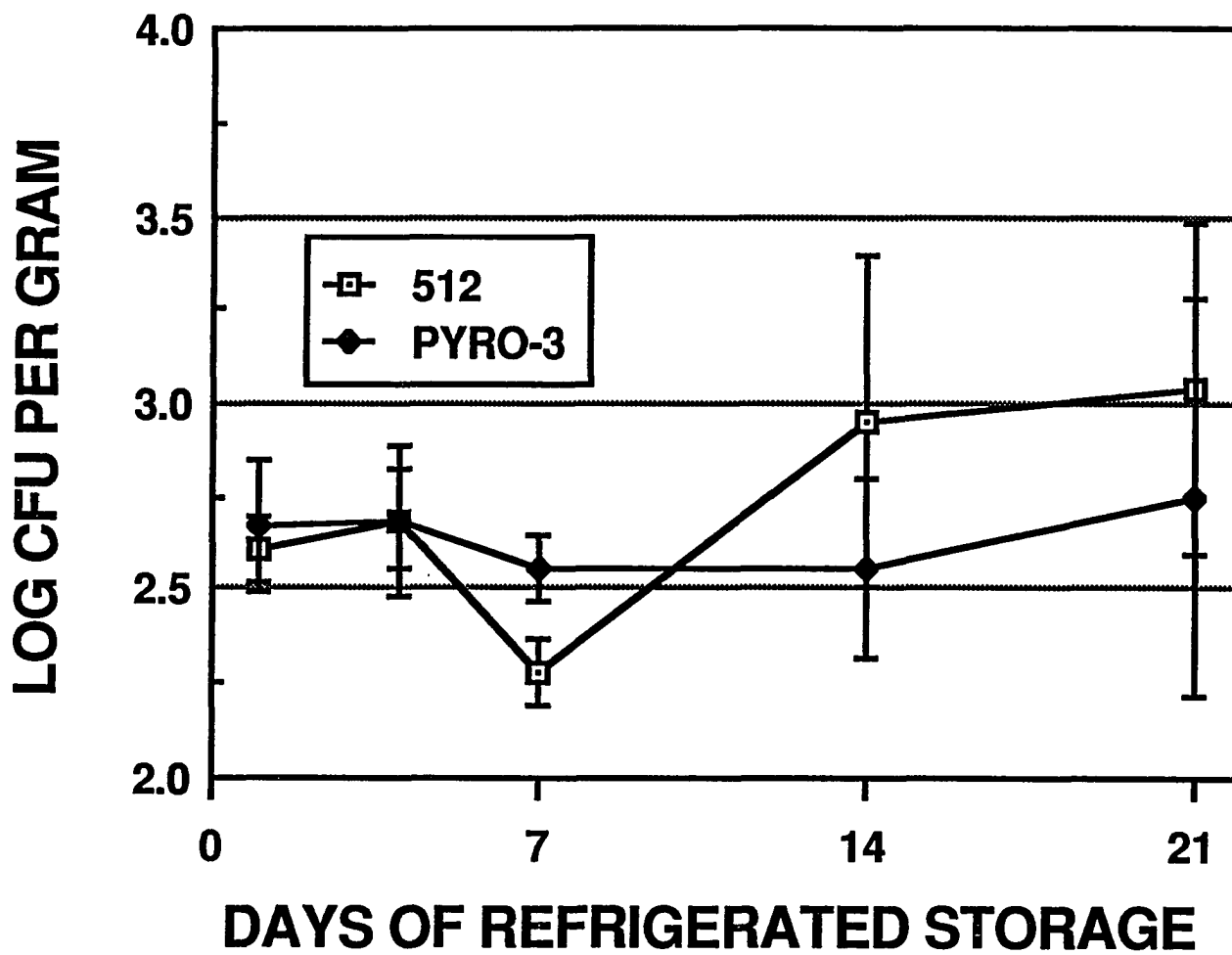


Figure 3. Mean responses of mesophilic bacteria for Brifisol 512 and Pyro-3 during refrigerated storage at 5°C. Overall S.E.M.=0.2514

