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Microbiological effects on poly- and pyrophosphates added to meat and meat products

Ricardo A. Molins
Iowa State University

Molins, Ricardo A.

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Microbiological effects of poly- and pyrophosphates added to meat and meat products

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Ricardo A. Molins

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DEDICATION

To my dear wife and children, who shared with me the joys and hardships of the search for the land of milk and honey.

INTRODUCTION

Renewed interest in studying the qualitative effects of condensed phosphates on meat and meat products has resulted from the approval granted their use in processed meats by the United States Department of Agriculture (USDA, 1982), as well as from the search for additives that may partially or totally substitute for sodium nitrite and/or allow a reduction in the levels of sodium chloride in meat products. That search was spurred by the National Academy of Sciences reports on the risk of nitrosamine formation from nitrite (NAS, 1981) and on the excessive sodium content of the average American diet (NAS, 1982).

Inorganic phosphates have gained widespread industry acceptance, in clear recognition of the economic and quality advantages that their inclusion in processed meat formulations offer. However, most research concerning the use and effects of poly- and pyrophosphates in meats has been related to physical, chemical and sensory aspects, while the equally important relation of phosphates to microbiology has largely been neglected in meats.

This study constitutes a modest attempt to contribute to the limited body of scientific knowledge available on the antimicrobial properties of inorganic polyphosphates and their possible application in improving the quality and safety of meat products.

Structure and Nomenclature of Inorganic Sodium Phosphates Allowed in Meat Products

The chemical structures of the various sodium phosphates that may be added to processed meats and poultry products to a maximum total level of 0.5% of product weight (USDA, 1982) are contained in Figure 1.

The nomenclature and classification of inorganic phosphates is based on the number of phosphorous atoms present in the molecule: orthophosphate (1), pyrophosphate (2) and polyphosphates (3 or more). All phosphates permitted in meats have straight chain molecules, as opposed to the more complex ring phosphates.

Orthophosphates are mono- or disodium salts of orthophosphoric acid (H_3PO_4): NaH_2PO_4 and Na_2HPO_4, respectively, and are 100% ionized in aqueous solutions (Batra, 1965). Our study refers to these compounds as orthophosphates or ortho-P, indistinctly.

Pyrophosphate molecules contain two phosphorus atoms linked by one of oxygen and constitute di- or tetrasodium salts of pyrophosphoric acid ($H_4P_2O_7$): sodium acid pyrophosphate ($Na_2H_2P_2O_7$) and tetrasodium pyrophosphate ($Na_4P_2O_7$), which are commonly referred to by the initials SAPP and TSPP, respectively (Bell, 1947a; Van Wazer and Callis, 1958).

Polyphosphates are chain phosphates containing three or more P atoms per molecule. As with other forms of phosphates, the link between two adjacent phosphorus atoms is oxygen. The most commonly used polyphosphates among those allowed in meat products are sodium tripolyphosphate ($Na_5P_3O_{10}$) - STPP in our study - and sodium

polyphosphate, glassy $(NaPO_3)_6$, formerly known by the misnomer hexametaphosphate, oftentimes referred to as SPG.

Because of their chemical structure, poly- and pyrophosphates have been grouped together under the term "condensed" phosphates or, somewhat incorrectly, as "polyphosphates".

Physical and Chemical Changes Induced by Inorganic Phosphates in Meats

A discussion of the microbiological effects of phosphates added to meats and meat products can hardly be undertaken without a review of some of the characteristic physical, chemical and sensory changes imparted to animal muscle by such compounds.

Phosphates and the pH of meats

Condensed phosphates can be divided into alkaline and acid depending on their pH effect. In meats the former group includes STPP, TSPP and SPG, while the only member of the latter is SAPP. pH changes due to phosphate addition to meat were noted early by Swift and Ellis (1956), who stated that pH and ionic strength influenced the extent to which pyrophosphates increased the water holding capacity (WHC) of ground pork meat. Sherman (1961a) attributed such increased meat WHC not to pH itself but to solubilization of actomyosin by phosphates, which increased along with meat pH values over 6.25. Working with

combinations of STPP and SPG, Tsai and Ockerman (1981) have demonstrated the direct relationship between ionic strength, pH and WHC of meats. Awad (1968) found that polyphosphates decreased the acidity of meat in relation to the degree of hydrolysis that they undergo in the meat and attributed pH increases to the buffering capacity of the orthophosphates formed. According to Shults et al. (1972), the order of effectiveness of condensed phosphates in increasing the pH of raw beef coincided with that of increased water holding capacity: TSPP>STPP>SPG. This order was also valid in chicken muscle (Shults and Wierbicki, 1973) and partially confirmed in frankfurter emulsions by Knipe (1982), who also noted that although TSPP induced a higher increase in emulsion pH than SPG, it allowed a lower pH increase upon cooking than SPG, SAPP or no phosphate. In contrast, SAPP is known to cause a decrease in the pH value of comminuted pork (Ivey and Robach, 1978) and frankfurters (Hargett et al., 1980). Furthermore, such a SAPP effect was observed in frankfurters whether the meat used in their manufacture was beef/pork, chicken or turkey (Terrell et al., 1982). Commercial blends of alkaline phosphates have been reported to increase the pH of hot cut chicken breasts from 5.81 to 6.15 when injected as a 4% solution to 0.5% of total meat weight (Peterson, 1977), or added to canned broiler meat (Rao et al., 1978).

Phosphates and the water holding capacity (WHC) of meats

Observed increases in the water holding capacity of fresh and processed meats resulting from addition of alkaline phosphates have been

explained in terms of induced solubilization of actomyosin (Swift and Ellis, 1956; Sherman, 1961a). In frankfurter emulsions, however, only a slightly higher soluble protein content was found to result from addition of 0.3% TSPP (Knipe, 1982). Berman and Swift (1964) suggested that the role of phosphates in increasing the hydration of meat in the presence of NaCl was related to zinc removal, this being the only electrolyte found by the authors to be firmly associated or bound to meat proteins. On the subject of meat swelling, a synergistic effect between low concentrations of sodium chloride (0.5-1.5%) and diphosphates (i.e., pyrophosphates) was documented by Tsai and Ockerman (1981). A similar synergism was previously observed by Shults et al. (1972), who suggested that meat swelling was the result of successive replacement of Ca^{++} and Mg^{++} ions for Na^{+}, although Inklaar (1967) had earlier stated the role of phosphates in meats was unrelated to calcium ion chelation, since most Ca^{++} is firmly bound to meat proteins and not available for reaction with phosphates.

Increased water absorption and retention brought about by alkaline phosphate addition has been reported in chicken carcasses chilled in polyphosphate solutions (May et al., 1963; Klose et al., 1963), pork loins (Hoes et al., 1980), frankfurters (Hargett et al., 1980) and cod tissue (Tanikawa et al., 1963). As a result of increased WHC, a 5% solution of an unspecified alkaline phosphate blend injected into chicken carcasses in combination with 10% NaCl greatly decreased cooking losses (Farr and May, 1970). Marinating chicken quarters in 6 and 12% solutions of similar commercial blends of unspecified phosphates was

found to be effective in minimizing cook losses (Landes, 1972). Yields of chicken white meat, on the other hand, greatly increased when the cooking was carried out in phosphate/NaCl solutions, particularly when a 0.5% TSPP and 1.0% NaCl combination was used (Shults and Wierbicki, 1973). Peterson (1977), however, was unable to obtain any cook yield improvements by injecting 0.5% of a STPP-SPG blend into hot cut chicken breast meat at 20 minutes post-mortem. The same blend, used in chilling water at 5%, had been earlier observed to increase water retention in cooked chicken by 2-5% (Klose et al., 1963).

Most of the published work concerning the effects of alkaline poly- and pyrophosphates in meat and meat products cook yields provide overwhelming evidence that such effect is positive. Early studies by Tims and Watts (1958) demonstrated that STPP, TSPP and SPG decreased cooking losses in a variety of meats, while Swift and Ellis (1957) had concluded that the addition of a 0.5% blend of SPG (65%), TSPP (10%) and SAPP (10%) had no effect on shrinkage of bologna if common industrial cooking and smoking protocols were followed, but decreased shrinkage resulted if cooking was done at 160°F. Increased yields of cooked beef rolls from 93 to 98% of uncooked weight were attained by Moore et al. (1976) when 0.25% STPP was added to the product with 3% instead of 1% NaCl. A similar interaction between STPP and sodium chloride was reported to occur in a flaked, cured pork product, such that increased concentrations of both additives resulted in higher smokehouse and cook yields (Neer and Mandigo, 1977). Sectioned and formed hams subjected to massaging experienced a 3.24% decrease in cook losses when 0.5% of an

SPG-STPP blend was added with 1.0% NaCl (Siegel et al., 1978). Vollmar and Melton (1981) measured greater cooking losses in cured hams when the pumping brine contained no phosphates than when 5% SPG had been added. Reports on the advantageous use of alkaline phosphates to increase cook yields also include frankfurters (Hargett et al., 1980) and pork loins injected to 110% of initial weight with 5% solutions of SPG-STPP, although in the latter case such treatment also produced a greater thaw loss and less browning on cooking (Hoes et al., 1980).

In contrast to that of alkaline, condensed phosphates, the effect of sodium acid pyrophosphate (SAPP) on cook yields of meat products has not been widely reported. Hargett et al. (1980) found no improvement in the WHC of frankfurters when SAPP was added to the formulation at various levels. Terrell et al. (1982) attributed a greater cooking shrinkage of pork/beef or chicken frankfurters to SAPP, but could not demonstrate the same effect in turkey frankfurters. Knipe (1982) concluded that all the phosphates tested in frankfurter emulsions - SPG, STPP, TSPP and SAPP - equally increased cook yields.

Interactions between phosphates and meat proteins

Swift and Ellis (1956) carried out pioneer work on the effects of poly- and pyrophosphates on meat proteins and first advanced the theory that pyrophosphates dissolve proteins, specially actomyosin, to an extent dependent on pH and ionic strength. Their study contained statements rejecting the hypothesis that pyrophosphates exert effects on meat proteins different or superior to those of orthophosphates.

Sherman (1961a) confirmed the role of TSPP and SPG in increasing the water holding capacity of meat by increasing actomyosin solubility, concluding that the effect of phosphates was not related to calcium or magnesium ion chelation but to phosphate anion absorption by meat proteins. pH and ionic strength, in his view, influenced the rate of anion absorption by meat proteins in direct proportionality. The same author showed later that at 0 or 100°C, cations and anions from neutral salt solutions were equally absorbed by lean pork to a degree dependent on the concentration of the solution; water absorption was found to be linearly related to the magnitude of ion absorption, since the latter would effect an opening of the protein molecule through repulsion of similarly charged groups. With alkaline phosphates, however, cations were preferentially absorbed over anions at lower but not at higher pH values, so that phosphate anion absorption became important (Sherman, 1962). Maesso et al. (1970) confirmed these results using chicken meat and a commercial blend of alkaline phosphates: adding NaCl reduced the negative charge of the proteins so that phosphates - also negatively charged - were not repelled as strongly. As more sodium chloride was added, more Na^+ ions were absorbed until the net charge of the proteins became positive, strongly attracting negatively charged phosphate ions. These findings also supported those of Inklaar (1967) in downgrading the importance once attributed to Ca^{++} and Mg^{++} ion chelation as the main factor relating phosphates to increased WHC of meats.

The ability of poly- and pyrophosphates to increase myosin solubility in muscle tissue as a consequence of dissociation of the

actomyosin complex has been documented by many researchers. Nikkila et al. (1967) observed that pyrophosphates had the ability to prevent NaCl-induced insolubilization of actomyosin in Baltic herring fillets at 4°C; tracer-marked TSPP rapidly migrated and accumulated in the acid soluble fraction after 24 hours, with some incorporation of marked phosphorus in lipids and nucleic acids. Galluzzo and Regenstein (1978) reported that the emulsifying capacity of natural actomyosin from chicken breast muscle was as high as that of myosin if adenosine triphosphate (ATP) or pyrophosphates were added, and that actin remained in the aqueous phase while myosin participated in the emulsion. Similarities between the chemical changes induced by polyphosphates and ATP on proteins had previously been seen in cod fillets by Tanikawa et al. (1963). In beef muscle, increased binding was found to be not a function of pH and ionic strength but of phosphate-protein interaction caused by solubilization of actomyosin, in turn a result of dissociation of actomyosin by added STPP (Siegel and Schmidt, 1979). Breaking the actomyosin complex by phosphates also resulted in increased migration of NaCl and sodium nitrite in tumbled hams, which contributed to more uniformly cured and colored hams (Krause et al., 1978). Electron microscopic examination of massaged hams confirmed that a blend of SPG and STPP caused a massive disruption of myofibrils and formation of interfilament spaces, due to breakage of bonds between actin and myosin and to increased ionic strength of the sarcoplasm (Theno et al., 1978). The capacity of the sarcoplasmic reticulum (SR) to accumulate Ca^{++} was found to increase in direct proportion to added inorganic phosphorus concentration, so that

cold-induced release of calcium from beef muscle SR was partly reduced by 50 mM P (20 mM P in rabbit muscle) (Newbold and Tume, 1981).

Phosphate-protein complex formation was studied by Tenhet et al. (1981a) in fresh and prefrozen, peeled shrimp, where P^{32}-labeled STPP was found to form a phosphate gradient in muscle and to accumulate on the surface when applied in solutions of low concentration (0.5-1.0%), thus preventing further uptake by the muscle; at higher concentrations the gradient was overcome with time and STPP became evenly distributed. Such an effect may play an important role in the activity of phosphates against bacteria on the surface of chicken or meat cuts as discussed later in this review. A surface reaction between phosphates and proteins had been suggested earlier by Nikkila et al. (1957). Increased phosphorus content of muscle in broilers marinated in 6 and 12% solutions of an alkaline phosphate blend (STPP-TSPP-SPG) had also been noticed by Landes (1972). Additional evidence of phosphate-protein interactions is derived from the study of Neer and Mandigo (1977), who concluded that increasing the concentration of STPP added to a flaked, cured pork product upward of 0.25% caused a decrease in % protein. Later, Rao et al. (1978) observed that 0.75 and 1.0% concentrations of an STPP-TSPP-SPG blend added to canned poultry meat reduced hydrolysis of dicarboxylic amino acids during retorting, thereby retarding the formation of free ammonia normally produced in canned meats. Gumpen and Fretheim (1983) have applied some of this knowledge to the development of a rapid, simple method for total meat extractable protein determination based on a Na-phosphate-KCl buffer extraction.

Phosphates and the ionic strength (μ) of meats

The already mentioned work of Swift and Ellis (1956) pointed to the dependence between solubility of actomyosin in phosphate-treated meats and ionic strength (μ). Sherman (1962), however, reduced the role of μ in fresh pork containing polyphosphates to one of limiting the rate of ion absorption by meat proteins: the greater the ionic strength of the added phosphate solution, the greater the absorption of ions. An inverse relationship between the degree of dissociation of phosphates and the number of P atoms in the molecule had been studied by Batra (1965), who pointed out that orthophosphates were 100% dissociated in aqueous solutions, that SPG was least dissociated and that Ca^{++} ions greatly enhanced phosphate dissociation. The degree of such dissociation would determine increases in ionic strength of a muscle food by phosphates. Ivey and Robach (1978) attributed increased μ of canned, comminuted pork to SAPP and SPG, but Seman et al. (1980) could not find differences between low (0.21) or high (0.41) ionic strength bologna prepared with 0.13% solutions of K_3PO_4. Increased μ of the sarcoplasmic reticulum of porcine muscle - brought about by 0.5% addition of a blend of STPP and SPG - was used by Theno et al. (1978) to explain concurrent increases in water holding capacity, while Siegel and Schmidt (1979) stated that increased ionic strength could not explain parallel increases in the binding capacity of crude myosin from bovine trapezius muscle.

Metal ion chelating and antioxidant properties of phosphates in meat and meat products

Phosphates are well-known metal ion chelators, their ion sequestering effectiveness being a function of the degree of molecular polymerization. Therefore, complexes formed by tripolyphosphate with alkali (Li^+, Na^+, K^+) and alkaline earth metals (Ca^{++}, Mg^{++}, Mn^{++}) are stronger than those of pyrophosphates. The latter, in turn, are more stable than the ortho-P chelates, although the orthophosphate-ferric ion complex - like those of longer chain phosphates and alkaline earth metals - may involve covalent bonding and thus be several-fold stronger than other orthophosphate chelates (Van Wazer and Callis, 1958). The previous observation may be of great importance in terms of phosphate inhibition of Clostridium botulinum growth in meat systems, which is opportunely discussed in this review. The order of effectiveness of phosphates as Ca^{++} ion chelators has been found to be inversely related to molecular chain length and degree of dissociation, ortho-P being 100% dissociated in aqueous solutions (Batra, 1965). Chelation of Ca^{++} and Mg^{++} ions, however, had been shown to be of little significance in relation to meat fluid retention (Sherman, 1961a), particularly since 60% of the calcium (and 20% of Mg^{++}) present in meat was found firmly bound to protein and not available to react with phosphates (Inklaar, 1967). Earlier, Berman and Swift (1964) had concluded that Zn^{++} was the only electrolyte strongly bound to or associated with meat soluble proteins and as a consequence, the sequestering role of phosphates in relation to meat hydration was minimal and limited to zinc removal.

These authors also determined that free Ca^{++}, Mg^{++} and to a lower extent Zn^{++}, increased in meat mixes held at 3°C upon addition of NaCl, while at 70°C free Ca^{++} concentrations increased but free Zn^{++} decreased; if no NaCl was present all Mg^{++} was free at 70°C, while free Ca^{++} and Zn^{++} increased. This information is discussed later in terms of the probable implication of such metal ions in the antibacterial activity of phosphates. Orthophosphates have been implicated in decreased Zn^{++} solubility, while STPP and SPG had the opposite effect (Zemel, 1984). In relation to the bioavailability of iron from meat products, TSPP and STPP are reported to impair Fe^{++} metabolism in growing rats more severely than disodium ortho-P or SPG, decreasing intestinal iron absorption as well as hemoglobin concentration and iron liver values (Mahoney and Hendricks, 1978). STPP is also known to inhibit catalysis of linoleic acid peroxidation by hemoprotein metmyoglobin and by a ferrous iron-EDTA (ethylene diamine tetraacetic acid) complex at pH 5.6 or 6.2, pointing to strong iron chelation by that phosphate (Hsiao-Ping, 1970). Zemel (1984), on the other hand, demonstrated that ortho-P, SPG and particularly STPP depressed the level of ionized iron following in-vitro peptic and peptic-pancreatic digestions, but did not affect the level of total soluble iron.

Metal ion chelation is believed to account for the ability of phosphates to delay the onset of oxidative rancidity and associated off-flavors in meat products. Reports to this effect include a study done in cooked meats, where STPP, TSPP and SPG but not ortho-P had antioxidant properties synergistic with those of ascorbic acid (Tims and

Watts, 1958). Less unsaturated carbonyl compounds were also found in canned broiler meat treated with 0.75 and 1.50% levels of a STPP-TSPP-SAPP blend by Rao et al. (1978), who attributed the effect not to metal ion chelation by the phosphates but to their inhibition of dicarboxylic amino acid hydrolysis during retorting. Delay in oxidative rancidity development (measured by iodine numbers) as a result of STPP addition (0.375-0.250%) in combination with 3.00 or 2.25% NaCl was also shown by Neer and Mandigo (1977) to occur in a flaked, cured pork meat product, and in frozen pork patties (1% NaCl, 0.25% of a 75% STPP - 25% SPG blend) by Keeton (1983).

Other studies have confirmed the favorable antioxidant effect of phosphates in poultry. The addition of 0.35% of a commercial blend of STPP, TSPP and SAPP to ground turkey breast and leg meat delayed rancidity for 10 days at 5°C (Marion and Forsythe, 1962), while cooking in 3% or injecting 5% solutions of the same blend decreased TBA (thiobarbituric acid) numbers in cut-up broilers (Farr and May, 1970). Precooked, frozen chicken marinated in 6 or 12% solutions of STPP-TSPP-SAPP also exhibited lower rancid flavor score values; flavor improved in direct proportion to phosphate concentration (Landes, 1972).

Hydrolysis and ionization of phosphates in aqueous solutions and meats

Much of the available knowledge on the hydrolysis of poly- and pyrophosphates in aqueous solutions dates back to the 1940s and 1950s. The work of Bell (1947a, 1947b, 1949) contributed a clear understanding of the products formed by the hydrolytic breakdown of condensed

phosphates in water (Figure 2).

It is important to distinguish between ionization and hydrolysis. Weast and Astle (1981, p. F-98) define ionization as "the process by which neutral atoms or groups of atoms become electrically charged, either positively or negatively, by the loss or gain of electrons; or the state of a substance whose atoms or group of atoms have become thus charged". Hydrolysis, however, defined by the same authors as "a double decomposition reaction involving the splitting of water into its ions and the formation of a weak acid or base or both" (Weast and Astle, 1981, p. F-100) has attained broader and various meanings such as breakdown into lower molecular weight components and even "rehydration" (Bell, 1947a) when applied to substances other than water.

Sodium acid pyrophosphate (SAPP) was found to undergo rapid hydrolysis to orthophosphates in aqueous solutions at 100°C, while TSPP proved considerably more stable: no detectable hydrolysis occurred at 100°C in 1% NaOH solution for 96 hours and only 40% under similar conditions in water (Bell, 1947a). The same author found that STPP hydrolyzed to one molecule of each ortho- and pyrophosphate by as much as 90% after 12 hours in water at 100°C, but only by 20% after 60 hours at 70°C. Those values contrast with others obtained from studies of the nutritional effects of high phosphate diets on rats, which claimed the half-lives of TSPP and STPP under normal gastric conditions (pH 2, 39°C) to be 400 and 150 hours, respectively, or 5200 and 2100 hours under achlorhydric gastric conditions (pH 6, 39°C) (Draper et al., 1972).

Heat-induced hydrolysis of poly- and pyrophosphates was observed by

Sherman (1961b) in cooked pork sausage, and accelerated hydrolysis of these compounds by naturally occurring meat enzymes was extensively studied by Awad (1968), Sutton (1973) and Tenhet et al. (1981b). Inactivation of meat phosphatases by heat is reported to begin at approximately 40°C and to be complete at 60°C (Awad, 1968), although possible reactivation of such enzymes after cooking has not been examined in meats. Studies on the uptake and stability of STPP from 0.5 and 1.0% solutions by peeled, deveined shrimp have indicated that after 2 weeks at -26°C only 12% of total phosphorus activity in the muscle could be attributed to STPP; pyrophosphate accounted for 25% and ortho-P for 27% (Tenhet et al., 1981b). Prolonged frozen storage of the shrimp to 10 weeks produced a final, stable residual level of STPP corresponding to 12% of total muscle phosphorus, 2% to pyro- and 45% to orthophosphate.

Ionization of phosphates in water has been directly related to molecular chain length, so that under those conditions ortho-P exists in 100% ionized form and SPG as the least ionized polyphosphate used in meat products. Ca^{++} was shown to promote dissociation of phosphates, while the ability to chelate Ca^{++} at the expense of Na^{+} also decreased with ionization (Batra, 1965).

Phosphates as Antimicrobial Agents

An examination of the bactericidal and/or bacteriostatic properties of phosphates is given in this review according to whether phosphate effects on bacteria have been studied in laboratory media or in meat systems and products.

Antimicrobial properties of phosphates in laboratory media

Considering the extensive information available on a wide variety of bacterial inhibitory compounds, the amount of research devoted to the study of phosphates and their influence on bacterial survival and growth under laboratory conditions has been limited.

Long before poly- and pyrophosphates were approved as additives for use in meat products, Post et al. (1963) discovered that SPG had been incorrectly used as solvent for calcium alginate wool swabs in techniques designed for bacterial recovery from surfaces; wild populations of bacteria were reported to be inhibited by SPG in direct proportion to its concentration (0.01-2.00%) in nutrient agar. Gram-positive species tested in that study were inhibited by 0.1% SPG in the medium and appeared to be more susceptible to that phosphate than were gram-negative organisms, which could often withstand 10% SPG (a concentration that resulted in immediate lysis of gram-positives). Lysis and overall inhibition by SPG, however, could be prevented by NaCl or $MgSO_4.7H_2O$. This evidence moved those authors to advance the theory

that SPG interfered with divalent cation metabolism - notably Mg^{++} - blocking cell division and causing loss of cell wall integrity. Elliott et al. (1964) confirmed that 1.0% pure polyphosphate or mixture of phosphates (75% STPP/25% TSPP, <1% ortho-P) inhibited the growth of non-fluorescent pseudomonads in synthetic medium and that such inhibition was due to chelation of an essential metal ion, not to phosphate-induced pH changes. Fluorescent strains (pyoverdine producers) used in that study, however, were found to grow after a short lag; natural competitive metal ion chelators such as pyoverdine and bacteriological peptone, as well as Mg^{++}, were reported to reverse phosphate inhibition. Gray and Wilkinson (1965) noted that SPG had a bactericidal activity very similar to that of ethylene diamine tetraacetic acid (EDTA) against Pseudomonas aeruginosa at 0.1% (w/v) in borate buffer (pH 7.1), causing structural damage to cells. According to those researchers, SPG and EDTA were both able to form highly stable chelates and their bactericidal activity was not due to chelation of metal cations in the growth medium, but on the cell wall of bacteria. Displacement or removal of a metal cation (probably Ca^{++}) essential to cell wall integrity by SPG and EDTA was therefore theorized to be responsible for leakage of cell solutes, loss of viability and rapid lysis of bacterial cells. The Ca^{++} and Mg^{++} chelation theory continued to be supported by Kohl (1971), who patented a process by which egg white could be pasteurized at pH 9, 134°F in 3.5 minutes. The process involved the addition of recommended levels of 0.50-0.75% STPP and was based on decreased D values for salmonellae (S. seftenberg 775W, S. typhimurium

No. 13311 and S. oranienburg No. 9239) in the presence of phosphates. Previously, Garibaldi et al. (1969) had reported rapid death of salmonellae (10^6/ml after 60 h at 28°C) in egg white supplemented with EDTA or 4% Kena, a commercial phosphate blend (75% STPP/25% TSPP, <1% ortho-P); D values for S. typhimurium were reduced by a factor of 2 with Kena and of 3-4 with EDTA at pH 5.3, while those of S. seftenberg were lowered by 1.7 and 9.0 with Kena at pH values of 5.3 and 8.9, respectively.