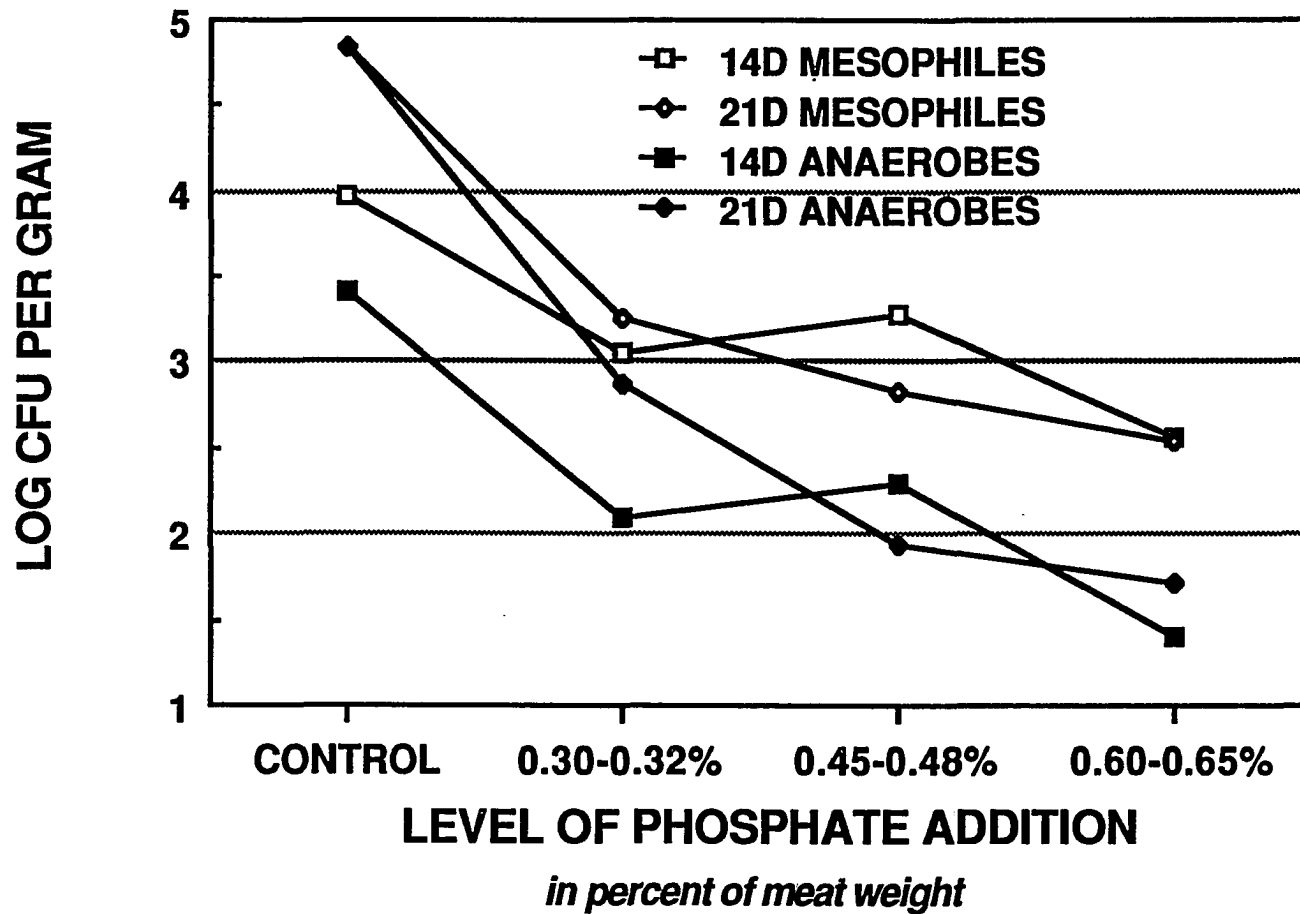


Figure 4. Mean responses of mesophilic and facultative anaerobic bacteria for each level of phosphate after 14 and 21 days of refrigerated storage at 5°C. Overall S.E.M. mesophiles=0.2248, facultative anaerobes=0.2382