Phosphate buffers are widely used in microbiological studies for bacterial cell and spore suspensions, washing, pH control, dilution and other purposes. Williams and Hennessee (1956) studied the heat resistance of spores of Bacillus stearothermophilus heated in disodium phosphate buffer and found that heat resistance increased with decreasing molal concentrations over a range of M/15 to M/120. Most of the effects were attributed by the authors to inhibitory activity of the phosphate carried over to the recovery medium in concentrations greater than M/120, not to the molality of the buffer in which the spores were heated. Later, Walker (1964) demonstrated that the buffer itself had no effect on the destruction of spores of either Bacillus megaterium 1A28 or Bacillus polymyxa 1A39, but dilution of the buffer spore suspension with peptone water prior to plating may have eliminated the carry over effect in this study. Carlsson et al. (1978) observed that media containing phosphate and glucose or alpha-hydroxy carbonyl compounds had a tendency to accumulate hydrogen peroxide upon being heated (autoclaved) to 120°C for at least 5 minutes under anaerobic conditions,

followed by exposure to atmospheric oxygen; the autooxidation reaction produced superoxide and was catalyzed by transitional metal ions, although the latter were not necessary for the reaction to occur. As a result, the rate of killing of Peptostreptococcus anaerobius VPI 4330-1 in such media was high (superoxide formation was confirmed by the fact that addition of superoxide dismutase to the medium eliminated the inhibitory factor). Similar generation of free radicals was observed by Miller (1969) in mixtures of H_2O_2 and ascorbic acid; as a result, gram-negative bacteria were lethally affected by loss of cell wall integrity and increased sensitivity to lysozyme. Field and Liechstein (1957) had earlier noted that many species of Lactobacillus and Streptococcus exhibited a prolonged delay in growth initiation when inoculated in synthetic media sterilized by filtration or autoclaved without a fermentable carbohydrate. Experimenting with Propionibacterium pentosauceum and other species of propionibacteria, those authors determined that autoclaving a medium composed of 1% yeast extract, 1% casitone, 1% glucose and 0.5% K_2HPO_4 (pH 6.7-6.9) enhanced the growth of those microorganisms, which grew even faster if preincubated (i.e., if the cultures were older). This result indicated that a metabolic product formed by the culture during preincubation in sufficient concentration was necessary before optimal growth and reproduction could proceed, something reminiscent of the reports by Firstenberg-Eden et al. (1981) that phosphates were more inhibitory to younger (24 h) than to older cultures (48 h) of Moraxella-Acinetobacter. The recent work of Schiavone and Hassan (1985) has shown that the synthesis of manganese-

containing superoxide dismutase (MnSOD) in gram-negative (but not in gram-positive) bacteria proceeded like in E. coli. When growing cells were exposed to elevated oxygen pressure (i.e., transferred from anaerobic to aerobic conditions), or to redox active compounds that increase intracellular concentrations of superoxide radicals (O_2^-), MnSOD synthesis took place. Iron chelators also induced synthesis of MnSOD. Therefore, the formation of superoxide radicals (and eventually, of H_2O_2) in heated phosphate/glucose containing media, combined with repression of MnSOD and/or catalase enzymes, may account for the stronger inhibitory effect of phosphates on gram-positive bacteria and on younger cultures than on gram-negative organisms and older cultures.

Firstenberg-Eden et al. (1981) found 0.5% trisodium orthophosphate (+ 0.8% NaCl) to be necessary for >99.9% inhibition of colony formation by Moraxella-Acinetobacter cells in plate count agar, while 0.12% STPP (+ 0.8% NaCl) or 0.4% TSPP - equivalent to 0.55% STPP - completely inhibited that microorganism. These authors also observed that heating the phosphates in the medium (70°C, 12 h) caused an increase in the amount of TSPP and specially of STPP that cells could tolerate, suggesting that hydrolysis of phosphates by heat was the factor responsible for the loss of inhibition. In no case could bacterial inhibition be related to pH in that study, which also showed synergistic interactions between NaCl and TSPP and additive effects in NaCl/STPP combinations. Additionally, defective or impaired cell division may have accounted for the observation that phosphate addition to growth media at sublethal levels resulted in formation of small, pin-point type

colonies by Moraxella-Acinetobacter. Selection of "petite" colony forming populations of Staphylococcus pyogenes - presently S. aureus - highly resistant to antibiotics and particularly virulent resulted from culture exposure to a variety of metal chelating chemicals (Shulman and Dwyer, 1964, pp. 419-426). Slower growth rates and increased glucose requirements by such miniature colony variants indicated a decreased capacity to utilize oxygen, due to impaired functionality of the cytochrome system. As a result, ATP formation was blocked. This mode of action is in general agreement with multiple studies of bacterial inhibition by metal chelators and may apply to phosphates. Nagai et al. (1961) showed that "petite" mutants of Saccharomyces sp. lacked the absorption bands of cytochrome a and b normally seen in spectroscopic examinations. Ycas and Starr (1953) had shown similar small colony forming yeast mutants to be deficient in catalase and hematin and to have a metabolic block in the pathway of glycine synthesis, which would hinder or stop synthesis of cytochrome c for lack of precursor. Supplying glycine and protoporphyrin to such mutants restored the formation of catalase and cytochrome c. This result bears a resemblance to observed instances of EDTA and $NaNO_2$ clostridial inhibition reversal by increased levels of available iron (Tompkin et al., 1979b).

The interactions between phosphates, other additives and metal ions have been the subject of various studies. Using microculture techniques, Seward et al. (1982) prevented Clostridium botulinum spore germination, outgrowth and vegetative cell growth in veal liver agar containing 1.5% sorbate (pH 7.1) and 0.5% STPP to a larger extent than

with sorbate alone. Earlier, Krishna Murty and Halvorson (1957) studied the effects of sequestering agents and metal ions on the germination and respiration of, and growth from Bacillus cereus spores. Concentrations of phosphate (K_2HPO_4) of M/3 or more inhibited the germination of spores that had been previously washed with phosphate or EDTA. Fe, Cu, Cr or Hg ions at 0.1M totally inhibited germination and washing the spores with water only partially reversed the inhibition. Adding arsenite, phosphate, EDTA or other chelators, however, completely reversed the inhibition by metal ions. The suggestion of that study was that phosphate, EDTA and some metal ions are individually inhibitory at high concentrations, while low amounts of one inhibitory agent or slight excess in the ratio of a metal ion over its chelator, or vice versa, may promote spore germination. Rowe and Koupal (1985) have recently reported that germination of heat-activated spores of Streptomyces antibioticus in a complex germination medium was enhanced by yeast extract or $CaCl_2/MgSO_4$ and monosodium orthophosphate (NaH_2PO_4). A similar conclusion was arrived at by Wilson and Baugh (1985) with spores of Bacillus thuringiensis var. kurstaki and israeliensis. However, incorporation of phosphate buffer into the plating medium reduced viable spore counts. Blankenship and Craven (1985) also reported that phosphate buffers only mildly induced the formation of coccoidal forms by Campylobacter jejuni at pH values between 6 and 9. Loss of cytoplasmic material (absorbing at 260 nm) occurred earlier at higher pH; added divalent cations functioned to stabilize ribosomes and membrane components essential for containment of cytoplasmic

constituents.

Like most animal tissue cells, bacteria possess enzymes involved in the breakdown of phosphates, extra- or intracellularly. Barnes and Morris (1957) quantitated the activity of a phosphatase from Micrococcus pyogenes var. aureus (Staphylococcus aureus) using p-nitrophenol phosphate as substrate (1g/liter H_2O). They determined that the hydrolysis of the phosphate monoester was linear for 2 hours, after which the velocity of reaction decreased. It is interesting to note that these researchers also found that the age of the culture had a pronounced effect on the magnitude of hydrolysis of p-nitrophenol-P. Cultures that were 48- and 144-h old had only 86 and 20% the activity of 24-h cultures, respectively; optimum pH for enzyme activity was 5.6 (range 5.5-7.0). A similar, non-specific acid phosphomonoesterase (pH optimum 5.5-6.0) was present in Saccharomyces mellis grown in a medium devoid of phosphate but hardly detectable when the yeast was cultured in phosphate-rich media (Weimberg and Orton, 1963). The latter enzyme required no metal cofactors and was inhibited by anions such as phosphate, arsenate, molybdate and borate. A pyrophosphatase (pH optimum 7.5) was present as a constitutive, non-repressible enzyme in the same yeast and required Mg^{++} for activity. Five times as much of any anion was necessary to inhibit hydrolysis of pyrophosphate than that of other substrates (ATP, glucose-6-phosphate or SPG) by the first, acid enzyme, indicating that those other substrates were competitive inhibitors of pyrophosphate hydrolysis. ATP and SPG were not broken down by the alkaline pyrophosphatase but stimulated the rate of

hydrolysis of pyrophosphate to ortho-P when present in concentrations slightly lower than that of Mg^{++}; otherwise, these compounds inhibited the reaction. Additionally, acid phosphatase was inactivated at pH <2.5 or >9.0, while the alkaline enzyme lost its activity even at pH 5.0 or >9.0 and both began denaturating at 40-45°C (completed at 50°C in 5 minutes).

These enzymes are undoubtedly important in the study of the mode of action of phosphates on bacteria, since such acid phosphatases have been found in many microorganisms (Weimberg and Orton, 1963). Since the pH decreases with SAPP in media as well as in meat systems previously discussed and, unlike alkaline pyrophosphatase, the acid phosphatase is repressed by external orthophosphate and does not require metal cofactors, it is conceivable that this type of enzyme might play a major role in bacterial inhibition by SAPP, whereas the antibacterial mechanism of alkaline phosphates might also be related to enzymatic phenomena primarily through metal ion chelation.

The presence of alkaline pyrophosphatase in Desulfovibrio desulfuricans was also documented by Akagi and Campbell (1963). They confirmed that Mg^{++} or Mn^{++} ions were essential for optimum activity (Co^{++} was only 65% as effective a cofactor as Mg^{++}). This enzyme had a 1:1 optimum ratio of magnesium to pyrophosphatase at pH 8.0, did not hydrolyze ATP, glycerol-P, diphenyl-P or p-nitrophenyl-P and was rapidly inactivated at temperatures above 40°C. That a Mg^{++}-requiring phosphatase may also be involved in the bacteriostatic or bactericidal activity of phosphates becomes equally apparent when the various reports

on reversal of alkaline phosphate inhibition by added magnesium are considered (Post et al., 1963; Elliott et al., 1964). Hugo (1967) and Weinberg (1957) reviewed the magnesium reversal of microbial inhibition by a host of antibiotics which are also chelating agents.

The knowledge that phosphatases, and other bacterial enzymes as well, require metal ion cofactors in concentrations so defined that levels above or below the optimum result in decreased activity, may provide an explanation to seemingly conflicting reports on the effect of phosphates on microorganisms. Foster et al. (1978) isolated a Bacillus sp. in soils that was capable of utilizing phosphite and hypophosphite anaerobically; the optimum concentration of hypophosphite for growth was found to be 60 μg/ml, above which it became toxic; optimum concentration of orthophosphate, in contrast, was >1000 μg/ml. The observation that in the presence of phosphate (ortho-P) the Bacillus was unable to metabolize either phosphite or hyphosphite may well have been the result of an enzymatic end-product inhibition of the type already discussed. It is also important to note that when utilization of either phosphite took place, the authors did not observe accumulation of phosphate in the medium and P-labeled hypophosphite was incorporated into the cells as organic phosphate. Harold and Harold (1965) studied two enzymes capable of degrading polyphosphates in mutants of Aerobacter aerogenes, a polyphosphatase and a polyphosphate kinase. The former mediated the main pathway of polyphosphate degradation from the cells' internal pool, required Mg^{++} ions and high levels of K^{+} and was inhibited by 0.05M but not 0.01M orthophosphate. A second mutant, constitutive for elevated

levels of polyphosphate kinase, polyphosphatase and alkaline phosphatase, was seen to exhibit transient accumulation of polyphosphate when the cells were transferred to fresh growth medium, which the authors concluded was due to shifting ratios of the biosynthetic and degradative enzymes during growth. In other terms, that nucleic acid synthesis inhibited that of polyphosphates and stimulated their degradation. Previous work by Harold (1963) had shown that the accumulation of polyphosphates by A. aerogenes took place in cells whose growth had ceased due to exhaustion of essential nutrients such as sulfur. The reciprocal relationship between growth and polyphosphate accumulation could be traced to competition by nucleic acid and polyphosphate syntheses for intracellular P. In terms of our area of interest, however, the most important observation in these studies was that the rate of intracellular polyphosphate synthesis in Aerobacter aerogenes was proportional to the level of polyphosphate kinase present in the cells and that the synthesis of polyphosphate kinase, in turn, was subject to repression by exogenous ortho-P. A similar polyphosphate kinase was reported in E. coli by Kornberg (1957) as an enzyme that catalyzed the formation of ATP from intracellular polyphosphate. Consequently, inhibition or repression of bacterial enzymes by orthophosphates in the extracellular environment has been documented for enzymes involved in utilization of external phosphorous sources, as well as in intracellular synthesis of ATP and phosphate reservoirs necessary for nucleic acid synthesis.

Microbiological effects of phosphates in meat and meat products

Most of the early work concerning the application of phosphates in improving the microbiological quality of meat and meat products and thus in prolonging shelf life was done on poultry. Spencer and Smith (1962) observed lower rates of bacterial spoilage and 1-2 days extended shelf life of refrigerated carcasses by chilling the broilers in iced water containing 10 oz/gal Kena (75% STPP/25% TSPP) - approximately 7% (w/w). The rate of spoilage was measured by total plate counts, UV fluorescence and development of off-odors. Immersion of chicken carcasses in 8% solutions of the same blend or of pure STPP diluted to 6% with ice, produced lower bacterial counts for as long as 20 days of subsequent storage at 5°C, and significantly ($P<0.05$) lower lipolytic but not proteolytic bacterial numbers in a first experiment; a second one failed to duplicate these results (Steinhauer and Banwart, 1963). Elliott et al. (1964), however, confirmed that the shelf life of chicken carcasses could be lengthened by 17 and 25% if chilled in ice slush containing 3 and 8% of a phosphate blend (Kena), respectively. Further, if the broilers were stored at 2.2°C in contact with the above-mentioned phosphate solutions, shelf life could be increased by 17 and 65%, respectively. Kena was also used at 3% for precooking or presoaking chicken parts by Chen et al. (1973), which eliminated naturally occurring microorganisms almost entirely but in the precooked poultry caused unappealing digestion of the skin. Among 17 pure broth cultures of microorganisms frequently associated with chicken, 10 species of gram-negative bacteria were found to be more resistant than 6 of 7 gram-

positive species tested, the notable exception being Streptococcus lactis. Foster and Mead (1976) studied the survival of salmonellae in minced breast and leg chicken meat containing 0.35% of a phosphate blend of unspecified composition (Puron); their finding of decreased Salmonella viability at -2°C and -20°C was not reproduced at -5°C or 1°C, but was conclusive in that survival was higher in breast (pH 5.8) than in leg (pH 6.4) meat. Working with frankfurter emulsions prepared from simulated mechanically deboned turkey breast meat, Mac Neil et al. (1973) obtained lower total counts during one week of storage at 3°C when 0.5% of an unspecified phosphate blend was incorporated into the formulation.

Although this review is by no means exhaustive, the available information on the microbiological effects of phosphates added to meat products other than poultry is not only sparse, but often includes combinations with other additives in such a way that bacterial inhibition directly attributable to phosphates is unclear. Fortunately, some exceptions do exist. Mol et al. (1971) could find no effect by 0.45% polyphosphates (Hamine OX) on the growth of an unclassified Streptobacterium LL3 inoculated on the surface of sliced berliner sausage held at 12°C. Similar studies were conducted by Nielsen and Zeuthen (1983) with cooked (75°C), sliced, vacuum packaged bologna formulated with 0.3%, low pH mixtures of sodium polyphosphates (unspecified), STPP or STPP in combination with SPG, combined with 55 ppm $NaNO_2$ and 168 ppm sodium ascorbate. Inoculation of the sliced bologna with a mixture of 3 strains of Brochothrix thermosphacta and an

atypical Streptobacterium, or 3 strains of Serratia liquefaciens followed by storage at 2°C resulted in no marked effect by phosphates on the lactic acid bacteria, but more growth took place in the absence of phosphates. Low pH mixtures of phosphates, however, did have a strong inhibitory effect on B. thermosphacta and S. liquefaciens, while TSPP alone, even at low pH, was ineffective.

A different approach to the study of phosphates was that of Snyder and Maxcy (1979), who effectively inhibited growth of radiation resistant strains of Moraxella-Acinetobacter isolated from meats by decreasing the water activity (A_w) of standard laboratory media. They used NaCl, yeast extract, dehydrated m-plate count broth or SPG to decrease A_w values to <0.99. Those microorganisms were also unable to grow in ground chicken or beef, or in fresh beef exudate containing 0.5% sodium hexametaphosphate (SPG), even with 20% added water, or 30% water if 1.0% SPG was present. The authors concluded that inhibition was due to decreased A_w by SPG, since no inhibitor could be detected in meat without SPG and growth occurred if water activity was raised to 0.99 in the absence of phosphate. Sikes and Maxcy (1980) studied the effect of meat protein hydration on the invasive capacity of the proteolytic Serratia marcescens. They reported that 0.5% STPP reduced the depth of bacterial penetration by 16 and 84% in comminuted beef and pork, respectively, and that a level of 0.25% STPP was nearly as effective.

A report by Hoes et al. (1980) indicated that injection of hot processed pork loins to 110% of weight with a 5% solution of SPG-TSPP resulted in significantly ($P<0.05$) higher microbial numbers (mesophilic

anaerobes) than conventional handling methods. This unfavorable result appears to be unique in the literature of phosphates reviewed.

Since the appearance of the National Academy of Sciences reports on possible health hazards derived from excessive NaCl intake (NAS, 1980) and on the health effects of nitrite in foods (NAS, 1981 and 1982), research oriented toward the search for total or partial substitutes for sodium nitrite as an antibotulinal agent in cured and processed meats has increased (Widdus and Busta, 1982). Similarly, efforts have increased to find additives that, like phosphates, may allow decreased levels of salt without impairing the quality and safety of meat products (Sebranek et al., 1983; Maurer, 1983; Terrell, 1983). The need for sodium nitrite in processed and cured meat products, besides aspects related to color, is due to its long recognized - and so far unequaled - antibotulinal activity (Sofos and Busta, 1980). The search for substitutes or potentiators of sodium nitrite in meats has covered such varied chemicals as sorbic acid and its salts, sodium hypophosphite, fumarate esters, acidulants, alpha-tocopherols and ascorbate (Widdus and Busta, 1982). More recently, poly- and pyrophosphates are beginning to receive attention.

Ivey and Robach (1978) stated that meat products safe from the dangers of botulism could be produced without nitrite. Working with canned, comminuted pork those authors found that SAPP and SPG did not significantly ($P>0.05$) interact with nitrite in preventing toxin production by Clostridium botulinum, but had a synergistic effect with sorbic acid while the latter had only additive effects in combinations

with $NaNO_2$. Later, Bowen et al. (1974) reported that sodium ascorbate did not decrease the antibotulinal effectiveness of nitrite but did not potentiate it either. Opposite results were obtained by Tompkin et al. (1978a) with perishable, canned cured meat inoculated with C. botulinum spores and held at 4.4 or 10°C; spores germinated at 10°C but cell counts remained stable for as long as 16-18 weeks in the presence of 0.02% sodium isoascorbate and 156 ppm $NaNO_2$, suggesting that inhibition was at the cell outgrowth stage (nitrite levels were unaffected by isoascorbate). An interesting observation by those authors was that the product became more prone to spoilage and toxicity development if it was temperature abused after a period of refrigerated storage. Additional work with the same product and conditions showed that isoascorbate, ascorbate and cysteine enhanced the antibotulinal properties of nitrite by sequestering a metal ion or ions in the meat (Tompkin et al., 1978b). This ion was subsequently determined to be iron, since adding hemoglobin or incorporating high-iron meats (i.e., beef or pork hearts) in the canned, cured meat product formulation resulted in no clostridial inhibition by even 156 ppm $NaNO_2$ (Tompkin et al., 1978c). As a corollary, these authors theorized that nitric oxide, formed from residual nitrite via nitrous acid, reacted with iron in the botulinal vegetative cell, thereby blocking some unspecified metabolic step essential for outgrowth. A fourth study by Tompkin et al. (1979a), indicated that it positively enhanced the antibotulinal activity of nitrite if temperature abuse (27°C) of the perishable, canned, cured meat product occurred at the time of manufacture, due to Fe^{++} chelation

by isoascorbate, but adversely affected inhibition if the product was refrigerated prior to temperature abuse or, if isoascorbate was used at levels >2000 ppm. In the case of product refrigeration prior to temperature abuse, isoascorbate seemingly induced depletion of residual nitrite at a faster rate. The similarity of this behavior and that of phosphate/metal ion ratios previously discussed must be highlighted. EDTA, a strong metal ion chelator was also found to enhance the antibotulinal properties of sodium nitrite in perishable, canned cured meat, in direct proportion to the level of EDTA used, while available iron decreased those properties also in direct proportion to its level in the meat (Tompkin et al., 1979b).

It is now apparent that $NaNO_2$ and sorbic acid may effect botulinal inhibition differently. As mentioned before, the reaction of sorbic acid with H_2O_2 to produce free radicals was attributed a potent antibacterial activity (Miller, 1969) and may have occurred in meats. C. botulinum type A and B spores germinated in chicken frankfurter emulsions containing 20, 40 and 156 ppm $NaNO_2$ during temperature abuse at 27°C, but failed to do so when 0.2% sorbic acid, alone or combined with nitrite, was present, although toxin production was delayed several days by either chemical alone and longer if combined (Sofos et al., 1979a). Other experiments showed that inclusion of mechanically deboned meat, known to have high K^+ and Ca^{++} contents (Essary, 1979), in the emulsion made the botulinal inhibitory effects of sorbic acid/nitrite disappear (Sofos et al., 1979b); total anaerobic counts, however, were not affected by $NaNO_2$ nor by sorbic acid in either one of the preceding

studies. In a third report, Sofos et al. (1980) showed that sorbic acid inclusion slowed depletion of residual nitrite in mechanically deboned chicken frankfurter-type emulsion, and that this, as well as delayed toxin production by inoculated Clostridium botulinum spores and total microbial anaerobic growth, were affected by pH. Spore germination, growth and toxin production were not delayed at pH values >6.2. Such a pH effect might partially explain the finding by Hargett et al. (1980) that SAPP does not negatively affect the level of residual nitrite in frankfurters.

The relationship between iron availability, its reduction by chelating compounds and the antibotulinal effectiveness of nitrite in processed meats, therefore, is well-documented. In a study with "low" pH meat (5.5-6.3) pork slurries, Roberts et al. (1981a) found no effect on spoilage by sodium nitrite nor by 0.3% of a polyphosphate blend (Curaphos 700), but observed that the phosphates significantly ($P<0.05$) increased toxin production by C. botulinum types A and B; $NaNO_2$ in this case reduced spoilage when increased from 100 to 200 ppm but not to 300 ppm. Identical experiments in pork slurries prepared from "high" pH meat (6.3-6.8) showed that least spoilage occurred when 4.5% NaCl and 0.3% phosphates were introduced, although NO_2/phosphate combinations also reduced spoilage; botulinal toxin production in this case was delayed by phosphates (Roberts et al., 1981b). Duncan and Foster (1968a) reported that nitrite concentrations below 0.06% (pH 6.0) allowed emergence and elongation of Clostridium sporogenes PA 3679 in veal liver agar, but blocked cell division and cells lyzed. Higher

$NaNO_2$ levels and higher pH prevented cell emergence. A second study (Duncan and Foster, 1968b) demonstrated that low nitrite concentrations (0.01-0.20%) stimulated C. sporogenes spore germination, particularly at low pH (<6.0), which made the spores more susceptible to inactivation by heat. Nelson et al. (1983) found 0.4% polyphosphates (SAPP>SPG>STPP) in combination with 40 ppm $NaNO_2$ and 0.20% sorbic acid or 0.26% K sorbate to delay toxin production by C. botulinum in chicken frankfurter emulsions, while phosphates, sorbic acid or K sorbate used alone did not (pH levels in this study were controlled with 1M KH_2PO_4 or K_2HPO_4 prior to cooking). Recent work by Wagner and Busta (1983) with beef/pork frankfurter emulsions inoculated with Clostridium botulinum spores and held at 27°C has shown that formulations containing 0.4% SAPP, 40 ppm $NaNO_2$ and 0.26% potassium sorbate delayed toxin production longer (12-18 days) than any other treatment (6-12 days); aerobic mesophilic counts and residual nitrite, however, showed little difference between formulations (the authors also controlled pH with 1M K_2HPO_4 or KH_2PO_4 and allowed a pH equilibration time of 2 hours between phosphate addition and cooking). The same authors later demonstrated that 0.2 or 0.4% SAPP and 0.13 or 0.26% K sorbate best inhibited growth of 10 strains of C. botulinum in peptone yeast extract glucose broth (PYEG) at pH 5.55 or 5.85, particularly in the absence of NaCl; no SAPP/NaCl interactions were found (Wagner and Busta, 1984). Although the PYEG medium in the latter study was not sterilized by autoclaving but by filtration, the presence of glucose and phosphates in it raise the possibility of superoxide radicals and eventual H_2O_2 formation studied

by Carlsson et al. (1978), while combinations of hydrogen peroxide and sorbic acid were similarly shown by Miller (1969) to yield superoxide and H_2O_2. Other experiments in prereduced PYEG medium have shown that a large variety of chemical compounds which inhibited growth of C. botulinum from inoculated spores were effective at lower concentrations in the presence of phosphate buffer (0.05M, pH 7.0 or 6.0) (Reddy and Pierson, 1982).

A complex pattern of conceivable phosphate bacterial inhibition mechanisms, which could take place either individually or simultaneously, can thus be hypothesized based on the available evidence:

a) Phosphate-induced pH changes could be expected to have a direct bearing on other bacterial optimum growth conditions such as temperature, water activity, availability of nutrients and redox potential.

b) Reduced water activity of the growth medium as a consequence of phosphate addition might affect not only bacterial growth itself but also related phenomena such as cell mobility and nutrient diffusion rates.

c) Ionic strength of the growth medium, most affected by the more ionizable phosphate species (ortho-P) and least by the more polymerized SPG, could also negatively influence bacterial growth.

d) Metal ion chelation which is likely to affect the availability of free metal ions necessary for cell wall biosynthesis and other metabolic processes, may bring about cell wall disruption and collapse

if such ions are chelated away from the cell wall by high phosphate concentrations, or otherwise impair cell wall permeability and nutrient transport characteristics. Most importantly, chelation may reversibly or fatally impair normal cell enzymatic processes. In the latter case, phosphate-induced superoxide and peroxide formation in the growth medium, along with bacterial superoxide dismutase and/or catalase synthesis repression through damaged cell respiration could lead to microbial death, as well as sensitize bacteria to other damaging factors or compounds. Also, penetration of lipophilic phosphates into cells, chelation of iron away from heme-containing proteins, lesion of the cytochrome system with subsequent effects on ATP and protein syntheses, are all possible. Impaired cell respiration could even result from release of bacterial mitochondria from the cytoplasm by phosphate removal of metal-linked barriers that hold the mitochondria within the cells. Aside from these physical effects of metal ion chelation, there is a distinct possibility of enzymatic activity disruption by cofactor metal ion chelation, which may be an important component of bacterial inhibition by alkaline phosphates.

e) Finally, enzymatic inhibition by poly- and pyrophosphates through their common breakdown end product, i.e., orthophosphate, may be another possibility. Exogenous ortho-P could repress synthesis of polyphosphate kinase in bacteria, hence affecting the formation of ATP from ADP and internal polyphosphate, which that enzyme catalyzes. Considering that SAPP addition lowers the pH of meat to values nearly optimum for meat acid phosphatase activity, SAPP breakdown to ortho-P

would be expected to take place at a high rate and, through ortho-P feed-back enzymatic repression, bacterial inhibition by SAPP might differ from that effected by alkaline phosphates.