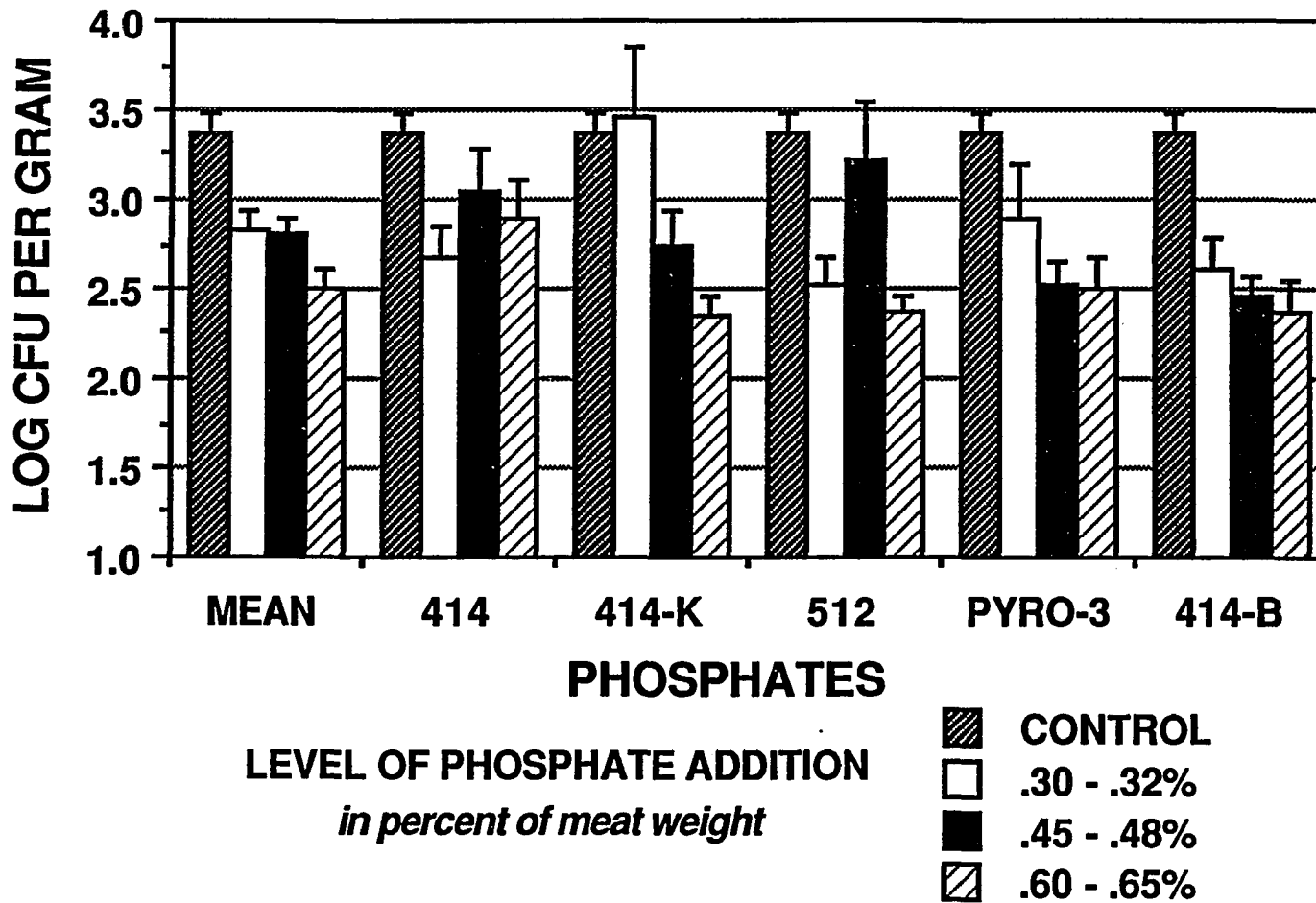


Figure 5. Mean responses of mesophilic bacteria for each phosphate and level, as well as the mean response for each level during refrigerated storage at 5°C. Overall S.E.M.=0.2248