Additional research is needed to determine whether the antimicrobial properties that poly- and pyrophosphates reportedly exhibit in laboratory media, could effectively and safely be used to control spoilage and pathogenic microorganisms frequently associated with meat and meat products. Further, research oriented toward elucidating the apparently conflicting findings on the antibacterial activity of phosphates in fresh and cooked meats seems justified, particularly in view of the ever-present danger of botulism and the general consensus on the health-related need to reduce sodium nitrite levels in meat products. Lastly, the study of the antibacterial characteristics of sodium acid pyrophosphate, so far the most promising antibotulinal adjunct of nitrite among the phosphates allowed in meats, as opposed to those of alkaline phosphates could be of great significance from economic considerations.

Explanation of dissertation format

The dissertation is divided in six parts, each being a complete paper already published (Parts I and II) or accepted for publication by (Part III), submitted (Parts IV and V) or to be submitted (Part VI) to, a professional journal. Part I examines the effect of 0.5% poly- and pyrophosphates on the survival and growth of selected bacteria in laboratory media, as well as the differences in the inhibitory

effectiveness of those compounds attributable to heat treatment. Part II examines the activity of food-grade phosphates on the survival and growth of an inoculated pathogen (S. aureus) and of the naturally occurring bacterial flora of uncooked, bratwurst-type sausage during refrigerated storage. Part III examines the effect of poly- and pyrophosphates on the survival and growth of inoculated Clostridium sporogenes PA3679 and natural aerobic and anaerobic bacteria in cooked, vacuum packaged bratwurst-type sausage under refrigerated and abuse temperature holding. Part IV focuses on the study of sodium acid pyrophosphate and sodium tripolyphosphate at a level of 0.5%, on the natural aerobic and anaerobic bacterial populations and on inoculated C. sporogenes PA3679 in similarly cooked, vacuum packaged bratwurst, with or without sodium nitrite added at low (50 ppm) or higher (100 ppm) levels. Part V examines the adaptation of a colorimetric method for measuring orthophosphates in meat and meat products, a necessary tool for the study of pyrophosphate hydrolysis in meats and its possible relationship to bacterial inhibition by such compounds. Part VI is a joint study by the Iowa State University Food Technology and Animal Science Departments. The study examines the microbiological and some physical, chemical and organoleptic effects of 0.4% addition of pure phosphates (sodium tripolyphosphate or tetrasodium pyrophosphate), or phosphate blends of unknown composition, to frozen ground beef patties.

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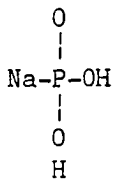
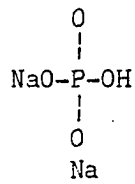
Orthophosphates

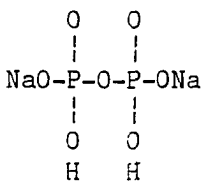


Monosodium orthophosphate

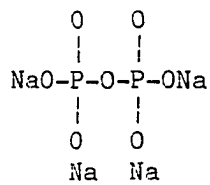


Disodium orthophosphate

Pyrophosphates

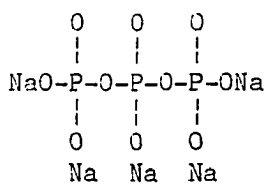


Sodium acid pyrophosphate

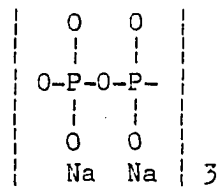


Tetrasodium pyrophosphate

Polyphosphates



Sodium tripolyphosphate



Sodium polyphosphate, glassy

Figure 1. Structure and classification of some ortho-, poly- and pyrophosphates allowed in meat and poultry products

1) $Na_5P_3O_{10} + H_2O$ ---------- $Na_3HP_2O_7 + Na_2HPO_4$

Sodium tripoly-P (STPP) — Trisodium pyro-P — Disodium ortho-P

2) $Na_2H_2P_2O_7 + H_2O$ -------- $2\ Na_2HPO_4$

Disodium pyro-P (SAPP) — Monosodium ortho-P

3) $Na_3HP_2O_7 + H_2O$ --------- $Na_2HPO_4 + NaH_2PO_4$

Trisodium pyro-P — Disodium ortho-P — Monosodium ortho-P

4) $Na_4P_2O_7 + H_2O$ --------- $2\ Na_2HPO_4$

Tetrasodium pyro-P — Disodium ortho-P

5) $3\ (NaPO_3)_6 + 12\ H_2O$ ---- $2(NaPO_3)_3 + 12\ NaH_2PO_4$

Sodium poly-P glassy (SPG) — Trimeta-P — Monosodium ortho-P

$+ H_2O$

$Na_3H_2PO_4$

Triphosphate

$+ 2\ H_2O$

$3\ NaH_2PO_4$

Monosodium ortho-P

Figure 2. Hydrolysis of poly- and pyrophosphates in aqueous solutions

PART I. RECOVERY OF SELECTED BACTERIA IN MEDIA CONTAINING 0.5% FOOD GRADE POLY- AND PYROPHOSPHATES

by

R. A. Molins, A. A. Kraft, D. G. Olson and D. K. Hotchkiss

Departments of Food Technology and Animal Science

Iowa State University

Ames, Iowa 50011

Running Title: Phosphate inhibition of meat bacteria

ABSTRACT

Pure cultures of Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus and two lactic starters were plated with laboratory media containing 0.5% of a food grade poly- or pyrophosphate. Heated or unheated tetrasodium pyrophosphate (TSPP) was highly inhibitory or lethal to all cultures tested, followed by unheated sodium tripolyphosphate (STPP) and sodium polyphosphate glassy (SPG), while sodium acid pyrophosphate (SAPP) was not inhibitory or enhanced recoveries. Heating reduced the effectiveness of phosphates as inhibitors.

INTRODUCTION

The approval of certain poly- and pyrophosphates as additives for use in a wide variety of processed meat products, granted by the USDA in 1982, has brought a considerable amount of research concerning physical and chemical effects of these compounds in relation to product quality characteristics such as texture, color, emulsion stability and water holding capacity. However, little work has been done on the microbiological effects of poly- and pyrophosphates in cured or otherwise processed meats. Earlier work has related to poultry processing. Spencer and Smith (1962) showed that chilling chicken fryers in a solution of polyphosphates for 6 h increased shelf life and decreased the rate of microbial spoilage. These results were confirmed by those of Steinhauer and Banwart (1963) in a first experiment, but could not be repeated in a second trial. Elliott et al. (1964), however, demonstrated that polyphosphates inhibited the growth of fluorescent pseudomonads in a synthetic medium. Subsequent growth of fluorescent strains after a short lag led these authors to suggest that chelation of metal ions essential for bacterial growth best explained the inhibitory activity of polyphosphates, a theory advanced earlier by Post et al. (1963) and Gray and Wilkinson (1965) for sodium hexametaphosphate (now sodium polyphosphate glassy, soluble). Garibaldi et al. (1969) also supported the metal ion chelation theory as a result of their research with S. typhimurium and S. senftenberg in egg white, as did that of Kohl (1971) in his patented process for egg white

pasteurization. Chen et al. (1973), using polyphosphate solutions to soak chicken parts prior to precooking demonstrated their effectiveness against gram-positive micrococci and staphylococci. Death rates of salmonellae were greater in chicken breast muscle stored at -2°C with addition of polyphosphate than they were at -20°C, but little effect was observed at 1°C or -5°C (Foster and Mead, 1976). More recently, Firstenberg-Eden et al. (1981) found sodium tripolyphosphate to inhibit colony formation by heat-stressed cells of Moraxella-Acinetobacter. A possible synergism between sodium acid pyrophosphate, potassium sorbate and sodium nitrite in delaying toxin production by C. botulinum in frankfurters has recently been reported by Wagner and Busta (1983).

The present study was intended to observe effects of heated and unheated food grade poly- and pyrophosphates on the survival and colony forming capacity of selected bacteria in laboratory media, with the possibility of applying this information to the microbial flora of cured and processed meats.

MATERIALS AND METHODS

Cultures

Pure cultures of Salmonella typhimurium, Pseudomonas aeruginosa and Staphylococcus aureus Z88 (Microbiology and Food Technology Departments, ISU) and two commercially available, frozen lactic starter cultures were used as inocula for preparing dilutions according to standard procedures. Twenty-four and 3-h old cultures were used to test possible differences in susceptibility attributable to age.

Media

Solutions containing 10% (w/w) of the selected poly- and pyrophosphates in distilled water were prepared immediately prior to use and sterilized by filtration through a 22 μ Millipore membrane (Millipore Corp., Bedford, MA). The phosphates chosen were: sodium acid pyrophosphate (SAPP), sodium polyphosphate glassy, soluble (SPG) (Stauffer Chemical Co., Westport, CT), tetrasodium pyrophosphate (TSPP) and sodium tripolyphosphate (STPP) (Monsanto, St. Louis, MO). Trypticase soy agar (TSA, BBL) was used for plating S. typhimurium, P. aeruginosa and S. aureus, while LBS agar (BBL) was used for the lactic starter cultures. Appropriate volumes of the sterile 10% solutions of poly- and pyrophosphates were added to the media prior to sterilization by autoclave (for TSA) or boiling (for LBS), in order to attain the

desired heated phosphate treatment, or after sterilization or boiling and cooling the media to 48°C in a water bath. The latter was labeled as the unheated phosphate treatment. In all cases, the volume of phosphate solution added to the media was calculated so as to obtain a final concentration of 0.5%. Dilutions of the 3 or 24-h cultures were plated and incubated at 37°C (S. typhimurium, P. aeruginosa, and S. aureus) or 30°C (lactic starters). Control plates were prepared in all cases with media that contained no phosphates and used as a reference index (100% recovery). The number of colony forming units (CFU) was determined after 48, 72 and 96 h of incubation, and the counts were calculated to give percent recovery with the control reference index as base. All experiments were replicated five times. The data were analyzed using an SAS computer program with ANOVA and GLM procedures.

RESULTS AND DISCUSSION

Bacterial counts were significantly higher ($P<0.05$) after 72 or 96 h of incubation than after only 48 h, but such increases, even though large enough for statistical significance, seldom represented more than 30% of the original inoculum. A consistent trend toward decreased bacterial colony size in media containing poly- and pyrophosphates was noticed, to a point where accurate counts at 48 h were impaired. Defective cell division as reported by Seward et al. (1982) might explain changes in colony size and morphology.

The extent of failure to form colonies varied for every microorganism, phosphate and treatment (heated or unheated phosphate), as shown in Table 1. S. typhimurium and P. aeruginosa were less inhibited by phosphates than S. aureus and the lactic starters, which is in agreement with the relationship between Gram reaction and phosphate susceptibility of bacteria reported by other investigators (Chen et al., 1973; Elliott et al., 1964; Post et al., 1963).

TSPP was found to be the most effective inhibitor of bacterial colony formation among the phosphates tested, and the only phosphate that consistently retained its inhibitory activity upon heating. Twenty-four h old cultures of S. typhimurium and P. aeruginosa, were less inhibited than 3 h cultures, but were nevertheless reduced in CFU numbers to 27-49% of control plates. All other cultures were almost totally inhibited by heated or unheated TSPP. The continued inhibitory activity of TSPP after being heated to temperatures of 100°C+ is

consistent with the known stability of pyrophosphates to high-temperature induced hydrolysis (Bell, 1947). SPG was highly inhibitory to Gram-positive cultures of either age and mildly inhibitory to Gram-negative S. typhimurium and P. aeruginosa, but its activity was lost upon heating, as was that of STPP for all the cultures except the lactics. SAPP was found to be only slightly inhibitory to 24-h old cultures in its unheated form, while it enhanced rather than inhibited recoveries (particularly of younger cultures) when present in heated form.

These results indicate that TSPP may be effective for microbiological applications in processed meat products. Initial inhibition of bacterial growth by the unheated form of the phosphate added to emulsions or coarse-ground mixes prior to stuffing and subsequent processing could later be continued in the cooked product by the heated TSPP.

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Table 1. Mean percent recovery of selected bacteria in laboratory media containing 0.5% poly- or pyrophosphates after 72 hours of incubation[a]

Treatment		Microorganism and culture age (hrs)									
		S.typhimurium		P.aeruginosa		S.aureus		Lactic 1		Lactic 2	
		24	3	24	3	24	3	24	3	24	3
TSPP	H[b]	49	1	48	12	<1	<1	<1	<1	<1	<1
	U[c]	27	3	34	2	<1	<1	2	5	1	7
SPG	H	108	105	93	88	87	101	87	41	39	19
	U	71	49	107	75	<1	<1	<1	<1	<1	<1
STPP	H	91	39	100	81	89	105	86	54	104	54
	U	42	3	56	10	<1	<1	75	3	85	4
SAPP	H	96	134	115	130	82	118	118	84	108	95
	U	73	87	74	121	87	114	84	114	116	100

[a] *S. typhimurium*, *P. aeruginosa*, *S. aureus*: TSA, 37°C. Lactics: LBS, 30°C.

[b] Heated.

[c] Unheated.

PART II. EFFECT OF PHOSPHATES ON BACTERIAL GROWTH IN REFRIGERATED UNCOOKED BRATWURST[1]

by

R. A. Molins, A. A. Kraft, and D. G. Olson
Departments of Food Technology and Animal Science
Iowa State University
Ames, Iowa 50011

Running Title: Phosphate inhibition of meat bacteria.

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ABSTRACT

The effects of 0.5% sodium acid pyrophosphate (SAPP), sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP) and sodium polyphosphate glassy (SPG) on aerobic mesophilic and psychrotrophic bacterial growth and on survival of inoculated Staphylococcus aureus Z88 were investigated in uncooked bratwurst stored at 5°C for 7 days. No significant microbial inhibition by phosphates was found, although SAPP addition resulted in consistently lower total aerobic plate counts. Phosphate-induced pH differences in the sausages had no effect on bacterial numbers. The possible role of meat enzymes in the hydrolysis of condensed phosphates to microbiologically inactive species is discussed.

INTRODUCTION

Antimicrobial effects of phosphates in poultry products was well-documented in earlier work of Spencer and Smith (1962), Steinhauer and Banwart (1963), and Chen et al. (1973) who demonstrated that dipping chicken parts or carcasses in phosphate-containing chilling water resulted in decreased bacterial counts and prolonged shelf life. Bacterial growth inhibition in laboratory media has been attributed to phosphates by Post et al. (1963), Elliott et al. (1964), Gray and Wilkinson (1965) and more recently by Snyder and Maxcy (1979), Firstenberg-Eden et al. (1981) and Seward et al. (1982). In this laboratory, 0.5% tetrasodium pyrophosphate (TSPP) was lethal or highly inhibitory to Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium and two commercial lactic starter cultures in laboratory media. Unheated sodium tripolyphosphate (STPP) and sodium polyphosphate glassy (SPG) were not as inhibitory and lost their antimicrobial properties when heated to 100 + ^{o}C. Unheated sodium acid pyrophosphate (SAPP) was only slightly inhibitory to the microorganisms tested and enhanced rather than decreased recoveries in the heated form. Furthermore, in most experiments, 3-hr old cultures and the Gram positive organisms used were more sensitive to phosphates than were older cultures (24-hr) and Gram negative bacteria (Molins et al., 1984).

Renewed interest in the applicability of phosphates as bactericidal or bacteriostatic compounds was raised by their approval as additives for use in a wide variety of cooked meat products by the USDA in 1982.

Although the physical and chemical contributions of phosphates to meat product quality with regard to texture, improved water holding capacity and binding, increased emulsion stability and other factors reviewed by Knipe (1982) have been determinant in their use and approval, only limited attention has been paid to the potential antimicrobial application of phosphates to meats and meat products. Ivey and Robach (1978) found SPG and SAPP to have no significant effects on the growth and toxin production by Clostridium botulinum when used alone or combined with nitrite in canned comminuted pork, but to have synergistic effects when sorbic acid was present. These findings were later confirmed with SAPP by Roberts et al. (1981), Wagner and Busta (1983), and Nelson et al. (1983). Nielsen and Zeuthen (1983) reported that 0.3% STPP alone had little influence on the growth of Brochothrix thermosphacta or Serratia liquefaciens in sliced, vacuum packed bologna under refrigerated storage (2°C), but that an acid mixture of SAPP, STPP and SPG markedly inhibited the growth of both spoilage microorganisms under the same conditions.

The present work was undertaken to determine effects of the highest legally allowed level (0.5%) of two food-grade poly- and two pyrophosphates commonly used in the meat industry on the survival of S. aureus and on total mesophilic and psychrotrophic bacterial counts in refrigerated, uncooked bratwurst-type sausages. Although the use of phosphates in uncooked meat products is not permitted, bratwurst was

selected for the present phase of a series of studies since this product is unique insofar as it is marketed in uncooked as well as cooked, vacuum packed forms and usually does not include nitrite in its formulation.

MATERIALS AND METHODS

Bratwurst was prepared from coarse ground pork butts (20% fat) obtained in frozen, 12-lb packages from the Iowa State Meat Laboratory. The meat was allowed to thaw 48 h at 2-4°C and the drip was reincorporated into the meat by blending with a Hobart C-10 mixer (The Hobart Mfg. Co., Troy, OH) for 2 minutes at low speed. Bratwurst spice mix (2%, w/w) (Saratoga Specialties, Elmhurst, IL) was similarly blended in, providing a concentration of 1.7% NaCl. Portions weighing 1000 g received the appropriate volume of a 10% aqueous solution (w/v) of one of the test phosphates to attain a level of 0.5% phosphate in the final product. After further blending, each 1000-g portion of phosphate-containing sausage mix was subdivided. One-half was inoculated with a diluted, 24-hr culture of S. aureus Z88 (Food Technology Department, ISU) grown in Brain Heart Infusion (BHI) broth (BBL) so as to number ca. 10^4 cells/gram while the other half received an equivalent amount of sterile distilled water. The final blending was followed by stuffing into 1-inch dia., edible collagen casings (Devro, Inc., Somerville, NJ). All sausages were packaged in polyethylene bags, twist-tied and stored in a display case at about 5°C. Inoculated and uninoculated controls were prepared with and without phosphates. Samples were taken on days 0, 1, 3, 5 and 7 for microbiological examination. Thirty g of sausage were weighed, blended with 270 ml of 0.1% peptone water, diluted and plated following standard methods. The numbers of surviving S. aureus were determined from inoculated samples (Baird-Parker medium, BBL, 24 h

at 37°C) and total mesophilic and psychrotrophic counts (Trypticase soy agar, TSA, BBL, 30°C and 5°C, respectively) were obtained from uninoculated sausages. The experiments were replicated four times and after logarithmic transformation the data were analyzed by using an SAS computer program with ANOVA and GLM procedures.

RESULTS AND DISCUSSION

None of the phosphates tested proved to have a significant ($P<0.05$) effect on the survival of inoculated S. aureus. S. aureus decreased in numbers by less than one log cycle throughout the 7-day storage period at 5°C for all samples including controls. This result is in sharp contrast with the lethality exhibited by 0.5% unheated TSPP, STPP or SPG on S. aureus when these phosphates were present in a laboratory medium in our previous work (Molins et al., 1984).

The growth of mesophilic (Fig. 1) and psychrotrophic (Fig. 2) bacteria was not significantly ($P>0.05$) reduced by any phosphate. However, SAPP and STPP caused a slight lengthening of the lag phase of growth of mesophiles. SAPP also resulted in consistently lower numbers of aerobic mesophilic and psychrotrophic bacteria than any other phosphate or controls throughout the experimental period, but these effects were not statistically significant ($P>0.05$).

The presence of the enzymes, poly- and pyrophosphatases in muscle cells may account for the loss of the inhibitory properties that condensed phosphates have on bacterial growth in systems other than meat, where no such enzymes are present. Rapid hydrolysis of poly- and pyrophosphates to the ortho forms and the important roles of pH, temperature, time and inactivation of phosphate-hydrolyzing enzymes by cooking temperatures on the retention of the active, unhydrolyzed forms of phosphates was demonstrated by Awad (1968). With ground or comminuted meat products and systems, additional release of phosphatases

by muscle cell rupture during grinding may further increase the rate of phosphate hydrolysis. Other complex interactions, such as those between phosphates and meat proteins (Nikkila et al., 1967; Newbold and Tume, 1981), may also explain the failure of phosphates as bacterial inhibitors in uncooked, processed meats.

Marked pH differences between bratwurst containing different phosphates existed throughout the 7-day storage period at 5°C without corresponding variations in bacterial numbers. Also, pH differences between treatments decreased only slightly after one week of storage when final bacterial counts were in excess of 10^8 CFU/gram.

Additional research is justified on possible effects of immediate cooking after formulation for retention of the microbial inhibitory properties of phosphates in meat products.

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

Figure 1. Effect of 0.5% phosphates on total mesophilic counts in uncooked bratwurst

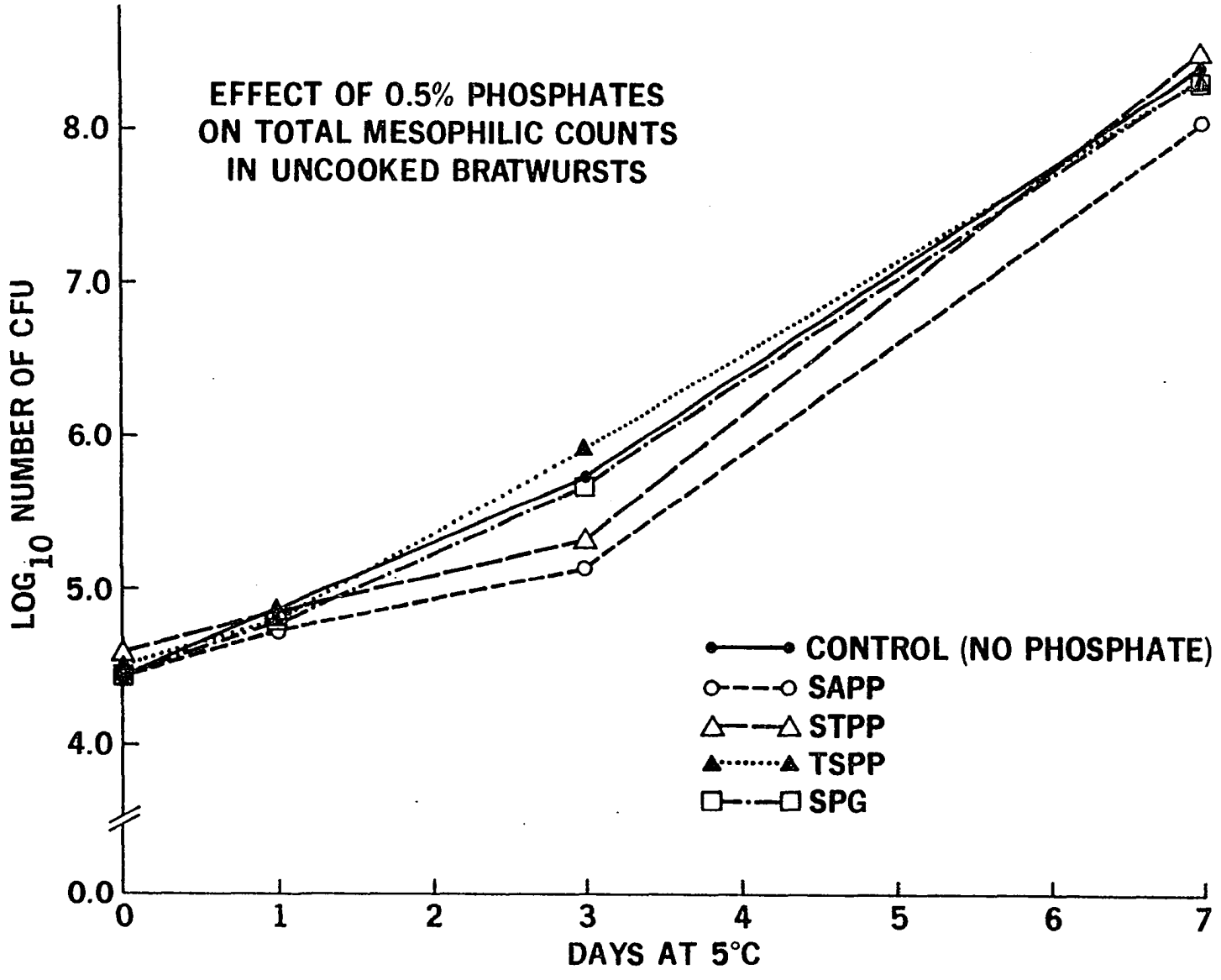
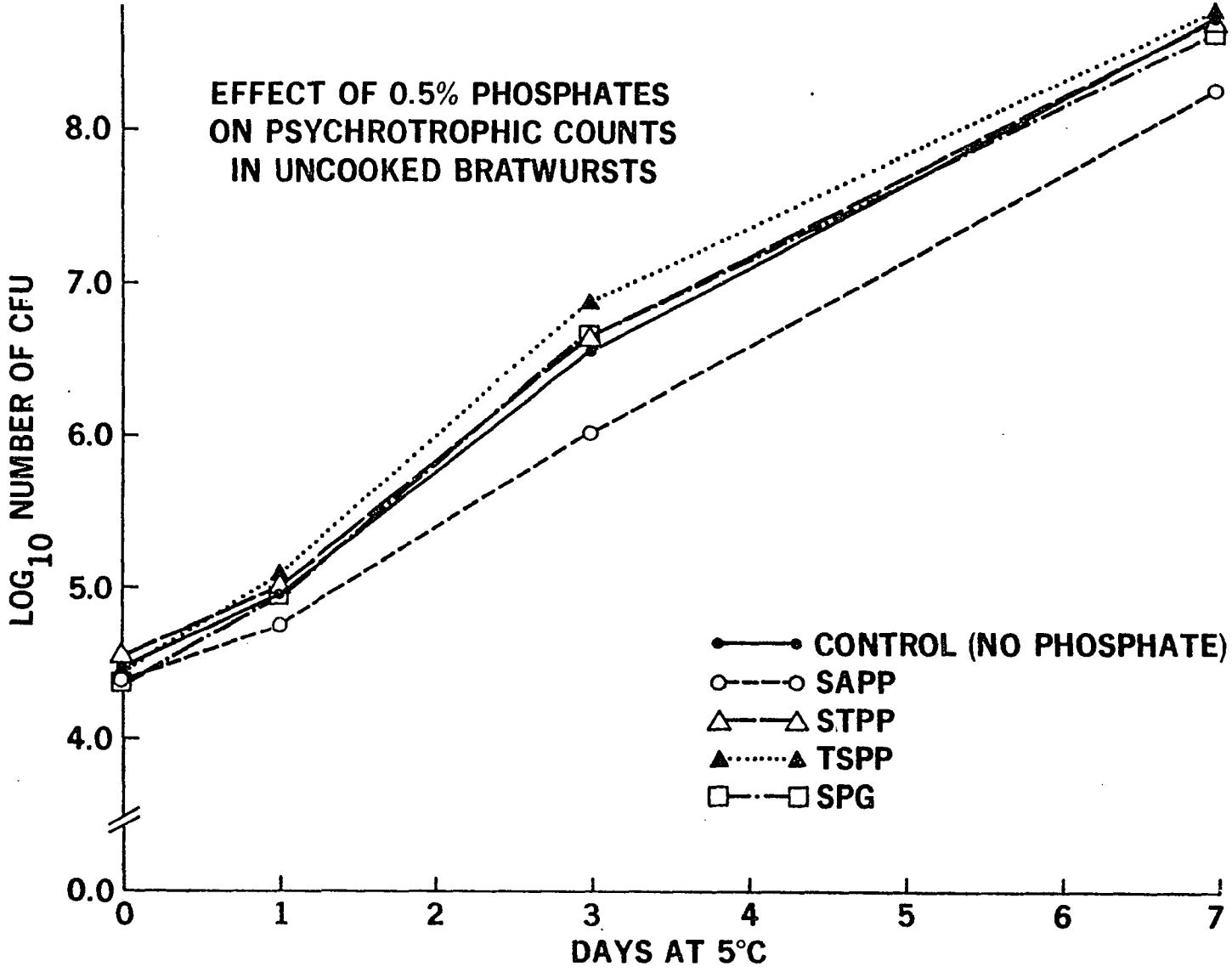

EFFECT OF 0.5% PHOSPHATES
ON TOTAL MESOPHILIC COUNTS
IN UNCOOKED BRATWURSTS
LOG10 NUMBER OF CFU
8.0
7.0
6.0
5.0
4.0
0.0
0
1
2
3
4
5
6
7
DAYS AT 5°C
CONTROL (NO PHOSPHATE)
SAPP
STPP
TSPP
SPG

Figure 2. Effect of 0.5% phosphates on psychrotrophic counts in uncooked bratwurst


EFFECT OF 0.5% PHOSPHATES
ON PSYCHROTROPHIC COUNTS
IN UNCOOKED BRATWURSTS
LOG10 NUMBER OF CFU
8.0
7.0
6.0
5.0
4.0
0.0
0
1
2
3
4
5
6
7
DAYS AT 5°C
CONTROL (NO PHOSPHATE)
SAPP
STPP
TSPP
SPG

PART III. EFFECT OF POLY- AND PYROPHOSPHATES ON THE NATURAL BACTERIAL FLORA AND INOCULATED CLOSTRIDIUM SPOROGENES PA 3679 IN COOKED VACUUM PACKAGED BRATWURST

by

R. A. Molins, A. A. Kraft, H. W. Walker and D. G. Olson
Departments of Food Technology and Animal Science
Iowa State University
Ames, Iowa 50011

Running Title: Phosphate inhibition of bacteria in cooked bratwurst.

ABSTRACT

Survival and growth of inoculated Clostridium sporogenes PA 3679 and of natural aerobic and anaerobic bacterial flora were studied in cooked, vacuum packed bratwurst containing 0.5% phosphates during refrigerated (5°C) and subsequent temperature abuse storage (24°C). Sodium acid pyrophosphate (SAPP), sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP) and sodium polyphosphate glassy (SPG) were tested. No significant ($P>0.05$) bacterial inhibition by any phosphate was observed during refrigerated storage, nor was there appreciable growth in the control bratwurst. However, SAPP significantly ($P<0.05$) inhibited aerobic and anaerobic bacteria (including C. sporogenes) upon temperature abuse, followed in effect by TSPP and STPP. Cooking to 65.5°C helped retain antimicrobial properties of phosphates to some extent. Enzymatic hydrolysis of phosphates is postulated as a major factor in loss of antimicrobial properties of phosphates in processed meats.

INTRODUCTION

Renewed interest in the possible use of phosphates as antimicrobial agents in meat products has derived from the search for compounds that may enable the meat industry to substitute or reduce amounts of long-accepted additives such as nitrites and sodium chloride. Protection must still be provided against the growth of pathogenic bacteria and possible production of microbial toxins. The approval given to the use of certain poly- and pyrophosphates in processed meats by the USDA in 1982 and the information available on the chemical and physical advantages derived from their use have resulted in the inclusion of phosphates in a large variety of meat products (Knipe, 1982).

In the microbiological field, however, the effects of phosphate addition to meat products is still a subject of some controversy. In studies with vacuum packed, sliced berliner sausage, Mol et al. (1971) found that the presence of 0.45% polyphosphates did not affect the growth of unclassified Streptobacterium LL3 during storage of 12°C. Recent work by Nielsen and Zeuthen (1983) with vacuum packed, sliced bologna to which a low pH mixture of sodium tripolyphosphate (STPP), tetrasodium pyrophosphate (TSPP) and sodium polyphosphate glassy (SPG) (0.3%) or STPP alone had been added in solution, showed that the blend had no influence on the growth of an atypical Streptobacterium inoculated on the surface, but had a pronounced inhibitory effect on similarly inoculated Brochothix thermosphacta and Serratia liquefaciens, while STPP alone was ineffective. Wagner and Busta (1983) observed that

sodium acid pyrophosphate (SAPP) delayed toxin production by Clostridium botulinum for 12-18 days when used in combination with 40 ppm of sodium nitrite and 0.26% potassium sorbate in beef/pork frankfurter emulsions at 27°C, but that mesophilic bacterial counts were no different from those in the absence of phosphates. Earlier experiments by Ivey and Robach (1978) involving canned comminuted pork showed no significant interactions between SAPP or SPG concentrations and sodium nitrite in preventing C. botulinum outgrowth and toxin production, but a synergistic relationship was found between those phosphates and potassium sorbate. The authors suggested that botulism-safe meat products could be produced without $NaNO_2$. Other researchers have reached similar conclusions in studies with pork slurries prepared from high pH meat (6.3-6.8) but not with slurries from meat in the pH range 5.5-6.3, in which 0.3% of a polyphosphate blend enhanced toxin production by C. botulinum (Roberts et al., 1981a,b).

Our work with uncooked refrigerated bratwursts packed in air tight bags and kept for seven days at 5°C led us to conclude that 0.5% SAPP, STPP, TSPP or SPG are ineffective as inhibitors of the natural aerobic mesophilic or psychrotrophic spoilage flora of uncooked bratwurst or against inoculated Staphylococcus aureus (Molins et al., 1985). The contrast between these negative findings and the strong inhibitory properties exhibited by phosphates on the growth of bacteria in less complex laboratory media as we reported earlier (Molins et al., 1984) and as stated by other authors (Post et al., 1963; Gray and Wilkinson, 1965; Seward et al., 1982; Firstenberg-Eden et al., 1981) might be due

to multiple interactions between these compounds and meat components. Reports by Awad (1968) and Sutton (1973) have demonstrated that phosphates added to meats and fish are hydrolyzed to orthophosphates by naturally occurring phosphatases. Mild heat treatment of meats to deactivate such enzymes may substantially decrease, though not eliminate, hydrolysis of the phosphates over time.

Consequently, the objective of this study was to determine the effect of commonly used, food-grade phosphates at the maximum level allowed in processed meats (0.5%) on the survival and growth of the natural bacterial flora and inoculated Clostridium sporogenes PA 3679 spores in bratwurst cooked and vacuum packed immediately following preparation.

MATERIALS AND METHODS

Bratwurst Preparation

A 300-lb batch of ground pork butts ground through a 1/8 in diameter plate was obtained from the Iowa State Meat Science Laboratory, packed in 12-lb portions in polyethylene freezer bags, twist-tied, frozen in an air blast freezer to -28°C and kept frozen until needed. Prior to preparation, 12-lb portions were allowed to thaw for 48 h in a walk-in cooler at 2-4°C. Bratwurst spice mix (Saratoga Specialities, Elmhurst, IL) was added to the meat and blended in for 5 minutes at medium speed by means of a Hobart C-10 mixer. The spice mix used was 2% (w/w), equivalent to 1.7% NaCl in the product. Portions of 1000 g of meat were weighed and each received 53 ml of a 10% (w/v) aqueous solution of a single phosphate previously sterilized by filtration through a 22 μ Millipore membrane filter (Millipore Corp., Bedford, MA), to obtain the desired concentration of 0.5%, the maximum limit allowed by the U.S.D.A., in the product. The phosphates used were sodium acid pyrophosphate (SAPP), sodium polyphosphate glassy (SPG) formerly known as sodium hexametaphosphate, (Stauffer Chemical Co., Westport, CT), tetrasodium pyrophosphate (TSPP) and sodium tripolyphosphate (STPP) (Monsanto, St. Louis, MO). Sterile distilled water was added to one of the 1000-g portions to be used for preparation of control sausages. After being blended for one minute at each low and high speeds in a Kitchen-Aid Model 4 mixer, the 1000 g batches were divided in half; one

was inoculated and the second received an amount of sterile distilled water equal to the volume of the inoculum added to the first portion. The inoculum consisted of 10 ml Clostridium sporogenes PA 3679 spore suspension in sterile, deionized water prepared according to the procedure outlined by Swank (1971) and targeted to give approximately 1000 spores/g sausage. The inoculum was introduced into the meat by blending, as described above. The culture was obtained from the ISU Food Technology Department collection and selected for rhizoid colony formation to facilitate differentiation from other anaerobic bacteria during plate counting. Uninoculated controls were balanced for added water as specified. The mixes were stuffed into 1 in-dia edible collagen casings (Devro, Inc., Somerville, NJ) by means of an Oster Food and Meat grinder (Oster, Milwaukee, WI). Immediately after stuffing and tying, each batch of sausages was cooked to an internal temperature of 65.5°C in a separate 3000-ml beaker containing distilled water and kept at 80°C in a Magni Whirl Constant Temperature Bath (Blue M Electric Co., Blue Island, IL) equipped with bottom paddle agitation. The cooking end-point was determined by inserting a copper-constantan thermocouple longitudinally though one bratwurst of each batch and monitoring the internal temperature with a Hewlett Packard 3476A Multimeter Potentiometer. Average cooking time was 11 min. Cooking losses that could have affected the level of phosphates were previously determined and found to be negligible (0.64 to 1.1%). After cooking, the bratwurst was wrapped in aluminum foil, rapidly cooled to an internal temperature of 4-5°C, separated and placed in small, individual, polyethylene bags

to avoid cross-contamination. One sausage from each treatment, for a total of five sausages, was placed in Curlon 892 (Curwood Inc., New London, WI) vacuum packaging bags (O_2 permeability < 1 ml/645 cm^2/24 h at 22.8°C and 0% RH) and vacuum sealed in a Multivac MG-2 packaging machine. Inoculated and uninoculated bratwurst were packed separately. The bags were stored for seven days in a display case at about 5°C, followed by 48 h of temperature abuse (24-25°C). The order of batch preparation was randomly assigned, and all experiments were replicated three times.

Sampling

For each of the three replications, one package each of inoculated and uninoculated sausages was taken from storage on days 0, 1, 3, 5 and 7 and on days 8 and 9 (corresponding to 24 and 48 h of temperature abuse at 24°C) for microbiological examination. The sausage was weighed in 30 g amounts and blended in a jar with 270 ml 0.1% sterile peptone water for 30 seconds at each of low and high speeds in an Osterizer blender. Serial dilutions were then made according to standard methods. Trypticase soy agar (TSA, BBL) was used to plate aerobic mesophilic (48 h, 30°C) and psychrotrophic (10 days, 5°C) bacterial populations. TSA was also used for total anaerobic counts, while TSA containing 0.1% soluble starch (Fisher Scientific Co., Fair Lawn, NJ) was the medium used to determine anaerobic viable spore counts after heat shocking the appropriate dilutions at 80°C for 20 minutes (Swank, 1971; Neal, 1976).

BBL Gas Pak Anaerobic Systems were used to obtain anaerobic incubation conditions (48 h, 37°C). C. sporogenes PA 3679 vegetative cell and viable spore counts were determined from the same plates used for total anaerobic and total viable spore counts, respectively, by counting typical rhizoid colonies characteristic of the culture used as inoculum, and which was determined to be a stable property. In order to confirm the identity of the typical rhizoid colonies, 10 isolated colonies were randomly picked from counted plates at each sampling time, examined under the microscope and subcultured in cooked meat medium (BBL, 48h at 37°C). Observations were made for gas production, proteolysis, and single terminal spores. No typical rhizoid colonies were observed from uninoculated bratwurst. The pH of the sausages was determined throughout the sampling period using a Radiometer 28 meter equipped with an Orion 9163 probe. Sampling order was randomized among treatments.

Plate count data were transformed to logarithms and analyzed by means of an SAS program with GLM option. Comparison of means was based on Duncan's multiple range test.

RESULTS

Figs. 1 and 2 show mesophilic and psychrotrophic bacterial counts in phosphate-containing samples. Bacterial numbers were not significantly different ($P>0.05$) from those of controls (no phosphate) throughout the 7-day refrigerated storage period. However, upon temperature abuse (24°-25°C), SAPP significantly ($P<0.05$) inhibited the growth of both mesophiles and psychrotrophs, limiting their numbers to less than 10^5 CFU/g of sausage even after 48 h at 24°C. SAPP was equally effective in inhibiting naturally occurring ("wild") anaerobes (Fig. 3), which were similarly inhibited by TSPP during the first 24 h of temperature abuse. SPG proved to be ineffective as an inhibitor of aerobic mesophiles, psychrotrophs or "wild" anaerobic bacteria, while TSPP provided no stable pattern of inhibition in any case.

Anaerobic growth curves obtained from samples inoculated with *C. sporogenes* PA 3679 are shown in Fig. 4. The addition of 0.5% SAPP resulted in significantly ($P<0.05$) lower numbers of total anaerobes as well as populations of *C. sporogenes* PA 3679 than those found in control sausages (Figs. 4 and 5, respectively) during the entire refrigerated storage period at 5°C. However, differences among phosphate treatments were not significant ($P>0.05$). After 24 h of elevated temperature (24°-25°C), all sausages formulated with STPP and TSPP contained significantly ($P<0.05$) lower numbers of "wild" anaerobes and PA 3679 vegetative cells than those with SPG or no phosphates, while SAPP allowed no anaerobic growth during that time. Prolonged holding at 24°-

25°C produced more difference, after 48 h, only SAPP remained strongly and significantly ($P<0.05$) inhibitory to all bacteria tested, although TSPP was most effective as an inhibitor of C. sporogenes PA 3679 at that time (Fig. 5). Fig. 5 shows approximately 10^5 organisms per gram initially, which includes vegetative cells as well as spores.

Viable spore counts in uninoculated bratwurst did not differ greatly during storage at 5°C (Fig. 6), and no significant ($P>0.05$) effects on "wild" anaerobic spore viability could be attributed to any phosphate tested during refrigerated storage at 5°C. After 24 h of temperature abuse (24°-25°C), however, SAPP-containing samples had higher total anaerobic spore counts than controls but the difference was not significant ($P>0.05$). At that time, use of SPG and SAPP also resulted in slightly higher numbers of PA 3679 viable spores than for controls (Fig. 7). At 48 h of abuse temperature, control sausages had significantly ($P<0.05$) higher total viable, but not PA 3679 spore counts than sausages treated with any of the phosphates, as shown in Fig. 6 compared with Fig. 7.

A comparison was made of bacterial and spore numbers prior to and after cooking the bratwurst to an internal temperature of 65.5°C. No significant ($P>0.05$) differences were observed among treatments, indicating that phosphates did not affect the heat resistance of vegetative cells or that of spores.

Determinations of pH demonstrated a strong buffering capacity by all phosphates (Table 1), since the pH values of bratwurst containing

phosphates decreased by only 0.02-0.15 unit even after 48 h at 24°C. At that time most bacterial counts were about 10^7-10^8 CFU/g of sausage (except for sausage containing SAPP) and the pH of control samples had decreased by 0.25-0.55 unit.

DISCUSSION

The effects attributable to the various phosphates tested are related to heating, storage temperatures and times. On day 0, cooking the bratwurst to an internal temperature of 65.5°C significantly ($P<0.05$) decreased vegetative cells and spore counts in all samples, but no additional effect by any phosphate on the heat resistance of vegetative cells or spores was observed.

SAPP caused a significant ($P<0.05$) reduction in numbers of anaerobic bacteria present in inoculated bratwurst during the first day of refrigerated storage at 5°C (Fig. 4). Anaerobic organisms that decreased were C. sporogenes PA 3679, shown in Fig. 5, and not "wild" anaerobes since populations of the latter were not affected in inoculated sausages (Fig. 3). In general, little bacterial growth occurred during refrigerated storage and no significant ($P>0.05$) antimicrobial effect of phosphates was observed during that period.

After 24 h of temperature abuse at 24°C, SAPP, STPP and TSPP significantly ($P<0.05$) decreased anaerobic counts in inoculated bratwurst (by approximately two log cycles) as well as mesophilic and psychrotrophic bacterial numbers. However, only SAPP and STPP were inhibitory to "wild" anaerobes. Viable spore counts were unaffected by STPP or TSPP, but significantly ($P<0.05$) higher numbers of viable C. sporogenes spores were recovered from samples treated with SPG or SAPP. The food safety implication of these findings is that if 24 h of temperature abuse occurred, TSPP and STPP might contribute to inhibition

of clostridia at the spore outgrowth and/or vegetative cell growth stages, while SAPP and SPG possibly inhibit spore germination.

After "temperature abusing" the bratwurst for 48 h, a very strong inhibitory effect on all bacterial growth by SAPP resulted in a reduction of total aerobic and anaerobic counts 3 to 5 log cycles below those of control samples.

The possible contribution of pH to our results cannot be overlooked, since we did not attempt to control it. In studies by Nelson et al. (1983) with 0.4% SAPP, STPP, or SPG, SAPP effectively inhibited growth and toxin production by Clostridium botulinum in temperature-abused (27°C) chicken frankfurter emulsions over a pH range of 5.87-6.19. Ingredients also included 40 ppm sodium nitrite and/or 0.26% potassium sorbate. Similar results were reported by Wagner and Busta (1983) for temperature-abused beef/pork frankfurter emulsions at various pH levels. In terms of enzymatic activity, Awad (1968) found that a pH range of 5.7-7.0 was optimum for tripolyphosphatase, while pyrophosphatase exhibited maximum activity at pH 7.0. He also demonstrated that 100 mg of STPP/100 g of meat would be hydrolyzed in fresh or cooked (80°C) beef homogenates by 47% and 33%, respectively, in less than one hour and by 88% and 33% after 4 h at pH 5.8 (fresh beef) or 6.0 (cooked beef) and 4-5°C. Equivalent values for TSPP hydrolysis in less than 1 h were 10% and 23% (pH values 6.0 and 6.2, 4-5°C), 27% and 30% at 4 h. For SPG, hydrolysis was 34% and 7% in less than 1 h (pH values 5.7 and 6.0, 4-5°C), and 41% and 13% at 4 hr. These factors may be of importance in understanding the differences in bacterial

inhibition observed in SAPP-containing formulations (pH 6.10) when contrasted with the apparent ineffectiveness of STPP (pH 6.65), TSPP (pH 6.80) and SPG (pH 6.38). Except for sausages containing SAPP, all other sausages had pH values that would optimize the activity of the hydrolytic enzymes corresponding to each phosphate. In our study, cooking the bratwurst to an internal temperature of 65.5°C could have decreased but not totally eliminated enzymatic hydrolysis of the poly- and pyrophosphates (Awad, 1968). Hydrolysis may still have occurred so that the concentration of SAPP, STPP, TSPP and SPG in the final product may have been considerably lower that the initial 0.5%. These results and those of previous studies in this laboratory (Molins et al., 1985) also suggest that any delay in cooking after addition of phosphates to bratwurst formulations would render these compounds useless or less effective as antimicrobial agents. However, the effects of hydrolysis of the condensed phosphate species to orthophosphates on microorganisms is still not clear. Many other factors are concerned with bacterial growth inhibition. Residual levels of added phosphates, pH, ionic strength, other inhibitors, temperature and presence of metal ions, are all involved in considering effectiveness of phosphates as bacterial inhibitors in processed meats.

CONCLUSIONS

Addition of SAPP, STPP, TSPP or SPG at a level of 0.5% to vacuum packaged, cooked bratwurst has no significant ($P>0.05$) effect on bacterial survival or growth during refrigerated storage for 7 days. However, the addition of 0.5% SAPP inhibits aerobic and anaerobic bacterial growth and may possibly contribute a safety margin against Clostridium spp in vacuum packed, cooked bratwurst in the absence of nitrite even when "temperature abused" at 24°C for 48 h. TSPP and STPP are also effective during 24 h of elevated temperatures, but their inhibitory action on mesophilic bacteria does not persist.

Cooking the product as soon as feasible after addition of phosphates to the formulation is suggested to help retain the antimicrobial properties of phosphates in bratwurst.

Consideration should be given to hydrolysis of phosphates, and hence residual poly- and pyrophosphates in relation to bacterial growth inhibition in future microbiological research involving these compounds and meat products.

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Roberts, T. A., Gibson, A. M., and Robinson, A. 1981b. Factors controlling the growth of Clostridium botulinum types A and B in pasteurized cured meats. II. Growth in pork slurries prepared from "high" pH meat (pH range 6.3-6.8). J. Food Technol. 16: 267.

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

Figure 1. Effect of phosphates on total mesophilic counts of cooked bratwurst

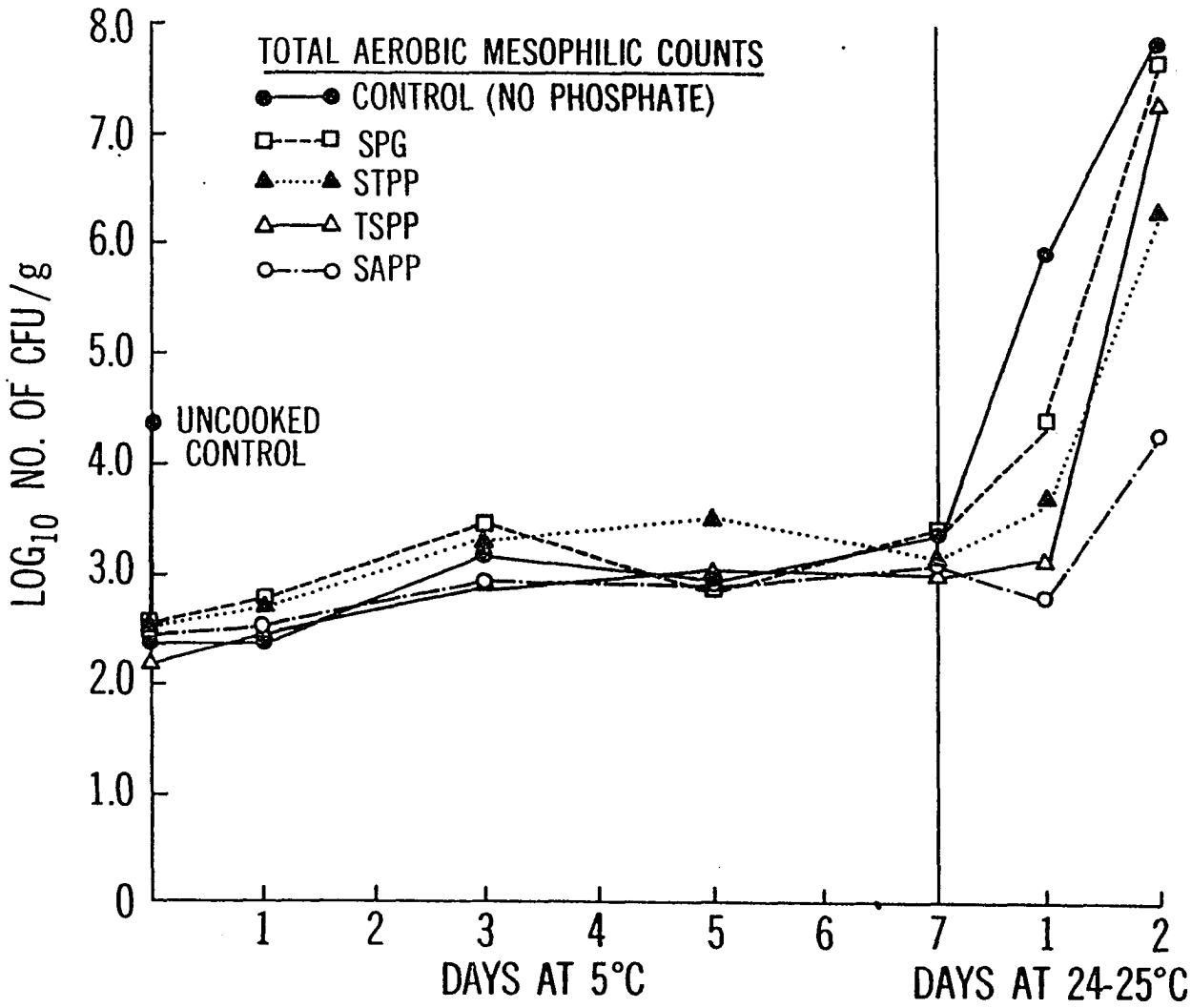

TOTAL AEROBIC MESOPHILIC COUNTS
CONTROL (NO PHOSPHATE)
SPG
STPP
TSPP
SAPP
UNCOOKED CONTROL
LOG10 NO. OF CFU/g
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1
2
3
4
5
6
7
1
2
DAYS AT 5°C
DAYS AT 24-25°C