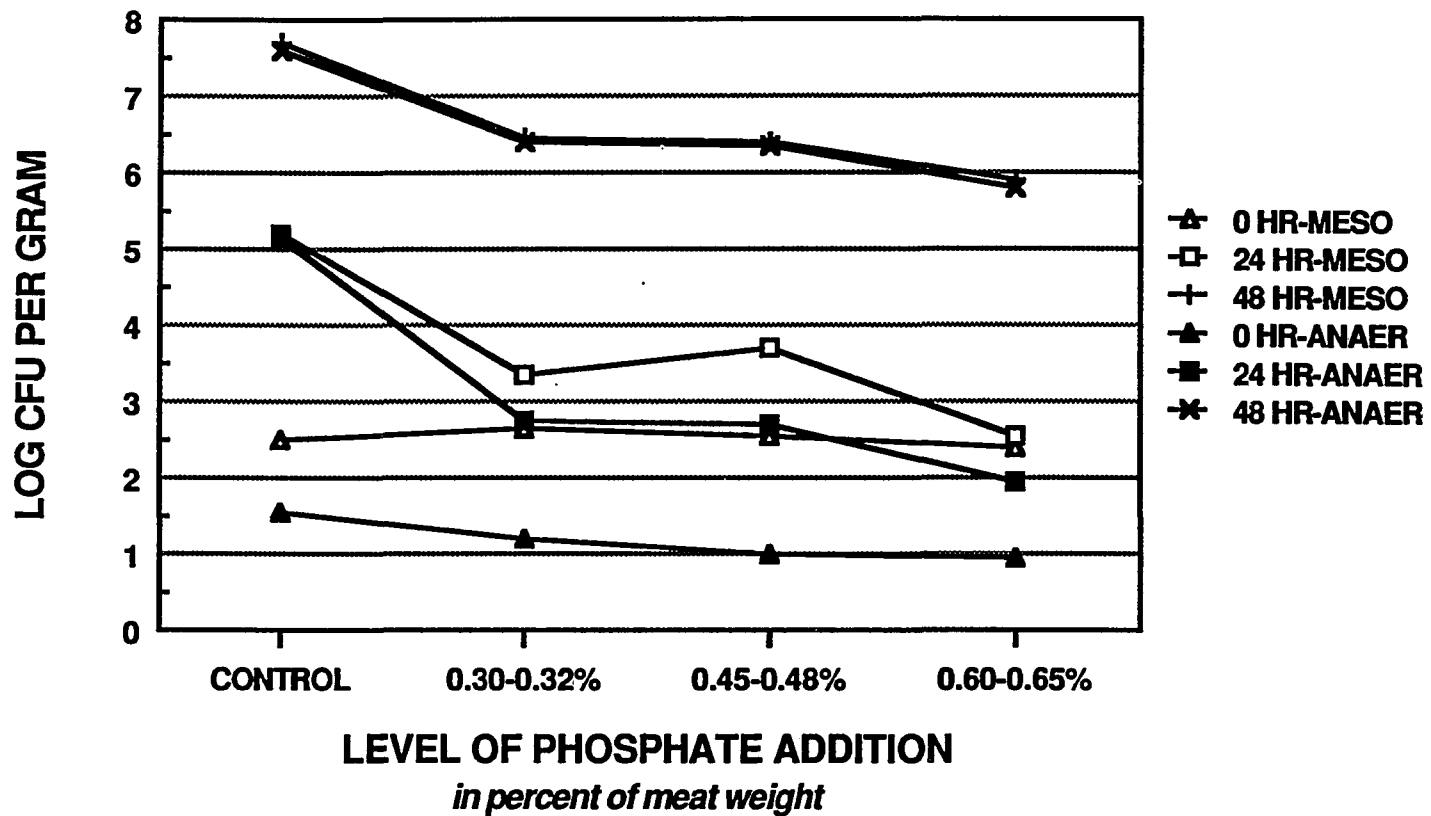


Figure 6. Mean responses of mesophilic and facultative anaerobic bacteria for each level of phosphate after 0, 24, and 48 hr temperature abuse at 20-22°C. Overall S.E.M. mesophiles=0.2254, facultative anaerobes=0.2345

SUMMARY

Microbial inhibition by phosphates added to cooked vacuum-packaged sausage has been demonstrated throughout this work. Sodium acid pyrophosphate, in particular, was effective in reducing the numbers of bacteria in pork sausage during short periods (48 hr) of elevated temperature holding (20-22°C).