Figure 2. Effect of phosphates on psychrotrophic counts of cooked bratwurst

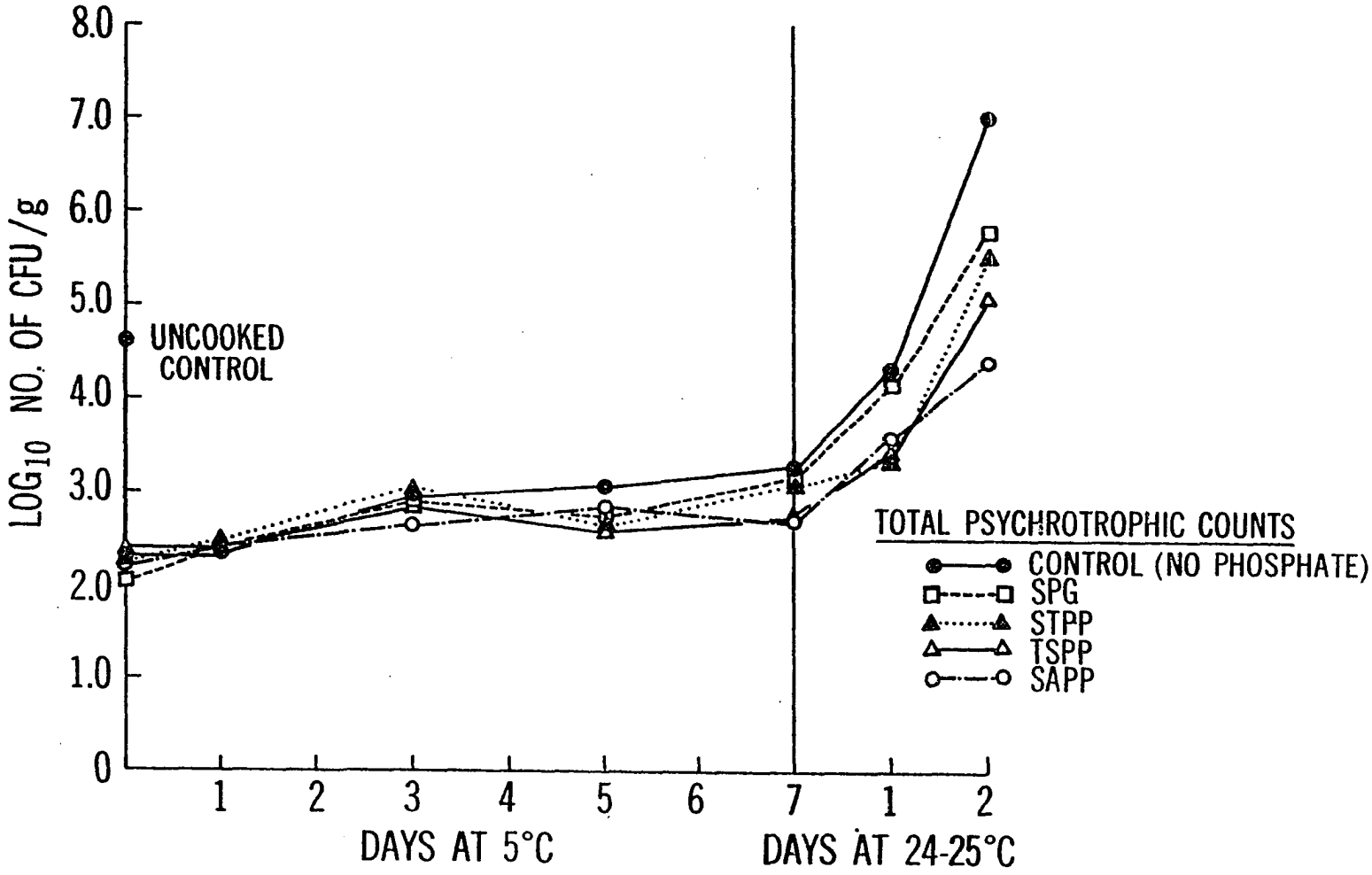

8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
LOG10 NO. OF CFU/g
UNCOOKED CONTROL
1
2
3
4
5
6
7
1
2
DAYS AT 5°C
DAYS AT 24-25°C
TOTAL PSYCHROTROPHIC COUNTS
CONTROL (NO PHOSPHATE)
SPG
STPP
TSPP
SAPP

Figure 3. Effect of phosphates on naturally occurring anaerobic bacteria in cooked bratwurst

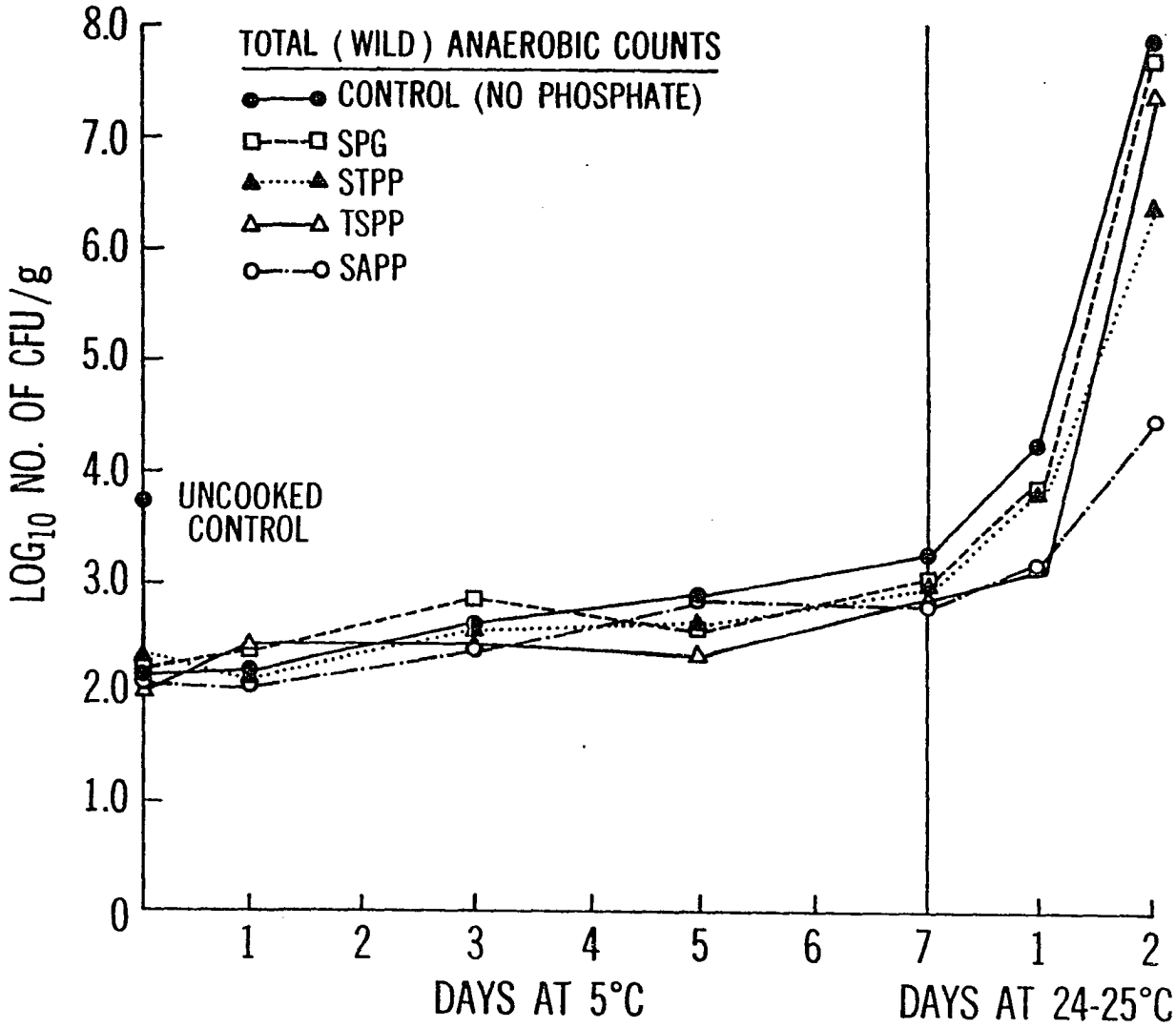

TOTAL (WILD) ANAEROBIC COUNTS
CONTROL (NO PHOSPHATE)
SPG
STPP
TSPP
SAPP
UNCOOKED CONTROL
LOG10 NO. OF CFU/g
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1 2 3 4 5 6 7 1 2
DAYS AT 5°C
DAYS AT 24-25°C

Figure 4. Effect of phosphates on total anaerobic counts of bratwurst inoculated with Clostridium sporogenes PA 3679

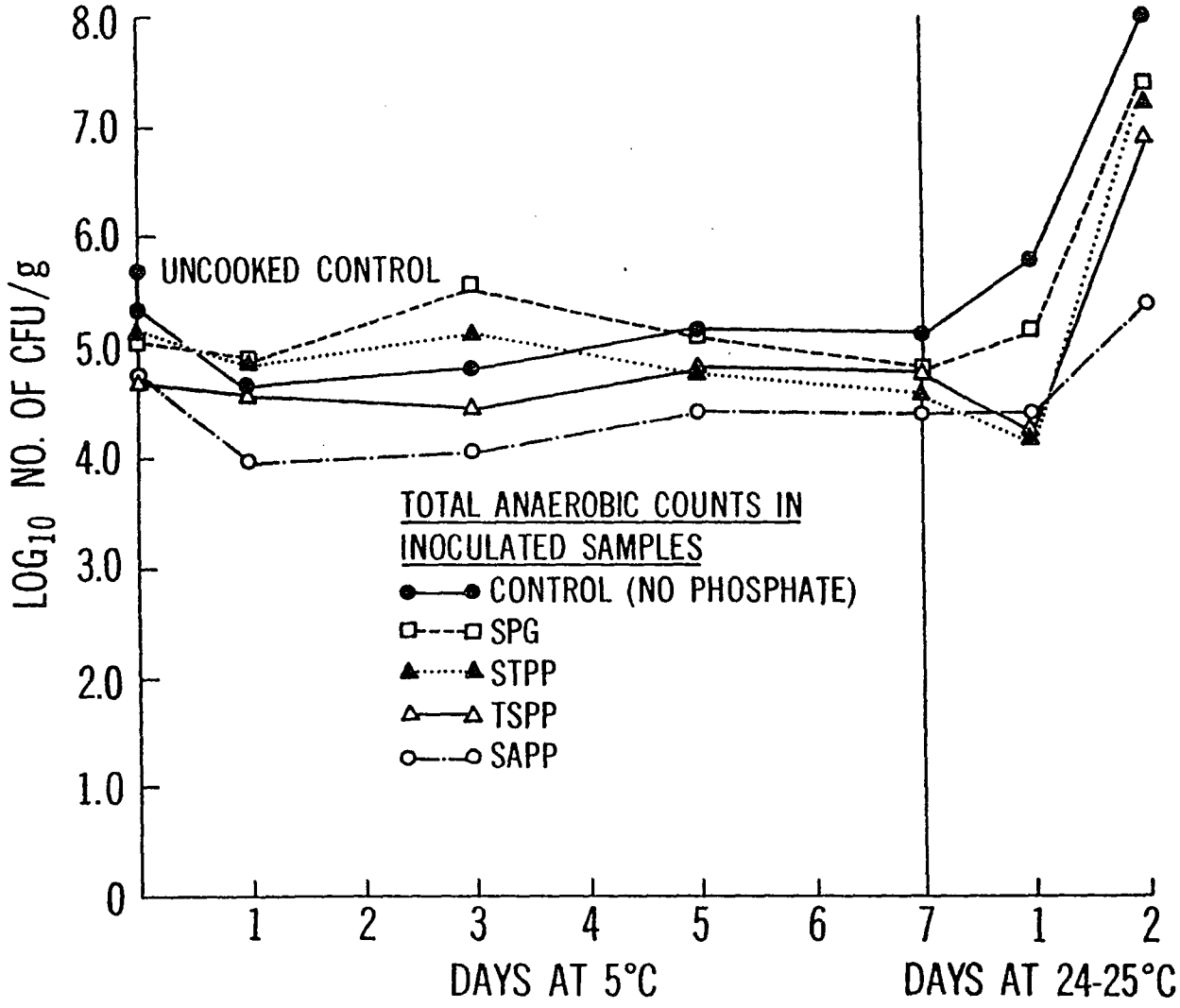
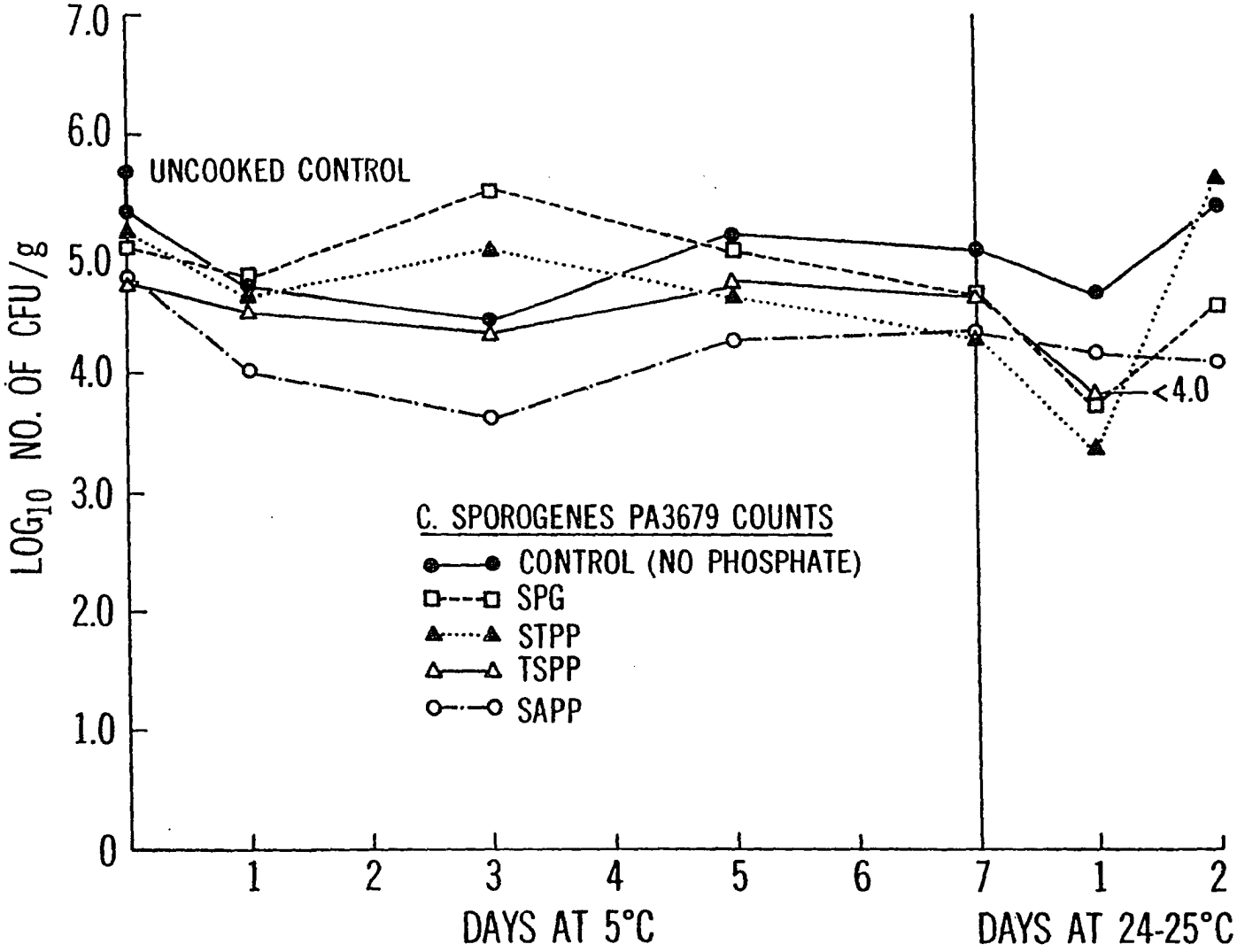

8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
LOG10 NO. OF CFU/g
UNCOOKED CONTROL
TOTAL ANAEROBIC COUNTS IN
INOCULATED SAMPLES
CONTROL (NO PHOSPHATE)
SPG
STPP
TSPP
SAPP
1
2
3
4
5
6
7
1
2
DAYS AT 5°C
DAYS AT 24-25°C

Figure 5. Effect of phosphates on counts of *C. sporogenes* PA 3679 in cooked bratwurst


7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
UNCOOKED CONTROL
LOG_{10} NO. OF CFU/g
<4.0
C. SPOROGENES PA3679 COUNTS
CONTROL (NO PHOSPHATE)
SPG
STPP
TSPP
SAPP
1
2
3
4
5
6
7
1
2
DAYS AT 5°C
DAYS AT 24-25°C

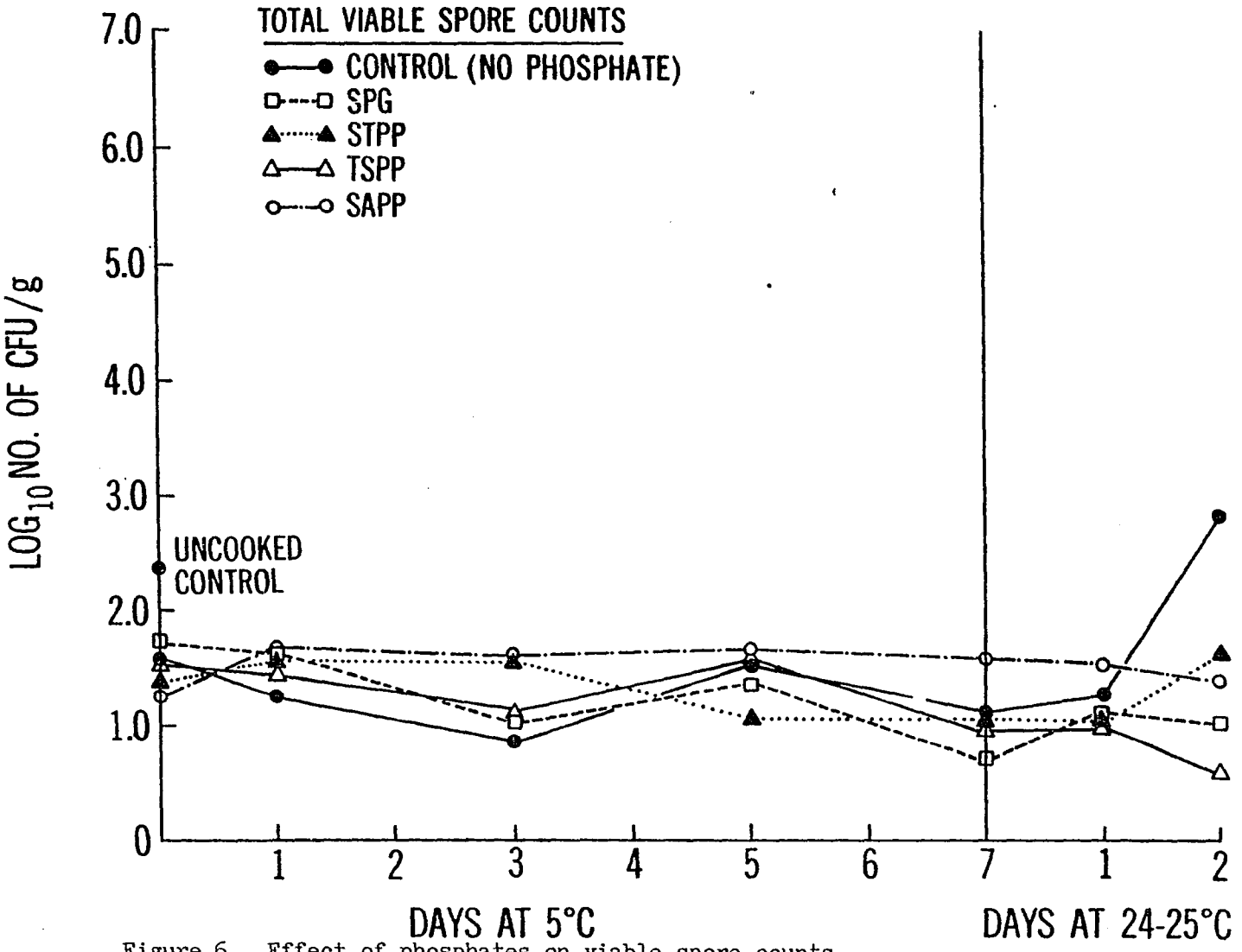


Figure 6. Effect of phosphates on viable spore counts

Figure 7. Effect of phosphates on counts of viable spores of C. sporogenes PA 3679 in cooked bratwurst

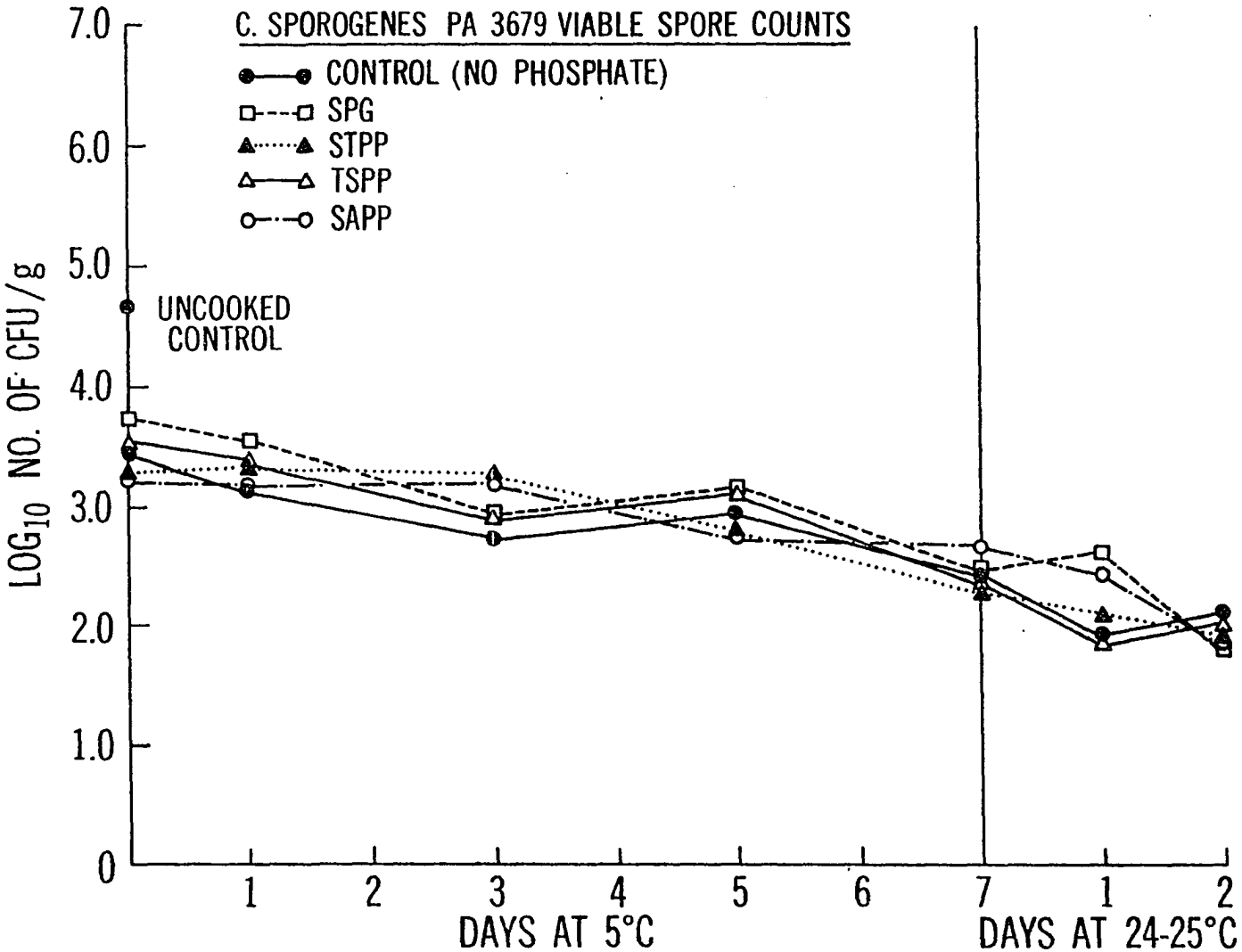

C. SPOROGENES PA 3679 VIABLE SPORE COUNTS
CONTROL (NO PHOSPHATE)
SPG
STPP
TSPP
SAPP
UNCOOKED CONTROL
LOG10 NO. OF CFU/g
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
1
2
3
4
5
6
7
1
2
DAYS AT 5°C
DAYS AT 24-25°C

Table 1. Mean pH values of cooked, vacuum packed bratwurst throughout refrigerated and abuse temperature storage

TIME (In days)	STORAGE TEMPERATURE (°C)	PHOSPHATE TREATMENT				
		NONE (control)	SAPP	STPP	TSPP	SPG
0[a]	5°C	6.45	6.10	6.65	6.80	6.38
1	"	6.44	6.08	6.60	6.78	6.33
3	"	6.40	6.10	6.60	6.83	6.33
5	"	6.43	6.08	6.60	6.80	6.35
7	"	6.38	6.08	6.60	6.78	6.35
8	24-25°C	6.39	6.08	6.58	6.78	6.33
9	"	6.08	6.08	6.50	6.75	6.28

[a]Uncooked control mix had a pH of 6.30.

PART IV. INHIBITION OF CLOSTRIDIUM SPOROGENES PA3679 AND NATURAL BACTERIAL FLORA OF COOKED VACUUM PACKAGED BRATWURST BY SODIUM ACID PYROPHOSPHATE AND SODIUM TRIPOLYPHOSPHATE WITH OR WITHOUT ADDED SODIUM NITRITE

by

R.A. Molins, A.A. Kraft, D.G. Olson and D.K. Hotchkiss
Departments of Food Technology, Animal Science and Statistics
Iowa State University
Ames, Iowa 50011

Running Title: Phosphate and nitrite inhibition of bacteria in cooked bratwurst.

ABSTRACT

Survival and growth of inoculated Clostridium sporogenes PA3679 and natural aerobic and anaerobic bacterial flora were studied in refrigerated (5°C) and subsequently temperature abused (24-25°C), cooked, vacuum packaged bratwurst containing 0.5% sodium acid pyrophosphate (SAPP) or sodium tripolyphosphate (TSPP) with or without sodium nitrite. Phosphates alone or combined with nitrite significantly ($P<0.05$) reduced numbers of anaerobes (including C. sporogenes) but not of aerobic bacteria at 5°C. Upon temperature abuse inhibition of all bacteria by SAPP was significant ($P<0.05$) and greatly enhanced by 100 ppm but not by 50 ppm sodium nitrite. Phosphate hydrolysis is discussed in relation to bacterial inhibition.

INTRODUCTION

Recent work on the antimicrobial properties of poly- and pyrophosphates added to cooked, vacuum packaged bratwurst has demonstrated that 0.5% sodium acid pyrophosphate (SAPP) and to a lesser extent sodium tripolyphosphate (STPP) effectively inhibited growth of aerobic (mesophilic and psychrotrophic) and anaerobic bacteria, including inoculated Clostridium sporogenes PA3679, when the bratwurst was removed from refrigerated storage at 5° C and placed under abuse temperature conditions (24-25°C) for up to 48 h, while no similar effect was observed when tetrasodium pyrophosphate or sodium polyphosphate glassy were used at the same level (Molins et al., 1985a). Nelson et al. (1983) had previously found that 0.4% SAPP used in combination with 40 ppm sodium nitrite and 0.2% sorbic acid or 0.26% potassium sorbate delayed toxin production by Clostridium botulinum in mechanically deboned chicken meat frankfurter emulsions at 27°C to a greater degree than nitrite-sorbate alone. Wagner and Busta (1983) later confirmed the added antibotulinal activity provided by 0.4% SAPP in beef/pork frankfurter emulsions containing 40 ppm sodium nitrite and 0.26% potassium sorbate. The reported aerobic mesophilic bacterial counts were not affected.

Most studies concerning the antimicrobial properties of poly- and pyrophosphates in meats, however, have overlooked the possible influence of naturally occurring phosphatases on the level of phosphates being tested. Rapid enzymatic hydrolysis of the condensed phosphates to

shorter chain compounds and eventually to orthophosphates has been documented by Awad (1968) and by Sutton (1973). Adaptation of a method developed by Dick and Tabatabai (1977) for the measurement of soluble orthophosphates in the presence of labile organic and inorganic phosphorous compounds enabled us to follow the hydrolysis of SAPP and STPP in meats. Even short delays in meat phosphatase inactivation by cooking temperatures reduced the level of SAPP or STPP considerably (Molins et al., 1985b).

The objectives of the present study were to determine and compare the effects of SAPP and STPP alone or in combination with high and reduced levels of sodium nitrite on the survival and growth of C. sporogenes PA3679 and of the spoilage flora of cooked, vacuum packaged bratwurst under refrigeration and abuse temperature conditions, and to relate the effects to residual levels of the condensed phosphates.

MATERIALS AND METHODS

Bratwurst Preparation

A 300-lb batch of ground pork butts in 12-lb portions was obtained from the Iowa State Meat Science Laboratory, packaged in polyethylene freezer bags, twist-tied, frozen in an air blast freezer to -28°C and kept frozen until needed. Prior to preparation, 12-lb portions were allowed to thaw for 48 h in a walk-in cooler at 2-4°C. Bratwurst spice mix (Saratoga Specialties, Elmhurst, IL) was added to the meat and blended in for 5 minutes at medium speed by means of an Hobart C-10 mixer. The spice mix used was 2% (w/w), equivalent to 1.7% NaCl in the product. Portions of 500 g of meat were weighed and each received 26.5 ml of a 10% (w/v) solution of a single phosphate previously sterilized by filtration through a 22 μ Millipore membrane filter (Millipore Corp., Bedford, MA), so as to obtain the desired concentration of 0.5% in the product. The phosphates used were sodium acid pyrophosphate (SAPP) (Stauffer Chemical Co., Westport, CT) and sodium tripolyphosphate (STPP) (Monsanto, St. Louis, MO). Batches of the mix then received either sodium nitrite as a filter-sterilized 10% (w/v) aqueous solution properly diluted with sterile distilled water to provide a concentration of 50 or 100 ppm in the product, or the equivalent volume of sterile distilled water to equalize the volume of added water in all batches. Control sausages were prepared with no additives, or with each level of nitrite and no phosphate and with each phosphate but no nitrite. After

being blended for one minute at each low and high speeds in a Kitchen Aid Model 4 mixer, the 500-g batches were divided in halves; one-half was inoculated and the second received an amount of sterile distilled water equal to the volume of inoculum added to the first portion. The inoculum consisted of 5 ml of a Clostridium sporogenes PA3679 spore suspension in sterile, deionized water prepared according to the procedure outlined by Swank (1971) and targeted to give approximately 1000 spores/g of sausage. The culture was obtained from the ISU Food Technology Department collection and selected for rhizoid colony formation to facilitate differentiation from other anaerobic bacteria during plate counting. Immediately after stuffing and tying, each batch of sausage was cooked to an internal temperature of 65.5°C in a separate 3000-ml beaker containing distilled water and kept at 80°C in a Magni Whirl Constant Temperature Bath (Blue M Electric Co, Blue Island, IL) equipped with bottom paddle agitation. The cooking end point was determined by inserting a copper-constantan thermocouple longitudinally through one bratwurst of each batch and monitoring the internal temperature with a Hewlett Packard 3476A Multimeter Potentiometer. Cooking losses that could have affected the level of phosphates and/or nitrite were previously determined and found to be negligible (<1.5%). After cooking, the bratwurst was wrapped in aluminum foil, rapidly cooled to an internal temperature of 4-5°C, separated and placed in small, individual, polyethylene bags to avoid cross-contamination. One sausage from each treatment, for a total of nine sausages, was placed in Curlon 892 (Curwood Inc., New London, WI) vacuum packaging bags

(O_2permeability <1 ml/645 cm^2/24 h at 22.8°C and 0% RH) and vacuum sealed in a Multivac MG-2 packaging machine. The bags were stored for seven days in a display case at about 5°C, followed by 48 h of temperature abuse (24-25°C). The order of batch preparation was randomly assigned and all experiments were replicated three times.

Sampling

Packages of sausages were taken from storage on days 0, 1, 3, 5 and 7 and on days 8 and 9 (corresponding to 24 and 48 h of temperature abuse at 24-25°C) for microbiological examination. The sausage was weighed in 30-g amounts and blended in a sterile plastic bag with 270 ml of 0.1% sterile peptone water for 2 minutes by means of a Stomacher Lab Blender 400 (Tekmar Company, Cincinnati, OH). Serial dilutions were then made according to standard methods. Trypticase soy agar (TSA, BBL) was used to plate aerobic mesophilic (48 h, 30°C) and psychrotrophic (10 days, 5°C) bacterial populations. TSA was also used for total anaerobic counts, while TSA containing 0.1% soluble starch (Fisher Scientific Co., Fair Lawn, NJ) was the medium used to determine anaerobic viable spore counts after heat shocking the appropriate dilutions at 80° C for 20 minutes (Swank, 1971). The C. sporogenes identity of typical rhizoid colonies, enumerated from total anaerobic, vegetative cell and spore count plates based on morphology, was ascertained by randomly taking 10 such colonies from counted plates on a daily basis, examining cells microscopically and culturing in cooked meat medium (BBL; 37°C, 48 h).

Gas formation, proteolysis, blackening of the medium and terminal, single spore formation were taken to indicate positive identification. No typical rhizoid colonies were found in uninoculated control sausages. BBL Gas Pak Anaerobic Systems were used to obtain anaerobic incubation conditions (48 h, 37°C). The pH of the sausage was determined throughout the sampling period using a Radiometer 28 meter equipped with an Orion 9163 probe. Soluble orthophosphate levels in the sausage were determined on every sampling day by the method of Dick and Tabatabai (1977) as adapted by Molins et al. (1985b). Sampling order was randomized among treatments.

Plate count data were transformed to logarithms and analyzed by means of an SAS program with GLM option. Comparison of means was based on Duncan's multiple range test.

RESULTS AND DISCUSSION

At the end of the 7-day refrigerated storage period at 5°C, control sausages containing no additives or only sodium nitrite had significantly ($P<0.05$) higher total anaerobic and PA3679 counts than bratwurst formulated with SAPP or STPP alone or combined with 50 or 100 ppm $NaNO_2$. This indicated that phosphates either inhibited *C. sporogenes* spore germination and/or outgrowth or had a lethal effect on clostridial cells after outgrowth, since no *C. sporogenes* vegetative cell growth would be expected at 5°C.

Figs. 1-5 depict the results obtained with control samples and SAPP/nitrite combinations. Fig. 1 shows that sausages containing SAPP had the lowest numbers of total anaerobes at the end of the 7-day refrigerated storage period. Concurrent decreases in clostridial vegetative cell (Fig. 2) and viable spore (Fig. 3) numbers suggest that spore germination and outgrowth followed by death of vegetative cells after outgrowth occurred in refrigerated bratwurst containing SAPP. Similar results were obtained for STPP. Although not presented graphically, sausages formulated with STPP alone or STPP/$NaNO_2$ combinations had the lowest aerobic mesophilic and psychrotrophic counts throughout the refrigerated storage period, but the differences with other treatments were not significant ($P>0.05$).

When the sausages were allowed to remain at 24-25°C for 24 h after refrigeration, those containing no additives or only nitrite at either level had significantly ($P<0.05$) higher numbers of total anaerobes (Fig.

1) and higher, although not significantly so, counts of PA3679 vegetative cells, aerobic mesophilic and psychrotrophic bacteria than phosphate-treated bratwurst. At 24 h, SAPP alone significantly ($P<0.05$) inhibited growth of anaerobes followed in decreasing order of effectiveness by STPP/50 ppm $NaNO_2$ (not shown) and SAPP/100 ppm $NaNO_2$ (Figs. 1 and 2). The presence of SAPP/100 ppm nitrite and of SAPP alone were also significantly ($P<0.05$) inhibitory to all aerobic bacteria at 24-25°C as shown in Figs. 4 and 5. During the first 24 h of abuse temperature holding, C. sporogenes growth was clearly seen only in sausages containing the lower level of sodium nitrite (50 ppm) and no phosphate, but after 48 h of elevated temperature storage clostridial growth was detected in all sausages except those treated with SAPP (alone or combined with nitrite), for which the opposite occurred.

Upon 48 h of temperature abuse, the combination SAPP/100 ppm $NaNO_2$ had a very strong, significant ($P<0.05$) inhibitory effect on the growth of all types of bacteria under study, particularly on anaerobic (including clostridial) and aerobic mesophilic microorganisms (Figs. 1, 2, 4 and 5). SAPP alone or combined with 50 ppm nitrite also significantly ($P<0.05$) affected all bacterial growth in the bratwurst but to a smaller extent than in combination with the higher level of $NaNO_2$. The magnitude of the antimicrobial activity of the SAPP/100 ppm nitrite treatment after 48 h of temperature abusing the bratwurst should be emphasized. Bacterial counts in sausages with that combination were 2-3 log cycles below those found in control samples and only 1-1.5 log cycles higher than bacterial numbers in the bratwurst at the end of the

refrigerated storage period at 5°C.

Soluble orthophosphate levels present in the sausage throughout the 5°C holding period and upon temperature abuse are shown in Fig. 6 for all treatments. A decrease in the level of soluble orthophosphates occurred in all samples except controls with no additives after 24 h of manufacture and storage at 5°C, followed by a gradual increase over time. The original level was reestablished and surpassed within 3-5 days in SAPP-containing bratwurst. Similar but slower increases were observed in treatments that included STPP, while the initial loss of soluble orthophosphates in sausages with only nitrite was not recovered until after 24 h of temperature abuse. Sausages containing SAPP had significantly ($P<0.05$) higher levels of soluble orthophosphates since manufacture and as a group than STPP-treated bratwurst. The latter, in turn, had significantly ($P<0.05$) higher levels than controls with no additives or only nitrite. A grouping of treatments according to type of phosphate added can be observed throughout the 7-day refrigerated storage at 5°C and beyond in Fig. 6. A negative correlation was found to exist between soluble orthophosphate content and the mean of all bacterial counts from day 1 through 8 (24 h at 24-25°C) in SAPP-treated bratwurst. Correlation coefficients were -0.87, -0.92, -0.68 and -0.78 for SAPP alone, SAPP/50 ppm $NaNO_2$, SAPP/100 ppm $NaNO_2$ and all SAPP-treated sausages as a group, respectively. Such correlations could not be obtained with STPP.

Bratwurst average pH values throughout the experimental period are contained in Table 1. A slow but continued increase in the pH of all

sausages took place during storage at 5°C and continued after 24 h at 24-25°C in phosphate-treated samples but not in controls or in bratwurst having only nitrite. In SAPP/$NaNO_2$ combinations, the pH increased even after 48 h of elevated temperature holding.

All treatments that included phosphates significantly ($P<0.05$) reduced the numbers of total anaerobes (including C. sporogenes) during the 7-day refrigerated storage of bratwurst. STPP alone (without added nitrite) was the most effective treatment under these conditions.

Upon temperature abuse, the presence of nitrite without phosphates resulted in significantly ($P<0.05$) higher clostridial, mesophilic and psychrotrophic bacterial counts than any treatment which included phosphates, but differences with control were small. After the sausages were held at 24-25°C for 24 h, STPP/50 ppm $NaNO_2$ effectively inhibited growth of C. sporogenes and other anaerobes, but the inhibitory characteristics of this combination dissapeared when the time under abuse temperature was prolonged. This finding suggests that STPP combined with a low level of nitrite (50 ppm) may provide protection against clostridial growth in cooked, vacuum packaged bratwurst exposed to short periods of temperature abuse. In terms of possible improvements in shelf life of vacuum packaged, refrigerated bratwurst, no effective protection against low temperature spoilage by psychrotrophic bacteria was obtained in this study.

The most effective treatment for bratwurst subjected to abuse temperature conditions (24-25°C), however, and the only one having significant ($P<0.05$) inhibitory effects on all bacteria studied after 48

h of such abuse was the 0.5% SAPP/100 ppm $NaNO_2$ combination. This was followed in decreasing order of effectiveness by SAPP alone, STPP/50 ppm $NaNO_2$ and SAPP/50 ppm $NaNO_2$. The low rates of growth of anaerobes (<1 log cycle), C. sporogenes (<1 log cycle), aerobic mesophiles (<2 log cycles) and psychrotrophic bacteria (<1 log cycle) allowed by the SAPP/100 ppm $NaNO_2$ treatment above the corresponding populations present in the bratwurst at the end of the refrigeration period and during 48 h of holding at 24-25°C are noteworthy. No similar effect on aerobic mesophilic counts was reported by Wagner and Busta (1983) in beef/pork frankfurter emulsions containing 40 ppm $NaNO_2$, 0.4% SAPP and 0.26% potassium sorbate. In the present work, if the total soluble ortho-P increase measured at the end of the experimental period were attributed to SAPP breakdown, 0.37% SAPP (w/w) would have remained in the bratwurst. This residual level of SAPP may, in fact, have been overestimated. In other experiments involving direct addition of orthophosphates to the same type of meat, as much as 25% of the added ortho-P became insolubilized shortly after addition (unpublished data). If this had also happened in the bratwurst, we would have failed to detect a considerable amount of orthophosphates, and possibly residual SAPP would be lower than the 0.37% previously mentioned.

Initial decreases and subsequent increases in soluble orthophosphate levels of cooked bratwurst containing added SAPP or STPP (Fig. 6) might indicate that only little or no hydrolysis of phosphates took place at 5°C (7 days of storage). In that case, a sizeable amount of soluble ortho-P initially present was bound or otherwise made

insoluble during the first 24 h and later released, so that the initial level of day 0 was reestablished. Hydrolysis of SAPP or STPP with subsequent increase in soluble orthophosphate content may have occurred only after the sausage was placed at abuse temperatures, when a higher rate of bacterial growth and/or possible reactivation of meat phosphatases occurred. That possibility, however, was not substantiated by the levels of soluble ortho-P found in bratwurst treated with only nitrite, which decreased after 24 h at 5°C similarly to those of phosphate-treated bratwurst but, unlike the latter, did not increase thereafter as they would if initially insolubilized orthophosphates had later been released. A more likely explanation of this phenomenon is that orthophosphates that were insolubilized by chelating, complexing or otherwise reacting with meat components (Sherman, 1961; Nikkila et al., 1967; Tenhet et al., 1981) remained bound. Steady returns to initial levels (and beyond in the case of SAPP) observed in phosphate-treated sausages stored at 5°C may therefore have been a result of continued breakdown of the condensed phosphates over time, possibly of intrinsic nature as described by Awad (1968), and/or of reactivation of meat phosphatases.

Also, the large increases in the level of soluble orthophosphates observed between 24 and 48 h at 24-25°C in control samples and sausages containing only nitrite (no phosphates), were absent in SAPP- and STPP-treated bratwurst, where the rate of soluble ortho-P formation remained constant (Fig. 6). This might indicate that bacteria growing in vacuum packaged, phosphate-treated bratwurst at 24-25°C did not metabolize SAPP

nor STPP to any large extent. A similar effect was reported by Foster et al. (1978) with a Bacillus sp. isolated from soil and capable of metabolizing phosphite and hypophosphite anaerobically if orthophosphates were absent, but not in their presence.

In general, antimicrobial effectiveness of the phosphates tested was related to soluble orthophosphate levels in the sausage. In treatments that included SAPP, a direct relationship between soluble orthophosphate content and bacterial growth inhibition was confirmed, suggesting an active participation by orthophosphates in the overall antibacterial properties exhibited by SAPP. This result contrasts with our previous studies in uncooked bratwurst held at 5°C, where bacterial inhibition by SAPP was present but not at statistically significant levels ($P>0.05$) (Molins et al., 1985c). However, we later confirmed that the soluble orthophosphate content of sausages thus treated increased at a much higher rate and reached values twice as high after 6 days than those in similar bratwurst cooked to 65.5°C internal temperature. In experiments with uncooked ground pork meat similar to that used to manufacture the bratwurst, held 7 days at 5°C, we also determined that soluble orthophosphate (K_2HPO_4) levels as high as 2000 μg/g of meat are only mildly inhibitory to psychrotrophic bacteria (unpublished data). Further, SAPP proved to be ineffective as an antimicrobial agent in laboratory media under conditions that might have resulted in total heat-induced hydrolysis of SAPP to orthophosphates (Molins et al., 1984). Therefore, we must conclude that bacterial inhibition by SAPP cannot be due solely to increased orthophosphate

content of the bratwurst.

Whether orthophosphates enhance the antibacterial properties of SAPP per se through interference with bacterial cell metabolic pathways (Kornberg, 1957; Harold and Harold, 1963, 1965), through increased ionic strength (Sherman, 1961) or otherwise, or simply prevent possible enzymatic, chemical and/or bacterial depletion of residual SAPP to noninhibitory levels remain to be elucidated. Possible interactions between SAPP and nitrite also is in need of study. Large increases in soluble orthophosphate content in sausages to which SAPP has been added might also be only indirectly related to bacterial inhibition. Unlike other phosphates, SAPP induces a decrease in pH values of meat and meat products (Terrell et al., 1982; Knipe, 1982; Hargett et al., 1980; Ivey and Robach, 1978) and its overall mode of action against bacteria may consequently be different from that of other phosphates. It is clear from this study, however, that cooking SAPP-containing bratwurst immediately after preparation is essential in retaining the antimicrobial properties of this pyrophosphate and that its use and that of STPP may provide a safety margin against clostridial growth in cooked meat products.

CONCLUSIONS

Addition of 0.5% sodium acid pyrophosphate (SAPP) or sodium tripolyphosphate (STPP) significantly ($P<0.05$) reduces the numbers of anaerobic bacteria (including Clostridium sporogenes PA3679) in vacuum packaged, cooked bratwurst held 7 days at 5°C, but does not significantly ($P>0.05$) affect psychrotrophic bacterial growth. Concurrent addition of 50 or 100 ppm of sodium nitrite does not enhance the effects of SAPP or STPP in refrigerated bratwurst and result in undesirable pink coloration.

A combination of 0.5% STPP/50 ppm $NaNO_2$ effectively inhibits anaerobic and clostridial growth in bratwurst exposed to abuse temperatures of 24-25°C for 24 but not for 48 h.

The combination 0.5% SAPP/100 ppm $NaNO_2$ is significantly ($P<0.05$) inhibitory to inoculated C. sporogenes PA3679 and to the natural anaerobic and aerobic bacterial flora of bratwurst exposed to 24 and 48 h of temperature abuse (24-25°C). Although less effective in this study, combinations of SAPP and lower levels of sodium nitrite appear worthy of future research as antimicrobials for use in cooked meat products.

Increased levels of soluble orthophosphates correlate with bacterial growth inhibition in SAPP-containing bratwurst, suggesting that orthophosphate formation from SAPP breakdown may be involved in the overall antibacterial activity exhibited by SAPP in cooked bratwurst.

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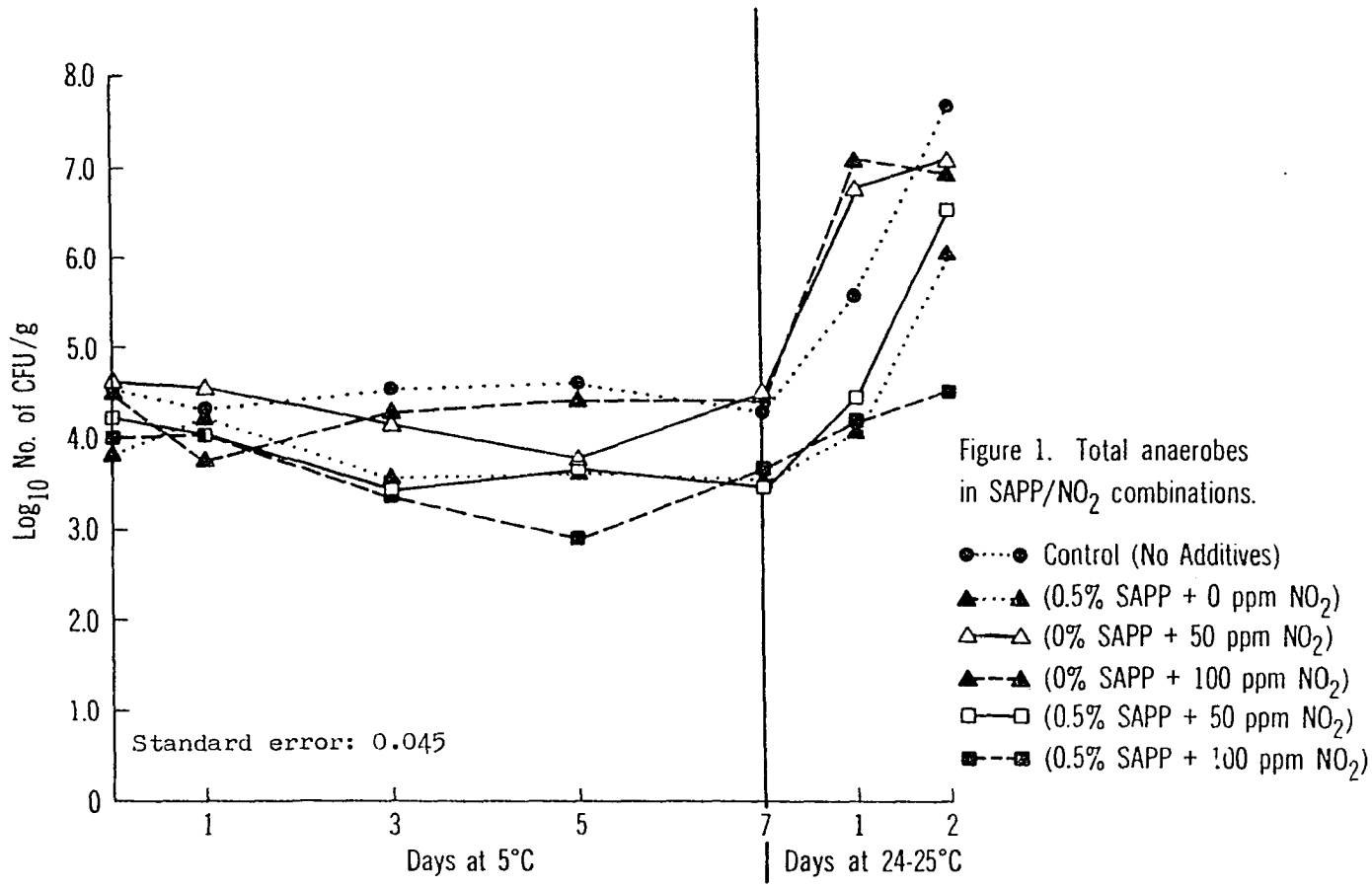

Figure 1. Total anaerobes in SAPP/NO_2 combinations.

Figure 1. Total anaerobic counts in bratwurst containing SAPP/$NaNO_2$ combinations

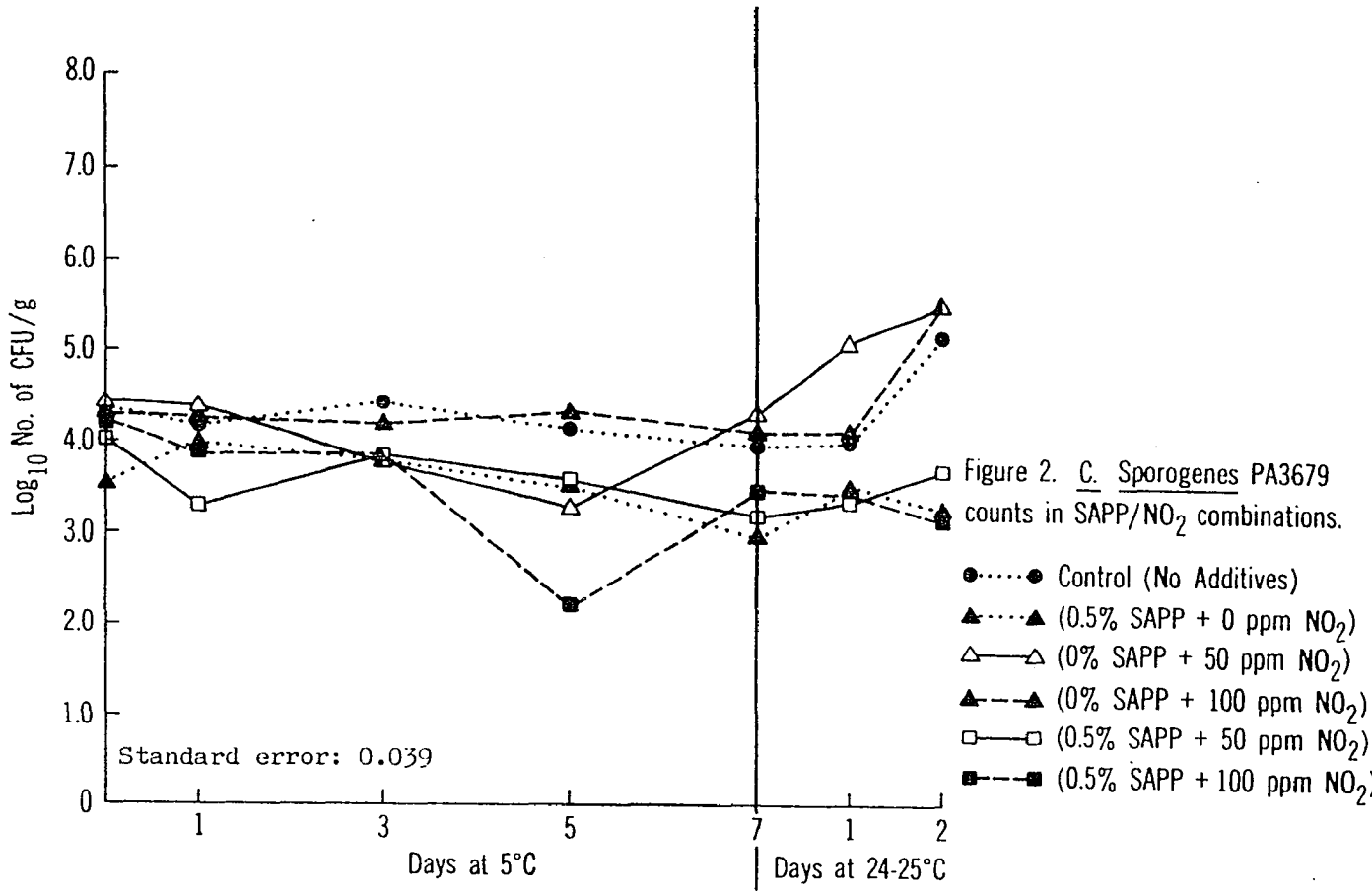

Figure 2. C. Sporogenes PA3679 counts in SAPP/NO_2 combinations.