Part I was a study of the antimicrobial effect of sodium acid pyrophosphate (SAPP) and/or tetrasodium pyrophosphate (TSPP) at 0.4% of meat weight, in comparison to control treatments containing no phosphate. SAPP was a much more effective inhibitor of total numbers of microorganisms during storage at 20-22°C than TSPP. Bacterial isolates identified indicated that the main organisms inhibited by SAPP in sausage during high temperature storage were streptococci. During refrigerated storage (5°C), the use of phosphates did not significantly alter microbial growth in cooked, vacuum-packaged pork sausage.

Based upon the two observations that the control treatment and the treatment containing 0.2% SAPP and 0.2% TSPP had identical pH values, and that the mesophilic and facultative anaerobic bacterial counts between those two

treatments were significantly different ($P < 0.001$) after 24 and 48 hours of temperature abuse, it was concluded that the pH change due to addition of SAPP to the meat was not a primary factor in the antimicrobial activity of SAPP.

A similar experimental design was used to study SAPP and trisodium pyrophosphate (Pyro-3) in cooked, vacuum-packaged sausage in Part II. The addition of 0.4% phosphate to sausage, in general, resulted in fewer mesophilic bacteria after 21 days of refrigerated storage at 7°C and also in lower mesophilic and facultative anaerobic bacterial numbers during 48 hours of temperature abuse (20-22°C) storage. There was no difference between the antimicrobial effects of SAPP or Pyro-3 in sausage at either low or elevated temperatures. The treatment consisting of SAPP alone at 0.4% resulted in a much lower percentage of Streptococcus isolations during temperature abuse storage than any treatment containing Pyro-3. The addition of any phosphate also reduced the incidence of streptococci dramatically when compared to control samples after 24 hours of temperature abuse. The difference during storage at 20-22°C between control treatments and treatments that contained only SAPP as added phosphate was not as great as in Part I, which might explain the inability to observe a difference between SAPP and Pyro-3.

There were differences between mesophilic bacterial counts in control and phosphate-treated sausages on days 0 and 1 in Part I, and day 1 in Part II. Those differences were very consistent and possibly due to phosphate carry-over in the diluent in very small amounts, which could aid the recovery of heat-stressed organisms (R. A. Molins, Animal Science Department, Iowa State University, personal communication). Another possibility is that increased water-binding in sausage containing phosphate resulted in reduced lethality of the cooking procedure (R. Flowers and D. Gabis, Silliker Laboratories, Chicago Heights, IL, personal communication). In either event, differences, although statistically significant, would not be meaningful in terms of product shelf life since mesophilic bacterial counts were equivalent by day 4.

A study of commercial phosphate blends and additional study of Pyro-3 was undertaken in Part III. Four blends from BK-Ladenburg Corp. were studied, as well as Pyro-3, at four levels; 0%, 0.30-0.32%, 0.45-0.48%, and 0.60-0.65%. Those levels were used to balance the different blends in terms of P_2O_5 content. This study provided information concerning the use of phosphates at levels higher than the 0.5% presently permitted in meat products. There were no significant differences between any of the phosphate types

during either refrigerated storage at 5°C or temperature abuse storage at 20-22°C. The most important finding was that the use of 0.30-0.48% phosphate resulted in lower mesophilic and facultative anaerobic bacterial counts in cooked, vacuum-packaged sausage after 14 days of refrigerated storage and after 24 hours of temperature abuse. In addition, increasing the level of phosphate from 0.30-0.48% to 0.60-0.65% resulted in significant, further reduction in bacterial numbers.

Throughout the course of all parts of the study, increased variance after 14 days of refrigerated storage was observed. Psychrotrophic organisms were found only occasionally and were less than 10 cfu/g, in general. However, some psychrotrophic bacteria did grow to large numbers in different treatments on different days in different replications without any regular pattern. This affected mesophilic counts because the psychrotrophic organisms are also capable of mesophilic growth. Accordingly, when psychrotrophs were detected, the mesophilic counts in those instances were usually higher than in other samples or other replications of the same treatment.

This work provides information concerning whether or not pH plays a critical role in the antimicrobial activity

of SAPP, and if phosphates have synergistic affects when used in combination. The results from treatments containing levels of phosphate over 0.5% provide a path for future research to optimize functional and antimicrobial properties. Sensory evaluation may be a critical issue when phosphate levels approach 1.0%.

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