Figure 2. Clostridium sporogenes PA 3579 counts in bratwurst containing SAPP/$NaNO_2$ combinations

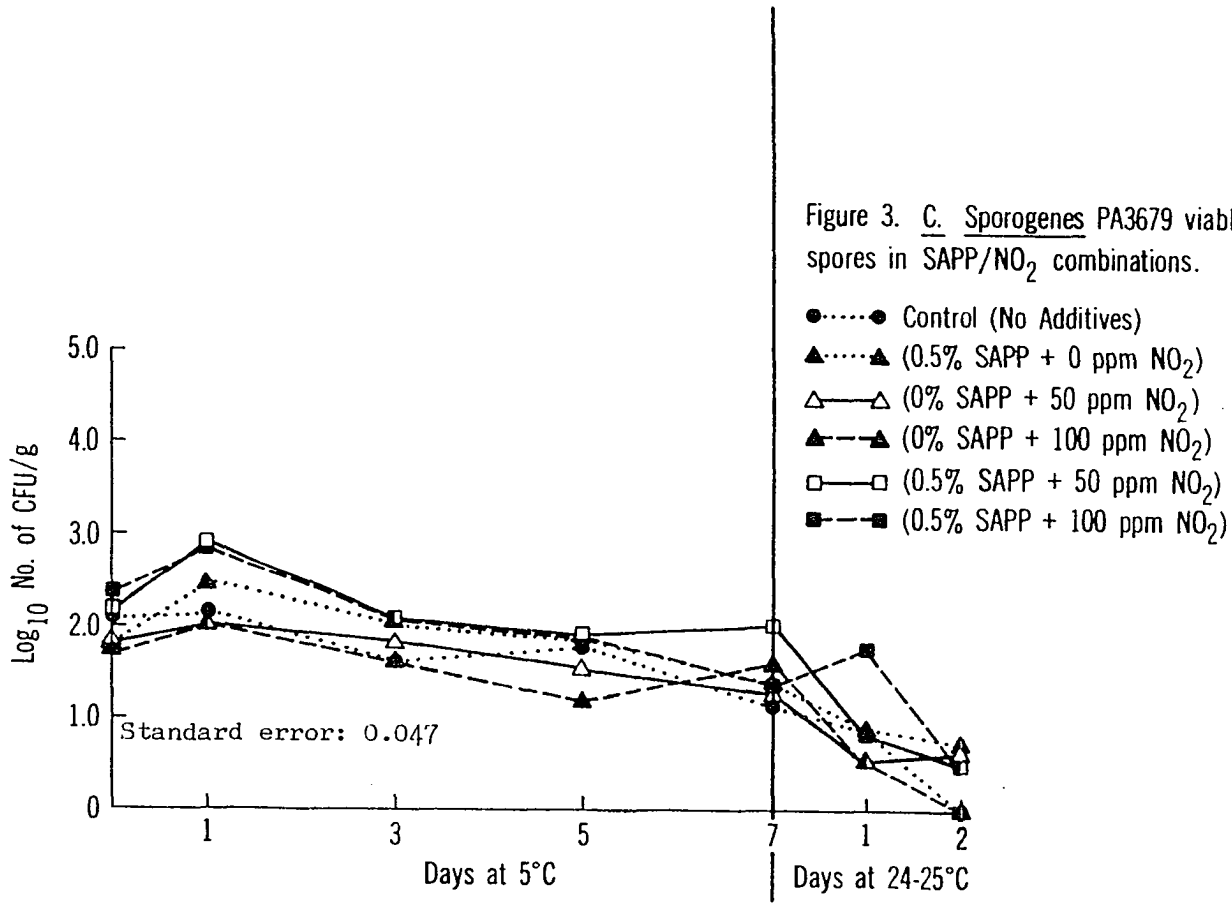

Figure 3. Clostridium sporogenes PA 3679 viable spore counts in bratwurst containing SAPP/$NaNO_2$ combinations

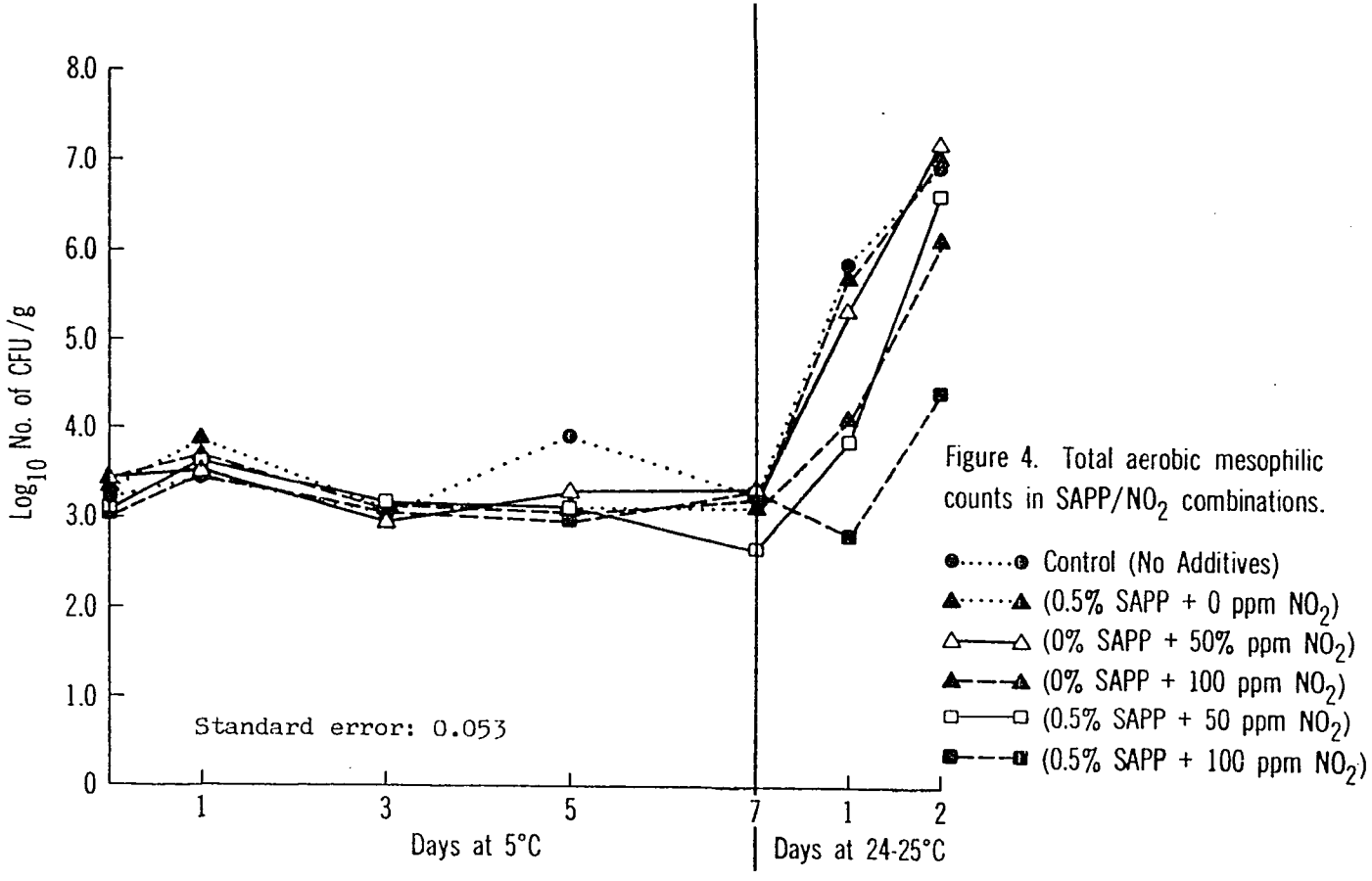

Figure 4. Total aerobic mesophilic counts in SAPP/NO_2 combinations.

Figure 4. Total aerobic mesophilic counts in bratwurst containing SAPP/$NaNO_2$ combinations

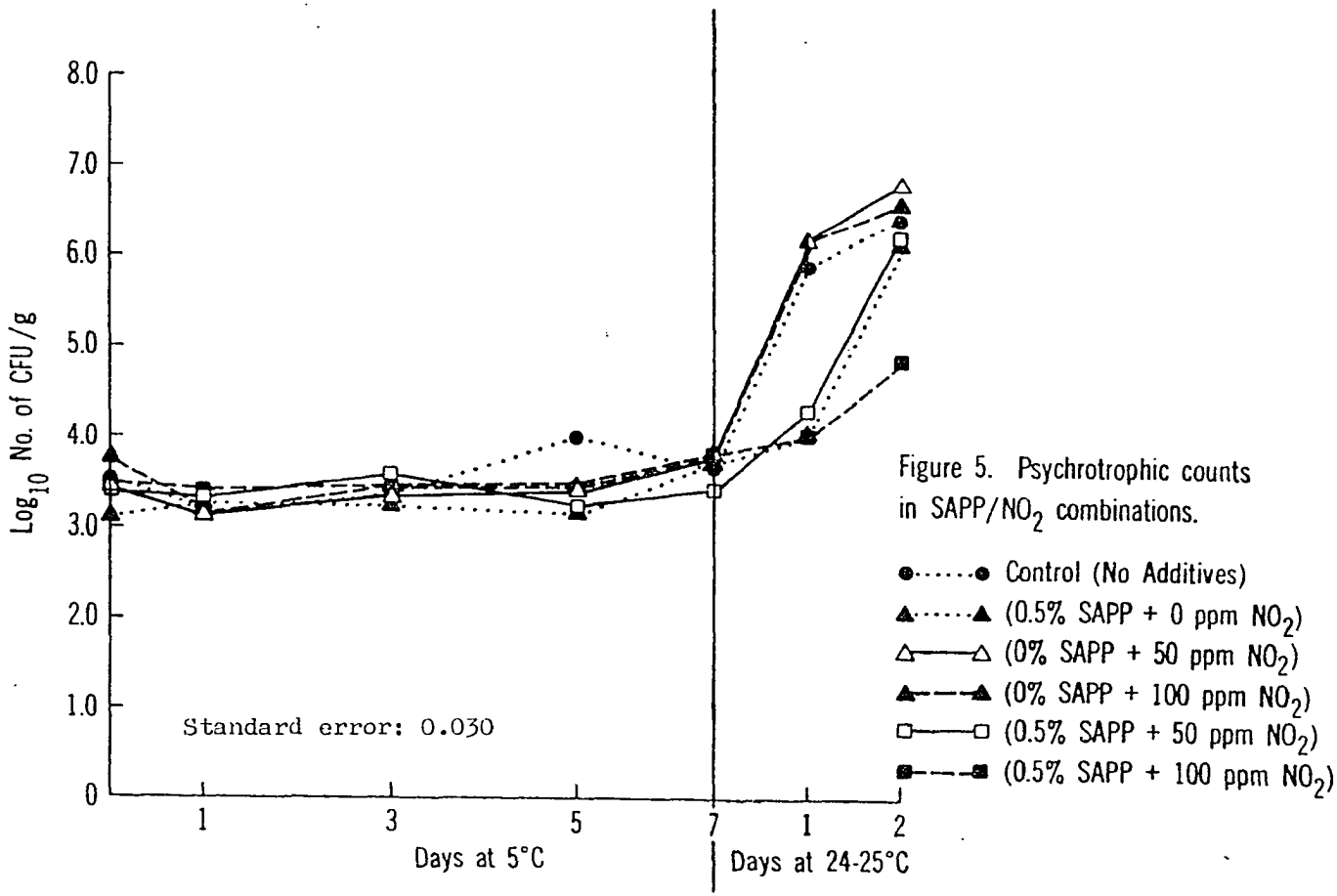

Figure 5. Psychrotrophic bacterial counts in bratwurst containing SAPP/$NaNO_2$ combinations

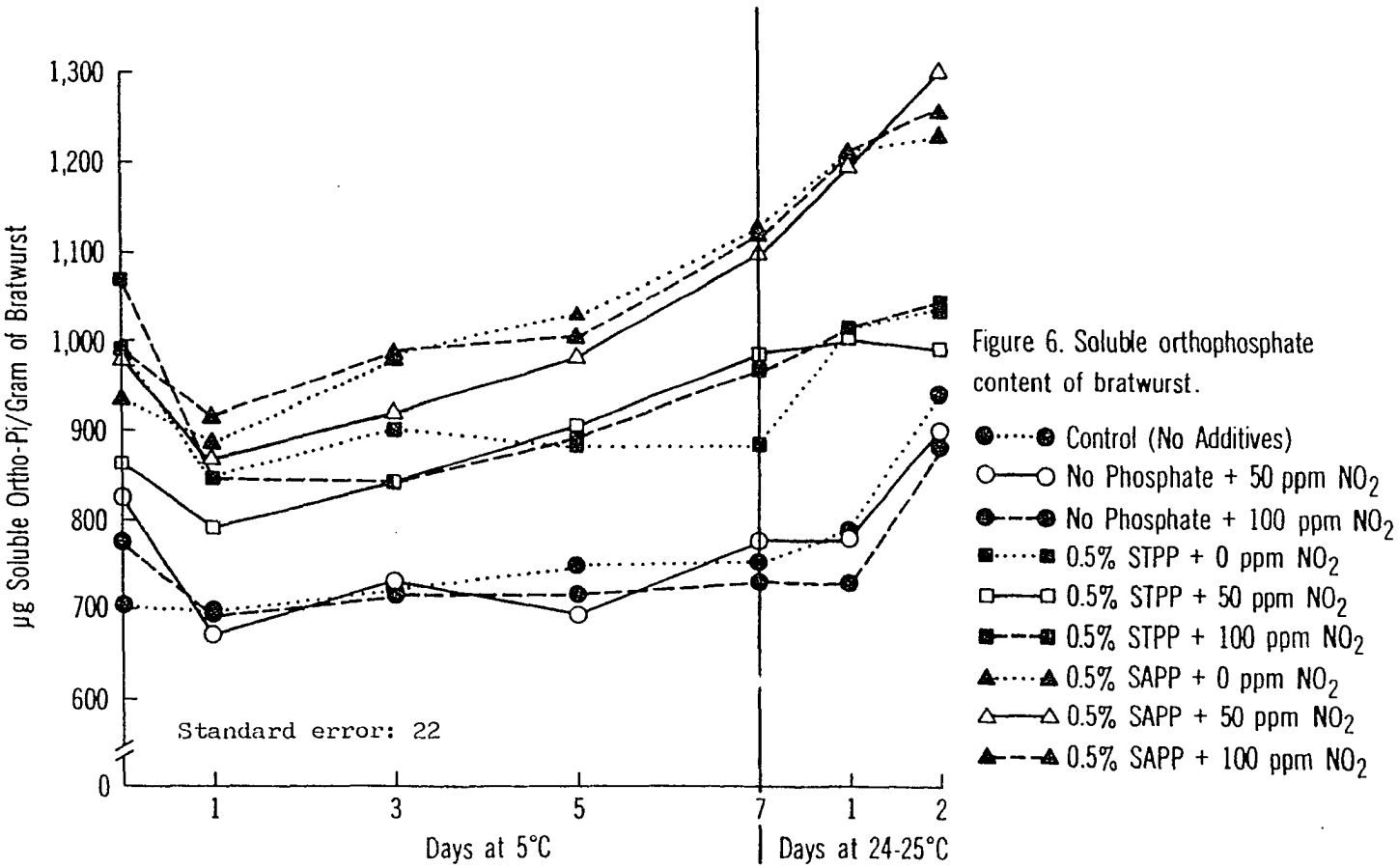

Figure 6. Soluble orthophosphate content of bratwurst.

Figure 6. Effect of SAPP or STPP addition on the soluble orthophosphate content of cooked bratwurst

Table 1. pH values of cooked vacuum packaged bratwurst

Days at 5°C	TREATMENT								
SAPP:	--	--	--	0.5%	0.5%	0.5%	--	--	--
STPP:	--	--	--	--	--	--	0.5%	0.5%	0.5%
$NaNO_2$[a]:	0	50	100	0	50	100	0	50	100
0	6.29	6.30	6.33	5.93	5.92	5.92	6.47	6.47	6.47
1	6.32	6.29	6.32	5.98	5.94	5.93	6.49	6.50	6.51
3	6.31	6.34	6.34	5.94	5.96	5.98	6.51	6.51	6.50
5	6.34	6.34	6.34	5.96	5.97	5.98	6.54	6.53	6.53
7	6.35	6.36	6.38	5.99	6.00	6.01	6.54	6.52	6.54
Days at 24-25°C									
1	6.31	6.33	6.32	6.03	6.01	6.03	6.55	6.54	6.54
2	6.36	6.31	6.27	6.02	6.03	6.05	6.48	6.52	6.49

[a]In ppm.

PART V. ADAPTATION OF A METHOD FOR THE DETERMINATION OF SOLUBLE ORTHOPHOSPHATES IN COOKED AND UNCOOKED PORK CONTAINING ACID-LABILE POLY- AND PYROPHOSPHATES

by

R. A. Molins, A. A. Kraft, and D. G. Olson
Departments of Food Technology and Animal Science
Iowa State University
Ames, Iowa 50011

Running Title: Phosphate analysis of meats

ABSTRACT

A colorimetric method developed for determining μg levels of soluble orthophosphates in soils was successfully used in cooked and uncooked pork to monitor hydrolysis and residual levels of added sodium acid pyrophosphate (SAPP). The method is based on the formation of blue color by reacting existing orthophosphate with molybdate ions in an acid medium and preventing further reaction by rapidly sequestering excess, unreacted molybdenum ions with a citrate-arsenite reagent. The usefulness of the method to monitor poly- and pyrophosphate hydrolysis in meat is discussed.

INTRODUCTION

Poly- and pyrophosphates, particularly sodium acid pyrophosphate (SAPP) have been found to be potentially inhibitory to bacteria in meat products (Nelson et al., 1983; Wagner and Busta, 1983; Molins et al., 1985). It would be beneficial for future studies to have a rapid, simple and accurate method to determine residual levels of the added phosphates. Hydrolysis of poly- and pyrophosphates has been shown to have orthophosphate as a common end product (Bell, 1947; Awad, 1968; Tenhet et al., 1981). Therefore, an increase in orthophosphate levels over time could constitute an indirect measurement of pyrophosphate hydrolysis. Unfortunately, most methods used to determine soluble orthophosphates are complex or, most importantly, hydrolytic (Netherton et al., 1955; Rieman and Beukenkamp, 1961; Murphy and Riley, 1962) and consequently more suited for total phosphorous analysis. Dick and Tabatabai (1977) modified a colorimetric method for determining soluble orthophosphates in aqueous solutions, based on the development of a measurable blue color by reacting ortho-P and molybdate ions in the presence of ascorbic and trichloroacetic acids. Unlike the original method of Murphy and Riley (1962), interference by orthophosphates originating from hydrolysis of acid-labile phosphorous compounds is prevented by chelation of excessive, unreacted molybdenum ions with a citrate-arsenite reagent. The present work was undertaken to test and

adapt this rapid method to the measurement of soluble orthophosphate levels in cooked and uncooked meat containing sodium acid pyrophosphate (SAPP) or sodium tripolyphosphate (STPP).

MATERIALS AND METHODS

Reagents and standard curve (Dick and Tabatabai, 1977): 1) Standard phosphate stock solution: dissolve 0.439 g of KH_2PO_4 in 700 ml of deionized water in a volumetric flask and take volume to 1 l (solution contains 100 μg/ml ortho-P). 2) Reagent A: dissolve 8.8 g ascorbic acid and 41 g trichloroacetic acid in 400 ml deionized water and complete volume to 500 ml (solution must be prepared daily). 3) Reagent B: dissolve 6.2 g ammonium molybdate in 400 ml deionized water and adjust volume to 500 ml. 4) Reagent C: dissolve 29.4 g sodium citrate and 26.0 g sodium arsenite in 800 ml of water, add 50 ml glacial acetic acid (99.9%) and adjust volume to 1 l. A standard curve was prepared by analyzing 1-ml aliquots of a series of dilutions of the standard solution containing known levels of 0, 5, 10, 15, 20 and 25 μg of orthophosphate/ml, determining the absorbance values at 690 nm as described under analysis and plotting μg of ortho-P/ml against absorbance values. Sample preparation and analysis: 500-g portions of pork butts (20% fat) ground through a 1/8 in. dia. plate were blended 2 min with a 10% (w/v) aqueous solution of sodium acid pyrophosphate (SAPP) (Stauffer Chemical Co., Westport, CT) or sodium tripolyphosphate (STPP) (Monsanto, St. Louis, MO) to give 0.5% (w/w) phosphate in the mix. A control batch received an equivalent volume of distilled water. Each mix was halved and placed in mason jars: one-half was cooked to 65.5°C (determined potentiometrically) by immersing the jar in a water bath at 80°C and the other half was left uncooked. Samples were stored

at 5°C and analyzed on days 0, 1, 2, 4 and 6. An extract was prepared by blending 30 g of meat with 270 ml of sterile distilled water in a Stomacher Lab Blender 400 (Tekmar Co., Cincinnati, OH) for 2 min. The extract (a 1:10 dilution of the meat) was further diluted 1:10 with deionized water to be within measurable range (1-20 μg ortho-P/ml), since previous trials had shown the natural soluble orthophosphate content of the meat to be 926-986 μg/g (3.1% maximum variation). The analytical procedure of Dick and Tabatabai (1977) was followed with some modifications: one ml of diluted extract was pipetted into a 50-ml beaker containing 10 ml of reagent A, followed immediately by 2 ml of reagent B and 5 ml of reagent C, carefully swirling after each addition. Blue color developed within 10 min and absorbance was determined at 690 nm within 1 h with a Bausch and Lomb Spectronic 10 spectrophotometer equipped with tungsten light source and red filter, zeroed with a blank prepared as before using 1 ml deionized water in lieu of sample. The corresponding soluble ortho-P content was found by multiplying the observed value derived from the standard curve by the dilution factor and expressing the result in μg soluble orthophosphate/g of meat. The sensitivity of the determination (1-20 μg ortho-P/ml) required that glassware be washed with unphosphated detergents or concentrated HCl. Experiments were replicated three times and the data analyzed by using an SAS computer program with GLM option.

RESULTS AND DISCUSSION

Fig. 1 depicts the changes in soluble orthophosphate content of cooked and uncooked ground pork meat with or without added phosphates during storage at 5°C. STPP-treated, cooked meat paralleled control samples throughout, while those containing SAPP showed values 300-350 μg/g higher than STPP or control samples after 1 day, possibly as a result of heat-induced hydrolysis of SAPP during cooking. After 4 days at 5°C, the soluble ortho-P content of all samples increased at constant but different rates, the highest being those of SAPP-treated meat. This increase could not be attributed only to SAPP or STPP hydrolysis because it also occurred in control samples. Rather, spoilage caused by bacterial growth (total psychrotrophic bacterial numbers were 10^7-10^8/g in uncooked samples on day 6) and decomposition of meat phosphorous compounds that coincided with the onset of off-odors may have been implicated. Constant increases were observed in uncooked meat containing SAPP or STPP since day 0, suggesting that enzymatic hydrolysis of phosphates by meat phosphatases occurred, as indicated also by Awad (1968) and Tenhet et al. (1981). One mole of STPP is known to hydrolyze to one mole of ortho-P and one of pyrophosphate (which can in turn be hydrolyzed to ortho-P), while one mole of SAPP directly yields two of orthophosphate (Tenhet et al., 1981; Awad, 1968; Bell, 1947). Therefore, unlike STPP, increases in the level of soluble ortho-P in uncooked meat containing SAPP could be translated into percent total SAPP hydrolyzed and by difference, into residual SAPP level in the

meat. The values obtained in cooked meat indicate that until day 4 at 5°C no hydrolysis of SAPP occurred beyond that apparently induced by cooking. Unlike the findings of Dick and Tabatabai (1977), the blue color was unstable one hour after the analysis due to precipitation, which was more noticeable the lower the dilution used (i.e., the more particulate matter present). Determining absorbance within 1 h of the analysis gave stable results (standard error: 11.6 μg/g). The optimum dilution protocol was found to be 1 part of meat to 10 parts of deionized water for extracting, followed by a second 1:10 dilution. A further 1:1 dilution would be necessary in samples with soluble ortho-P concentrations above 2000 μg/g. Considering the high natural soluble ortho-P level in pork meat and its variation and the need to dilute extracts to within range (1-20 μg/g), this analytical method would not detect ortho-P differences below 100 μg/g.

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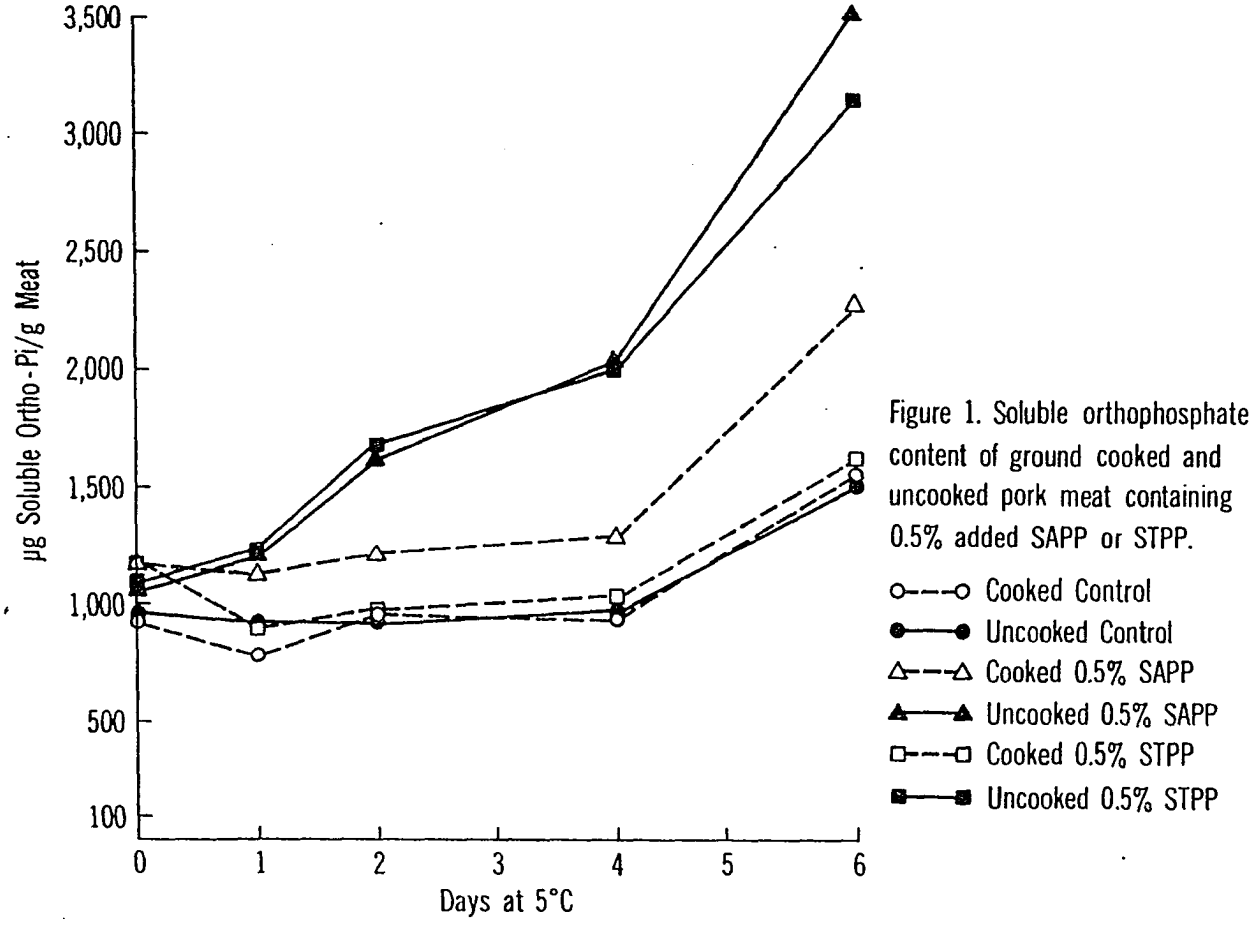

Figure 1. Soluble orthophosphate content of ground cooked and uncooked pork meat containing 0.5% added SAPP or STPP.

Figure 1. Soluble orthophosphate content of ground cooked and uncooked pork meat containing 0.5% added SAPP or STPP

Table 1. Soluble orthophosphate content of cooked and uncooked ground pork meat with and without added phosphates[a] (μg ortho-P/g of meat)

Days at 5°C	CONTROL		0.5% STPP		0.5% SAPP					
	U[b]	C[c]	U	C	U	% loss[d]	% res[e].	C	% loss	% res.
0	965	935	1096	1178	1062	21.3	0.39	1172	23.5	0.38
1	926	789	1238	892	1202	24.1	0.38	1023	20.5	0.40
2	935	956	1679	968	1625	32.5	0.34	1211	24.3	0.38
4	959	947	1998	1032	2020	40.5	0.30	1297	26.0	0.37
6	1521	1555	3146	1627	3511	70.3	0.15	2283	45.7	0.27

[a]Standard error: 11.6 μg/g.
[b]Uncooked.
[c]Cooked to 65.5°C internal temperature.
[d]Percent total SAPPP hydrolyzed.
[e]Residual SAPP as % of meat weight.

PART VI. EFFECT OF INORGANIC POLYPHOSPHATES ON GROUND BEEF CHARACTERISTICS

by

R. A. Molins, A. A. Kraft, H. W. Walker, R. E. Rust, and D. G. Olson

Departments of Food Technology and Animal Science

Iowa State University

Ames, Iowa 50011

ABSTRACT

Chemical, physical, organoleptic and microbiological effects of 0.4% sodium tripolyphosphate, tetrasodium pyrophosphate, and four phosphate blends were studied in frozen beef patties (90 days, -20°C). Phosphates had no significant ($P>0.05$) antimicrobial effects but reduced mesophilic, psychrotrophic and lactic acid bacterial numbers during frozen storage of the patties and significantly ($P<0.05$) inhibited bacterial growth upon subsequent elevated temperature holding. pH, soluble orthophosphates, Hunter color values, cook yields and overall acceptability scores significantly ($P<0.05$) increased with phosphate addition. Texture and flavor also improved. Phosphates significantly ($P<0.05$) lowered TBA numbers but seemed to interfere with the analysis. Overall quality of patties was improved most by adding phosphates in solution rather than in powder form.

INTRODUCTION

Since the addition of various poly- and pyrophosphates to cured and processed meat products was approved (USDA, 1982) their use has gained widespread acceptance within the industry because of possible economic and quality advantages. Improved water retention and subsequent higher cook yields, increased binding, higher palatability scores in terms of texture, juiciness and flavor, have been extensively reported to result from alkaline phosphate inclusion in the formulation of frankfurters (Hargett et al., 1980) and bologna (Swift and Ellis, 1956). Phosphates have also resulted in higher quality products when used in ham curing brine (Siegel et al., 1978; Vollmar and Melton, 1981), or when added to beef rolls (Moore et al., 1976) or to frozen pork patties (Keeton, 1983). Phosphates reportedly improved the physical, chemical and microbiological quality of chicken meat when used in solutions for chilling chicken carcasses (Klose et al., 1963; Landes, 1972; Shults and Wierbicki, 1973) or presoaking chicken parts (Chen et al., 1973). Quality improvements from phosphate addition were also reported by Knipe (1982) to occur in meat emulsions. Hot-processed pork loins injected to 110% of weight with a 5% solution of a sodium polyphosphate, glassy, (SPG) and tetrasodium pyrophosphate (TSPP) mixture resulted in juicier, more tender, heavier pork chops which, however, also exhibited more thaw loss and less browning on cooking (Hoes et al., 1980).

In terms of microbiological quality, phosphates have been shown to be inhibitory to various bacterial types and to enhance the

anticlostridial activity of sodium nitrite in cooked, vacuum packaged, refrigerated bratwurst (Molins et al., 1985a). Sodium acid pyrophosphate (SAPP) has also been reported to increase nitrite effects against inoculated Clostridium botulinum in beef/pork frankfurter emulsions (Wagner and Busta, 1983), while no significant phosphate effect on total bacterial counts was found in chicken frankfurter emulsions kept at 27°C (Nelson et al., 1983). In fresh meats, phosphates were only mildly inhibitory against the natural mesophilic and psychrotrophic bacterial flora of uncooked, bratwurst-type sausage held at 5°C (Molins et al., 1985b), possibly as a result of poly- and pyrophosphate hydrolysis to orthophosphates, as discussed by Awad (1968) and by Sutton (1973). Snyder and Maxcy (1979) examined the effect of 0.5% SPG on Moraxella-Acinetobacter in ground beef and pork meat, and concluded that the inhibitory activity of that phosphate was due to lowered water activity of the meat. Hoes et al. (1980) found higher total mesophilic microbial counts in phosphate-injected, hot-processed pork loins.

The study of phosphate effects in uncooked whole and ground muscle foods is lacking. Although it is not anticipated that phosphates will soon be approved as fresh meat additives, the present study was undertaken to determine the possible contribution of 0.4% pure phosphates or phosphate blends to some physical, chemical and microbiological quality characteristics of frozen beef patties prepared and stored for use similar to that for commercial fast-food operations.

MATERIALS AND METHODS

Preparation of Beef Patties

Beef trim (90/10) and fat (50/50) were obtained from a commercial source, ground through a 3/8 in. dia. plate, mixed for 2 min in a Blonco mixer in the necessary ratio to obtain 20% fat in the blend, ground for 2 min through a 1/8 in. dia. bone removal plate and divided into 7 25-lb batches. Each received the appropriate amounts of one phosphate treatment, either as a powder (to 0.4% of meat weight) or as a 16% (w/v) aqueous soln. (to 2.5% of meat weight) to obtain a 0.4% final concentration of phosphate in all batches except one that received no phosphate and was used as control. After mixing for 2 min in a Blonco mixer to throughly distribute the added phosphate, the meat was formed into 0.25-lb patties by means of a Hollymatic 500 patty making machine. The patties were separated from each other with waxed parchment paper squares, wrapped in plastic freezer bags in groups of 25, twist-tied, placed in cardboard boxes and labeled according to treatment:

1. Control (no phosphates added).
2. Brifisol 414B (added as powder, 0.4% of meat weight).
3. Brifisol 414B (added as 16% aqueous soln., 2.5% of meat weight).
4. Brifisol 414P (added as powder, 0.4% of meat weight).
5. Brifisol 614P (added as powder, 0.4% of meat weight).

6. Sodium tripolyphosphate (STPP) (added as powder, 0.4% of meat weight).
7. Tetrasodium pyrophosphate (TSPP) (added as 16% aqueous soln., 2.5% of meat weight)

Brifisol phosphate blends (unspecified composition) were provided by Benckiser-Knapsack Gm bH (Ladenburg, Fed. Rep. Germany). Sodium tripolyphosphate (STPP) and tetrasodium pyrophosphate (TSPP) were commercially available (Monsanto, St. Louis, MO).

The boxed patties were frozen in an air blast freezer and kept frozen at -20°C for the duration of the study (90 days). Freezing was considered to be complete after 48 h. Four replications of the experiment were initiated at one week intervals.

Sampling

Microbiological, pH and soluble orthophosphate determinations were done on fresh patties on the day of manufacture (day 0) and on frozen patties thereafter, while TBA numbers, Hunter color values and sensory evaluations began on day 2 after manufacture, considered to be the first day of frozen storage at -20°C. Thereafter, sampling was done on days 7, 30, 60 and 90 of frozen storage at -20°C.

Duplicate samples were obtained for microbiological evaluation on each sampling day. Approximately 15 g were aseptically taken from each patty, weighed to make a 30-g composite sample, placed in a sterile plastic bag containing 270 ml of sterile 0.1% peptone water and blended

in a Stomacher 400 Lab Blender (Tekmar Co., Cincinnati, OH) for 2 minutes. Serial dilutions were prepared from the resulting extract according to standard methods and used for microbiological and soluble orthophosphate determinations. Trypticase soy agar (TSA, BBL) was the medium used to enumerate total mesophilic (30°C, 48 h) and psychrotrophic (5°C, 7-10 days) bacteria. Lactic acid bacteria were recovered in lactobacilli selective agar (LBS, BBL) and counted after incubation at 30°C for 48 h (Rogosa et al., 1951). Baird-Parker agar base (DIFCO) was used to identify and enumerate presumptive Staphylococcus aureus (37°C, 24 and 48 h) (Baird-Parker, 1962). To determine viable anaerobic spore counts, 10 ml of the initial extract were pipetted into sterile tubes, heat-shocked in a water bath at 80°C for 20 minutes, allowed to cool at room temperature and plated with TSA containing 0.1% soluble starch using the plastic pouch method (37°C, 48 h) of Bladel and Greenberg (1965).

pH values were determined with a Radiometer 28 meter equipped with an Orion 9163 probe after thawing the remaining portions of the patties at room temperature.

Analysis of orthophosphates was done by the method of Dick and Tabatabai (1977) as adapted to meats by Molins et al. (1985c).

For comparative purposes, the remaining portions of the patties analyzed on day 90 of frozen storage at -20°C were repackaged in aluminum foil, kept at room temperature (24-25°C) for 24 h and reanalyzed for bacterial numbers, pH and soluble orthophosphates as before. This was done to evaluate effects of phosphates on

"temperature-abused" products.

The possible effect of phosphates in preventing lipid oxidation was determined by measuring thiobarbituric acid values (TBA) according to the method of Tarladgis et al. (1960).

Possible color changes in phosphate-treated patties were monitored throughout the entire frozen storage period by determining Hunter L, a and b values.

Proximate analysis (moisture, fat and protein) was done on the first day of frozen storage at -20°C (48 h after manufacture). A.O.A.C. (1980) methods were followed.

Sensory evaluation of the treatments included juiciness, texture, flavor and overall acceptability. On sampling days, 10 patties per treatment were cooked on a gas grill set at 400°F (204°C) for 2.5 minutes on one side, turned over and cooked for additional 2 minutes to simulate a normal fast-food restaurant cooking operation procedure. Cooking losses were determined by weighing 5 of the 10 patties before and after cooking and recording the difference. All patties were cut into 9 portions and presented on plates to an untrained taste panel consisting of an average of 35 members. Flavor, texture, juiciness and overall acceptability were evaluated on a 7-point hedonic scale ranging from "like extremely" to "dislike extremely".

Microbiological, pH and soluble orthophosphate determinations were performed at the Iowa State University Food Research Laboratory; all other chemical and organoleptic determinations were carried out by personnel of the Iowa State University Meat Science Laboratory.

Microbiological data were transformed into logarithms and all data from the four replications were analyzed using an Statistical Analysis System (SAS) computer program with Analysis of Variance and General Linear Model options. Comparison of means was based on Duncan's multiple range test.

RESULTS AND DISCUSSION

Although patties treated with phosphates other than Brifisol 614P had consistently lower bacterial numbers than controls, no significant ($P>0.05$) microbiological effect could be attributed to phosphate addition upon freezing the beef patties or throughout the 90-day storage period at -20°C. Bacterial counts decreased by less than one logarithmic cycle from day 0 to 90 (Figures 1-4) for all treatments. Brifisol 614P appeared to have some protective effect against bacterial freeze injury which manifested itself in higher bacterial recoveries than any other treatment.

In general, TSPP, STPP and Brifisol 414B added as powder, in that order, contributed best to the microbiological quality of the patties in that they had lowest bacterial counts throughout the frozen storage period. Brifisol 614P, in contrast, resulted in the highest total mesophilic counts and in significantly ($P<0.05$) higher psychrotrophic bacterial populations than the other Brifisol blends and control considered as a group. Phosphate addition had no effect on anaerobic spore viability after 90 days of storage at -20°C.

When the patties were temperature-abused at 24-25°C for 24 h after the 90-day frozen storage period, however, phosphates as a group significantly ($P<0.05$) inhibited the growth of mesophilic, psychrotrophic and lactic acid bacteria. Under those conditions, 0.4% Brifisol 414B added to the meat as a 16% (w/v) aqueous solution exhibited significant ($P<0.05$) antimicrobial properties against

mesophilic, psychrotrophic and lactic acid microorganisms, followed in decreasing order of effectiveness by TSPP and Brifisol 414B added in powder form. Similar to results for frozen storage of patties, Brifisol 614P resulted in the highest numbers of mesophilic and psychrotrophic bacteria and in significantly ($P<0.05$) higher populations of these microorganisms when contrasted against all other Brifisol treatments grouped together. Despite the inhibitory effects exhibited by some phosphates, it should be noted that after 24 h at 24-25°C total bacterial counts in all patties, regardless of treatment, were high enough (10^8-10^9/g) to indicate spoilage.

Because of its implications in terms of processing, it must also be noted that adding the phosphates in solution resulted in significantly ($P<0.05$) lower numbers of all bacterial types studied than incorporating the phosphates in powder form. This result might be related to improved distribution and diffusion of the added phosphate solution through the meat mass, while addition in powder form may cause accumulation of the polyphosphates on the immediate surface of contact, complexing with protein and resulting in slower rates of migration as advanced by Tenhet et al. (1981).

The pH value of beef patties throughout the experimental period (including the final 24 h under temperature abuse conditions) are contained in Table 1. TSPP increased the pH of the meat more than any other phosphate or blend tested, followed by Brifisol 614P>STPP>Brifisol 414B in solution>Brifisol 414P>Brifisol 414B powder>control.

Table 2 shows that there was also an immediate increase in the

level of soluble orthophosphates present in the meat after the addition of any of the phosphates tested. This increase was highest in patties treated with TSPP and continued in all patties containing phosphates during the entire experimental period, indicating that hydrolysis of the condensed phosphates proceeded even at a temperature as low as -20°C. The rate of phosphate hydrolysis markedly increased when the patties were kept at 24-25°C for 24 h. In agreement with the results of previous studies (Molins et al., 1985a), those phosphates that contributed the highest soluble orthophosphate levels were also the most effective bacterial inhibitors, although the nature of a possible relationship between these two effects has not yet been established.

There appeared to be a pattern in the arrangement of treatments according to soluble orthophosphate content and pH such that higher soluble orthophosphate levels in the patties corresponded to higher pH values, the parallel becoming more noticeable the longer the frozen storage time at -20°C. This result corroborates the findings of Awad (1968), who theorized that the ability of condensed phosphates to increase the pH of meat is related to the extent to which a particular phosphate is degraded to orthophosphates. In our study, the above-mentioned parallel can be extended to include higher Hunter L and Hunter b values (Tables 4 and 6, respectively), as well as higher texture (Table 9), flavor (Table 10) and overall acceptability scores (Table 11). In the case of Brifisol 414B added in solution and TSPP (also added in solution), this apparent relationship also included best (most desirable) effect in retarding microbial growth (i.e., lowest bacterial

numbers) upon temperature-abusing the patties as discussed earlier.

Thiobarbituric acid (TBA) values were significantly ($P<0.05$) lower in all phosphate-containing patties than in controls (Table 3). TBA values of Brifisol 414B (powder) were lowest, followed by TSPP<Brifisol 614P<Brifisol 414B (solution)< STPP<Brifisol 414P. However, it is important to observe that TBA numbers in patties treated with phosphates were lower than in control patties since the first determination, on day 0 of frozen storage, indicating that phosphate interference with the analytical method of Tarladgis et al. (1960) may have occurred.

No significant ($P>0.05$) differences in proximate analysis could be attributed to any phosphate treatment, although control samples had the highest moisture levels. Hunter L (lightness) and Hunter b (yellowness) values indicated that phosphates did not cause any significant ($P>0.05$) discoloration of beef patties (Tables 4 and 5, respectively). However, the three Brifisol 414 blends had lower Hunter L values than controls, suggesting darker patties. Redness, on the other hand, was significantly ($P<0.05$) higher in all phosphate-treated patties when measured by Hunter a values and highest with Brifisol 614P. An overall color evaluation indicated that frozen storage at -20°C significantly ($P<0.05$) affected color: lightness of the patties significantly ($P<0.05$) increased and redness significantly ($P<0.05$) decreased after one week at -20°C regardless of treatment.

Cooking losses were found to be significantly ($P<0.05$) higher in control samples than in any phosphate-containing patties, but no such differences existed among phosphate treatments (Table 7). Best cook

yields corresponded to Brifisol 414B (powder), followed by TSPP and Brifisol 414B (solution). Brifisol 614P gave the highest cooking loss among the phosphate blends tested; however, they were higher in STPP-treated samples. Storing the patties for 1 week at -20°C was found to result in significantly ($P<0.05$) lower cooking losses than keeping them frozen for 8 or 12 weeks. Frozen storage for 4 weeks resulted in the largest decrease in cook yields. Scores for juiciness (Table 8) were inversely related to those of cooking loss. Accordingly, the juiciest patties were judged to be those containing Brifisol 414B (powder), TSPP and Brifisol 414B (solution).

Although phosphates did not significantly ($P>0.05$) improve texture scores (Table 9) all phosphate treatments produced higher scores than obtained in the absence of phosphates (controls). Brifisol 614P scored highest in texture acceptability, followed by Brifisol 414B added in solution. This order was reversed when flavor scores were considered. All patties containing phosphates had higher flavor scores than patties with no phosphate, although the differences with control samples were not statistically significant ($P<0.05$, Table 10).

Overall, treating beef patties with phosphates resulted in significant ($P<0.05$) improvements in sensory quality (Table 11). Patties that received phosphates in solution had the highest overall acceptability scores and also the best microbiological quality: Brifisol

414B and TSPP ranked high. Brifisol 614P ranked third in overall acceptability scores but last in microbiological quality of frozen beef patties. Brifisol 414P or STPP addition did not provide sensory or microbiological advantages in this study.

CONCLUSIONS

Addition of 0.4% levels of the pure or blended phosphates tested does not significantly ($P>0.05$) cause a reduction in the numbers of mesophilic, psychrotrophic or lactic acid bacteria or presumptive Staphylococcus aureus or viable anaerobic spores in beef patties stored at -20°C for up to 90 days, but does result in lower bacterial populations than in the absence of phosphates.

All phosphates tested except Brifisol 614P have a significantly ($P<0.05$) inhibitory effect on the growth of mesophilic, psychrotrophic and lactic acid bacteria in patties subjected to 24 h of temperature abuse at 24-25°C. This inhibition is significantly ($P<0.05$) higher when the phosphates are added to the meat in solution. The most effective phosphate tested in terms of antimicrobial activity is Brifisol 414B (solution). Brifisol 614P adversely affects the microbiological quality of beef patties.

All phosphates studied increase the pH and the soluble orthophosphate content of beef patties. Hydrolysis of phosphates added to ground beef takes place even during frozen storage at -20°C.

Addition of phosphates appears to significantly ($P<0.05$) reduce the development of oxidative rancidity in frozen beef patties, but there are indications that phosphates interfere with the TBA analytical method.

The phosphates studied do not affect proximate analysis of frozen beef patties but result in significantly ($P<0.05$) higher Hunter a values, cook yields and overall acceptability scores. Texture and

flavor also improve with phosphate addition but not to a statistically significant ($P<0.05$) extent.

Among the phosphate treatments tested, overall quality of frozen beef patties can be best improved with addition of a 16% aqueous (w/v) solution of Brifisol 414B or TSPP to a level of 0.4% (w/w) of meat weight.

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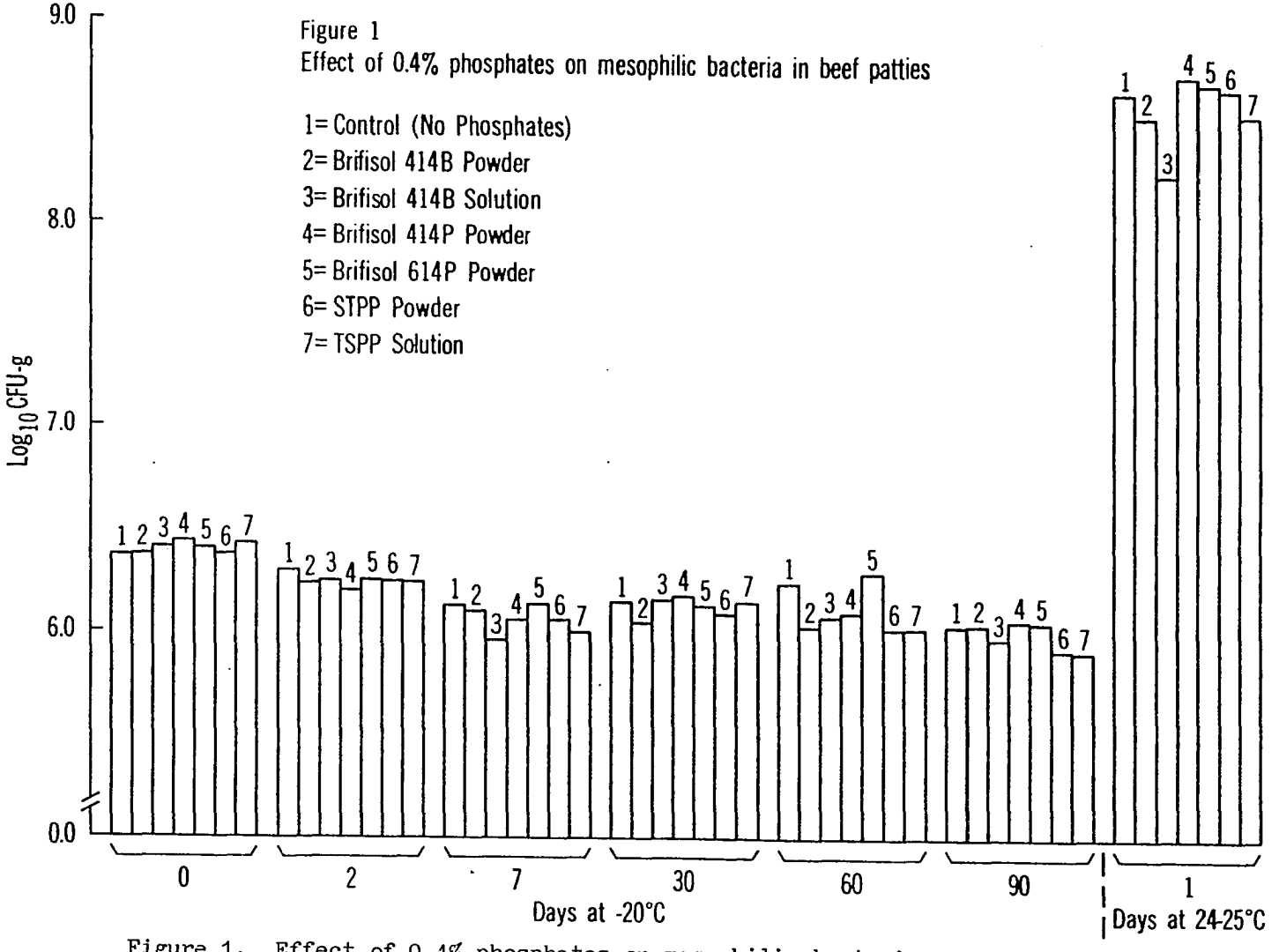

Figure 1. Effect of 0.4% phosphates on mesophilic bacteria in beef patties

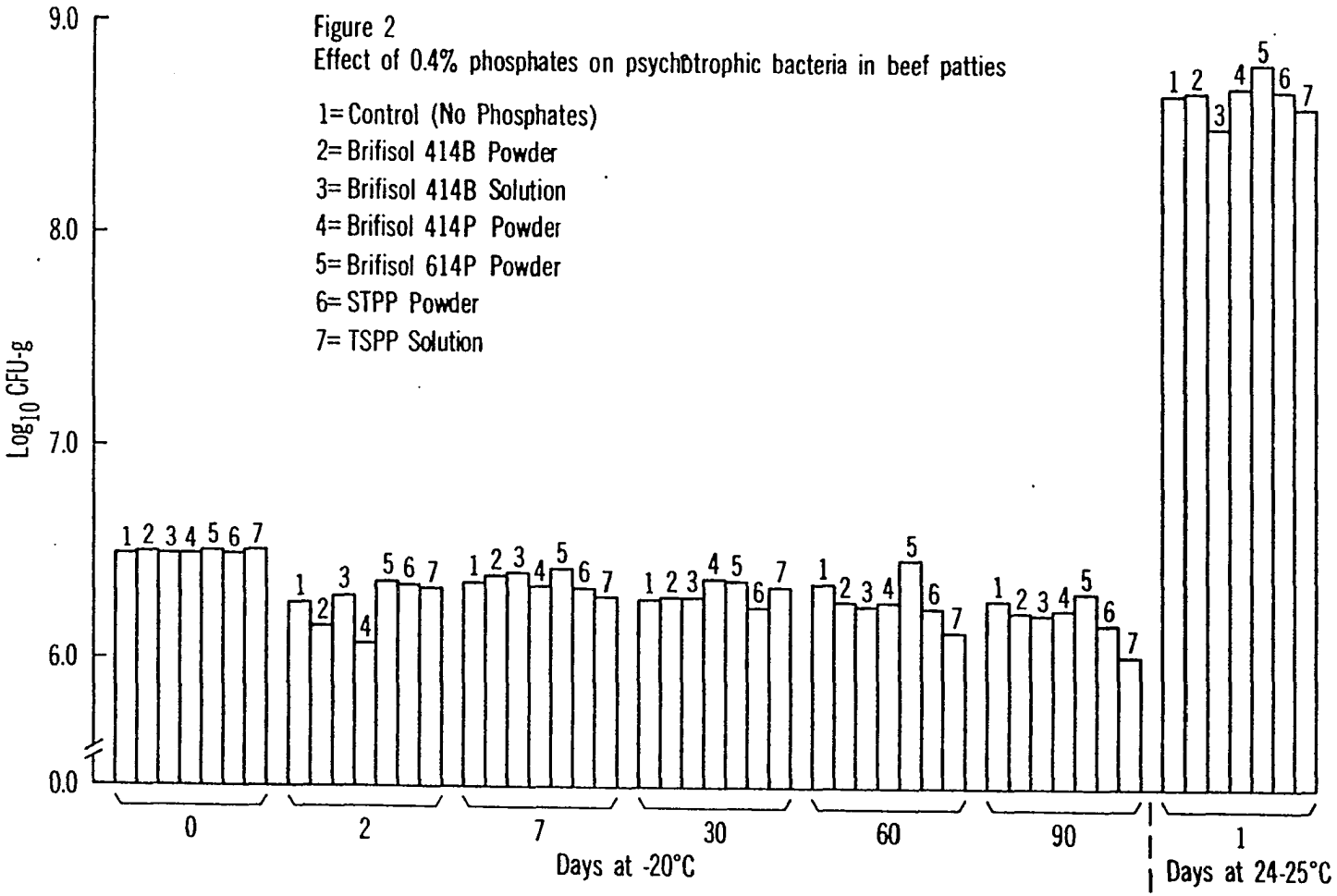

Figure 2. Effect of 0.4% phosphates on psychrotrophic bacteria in beef patties

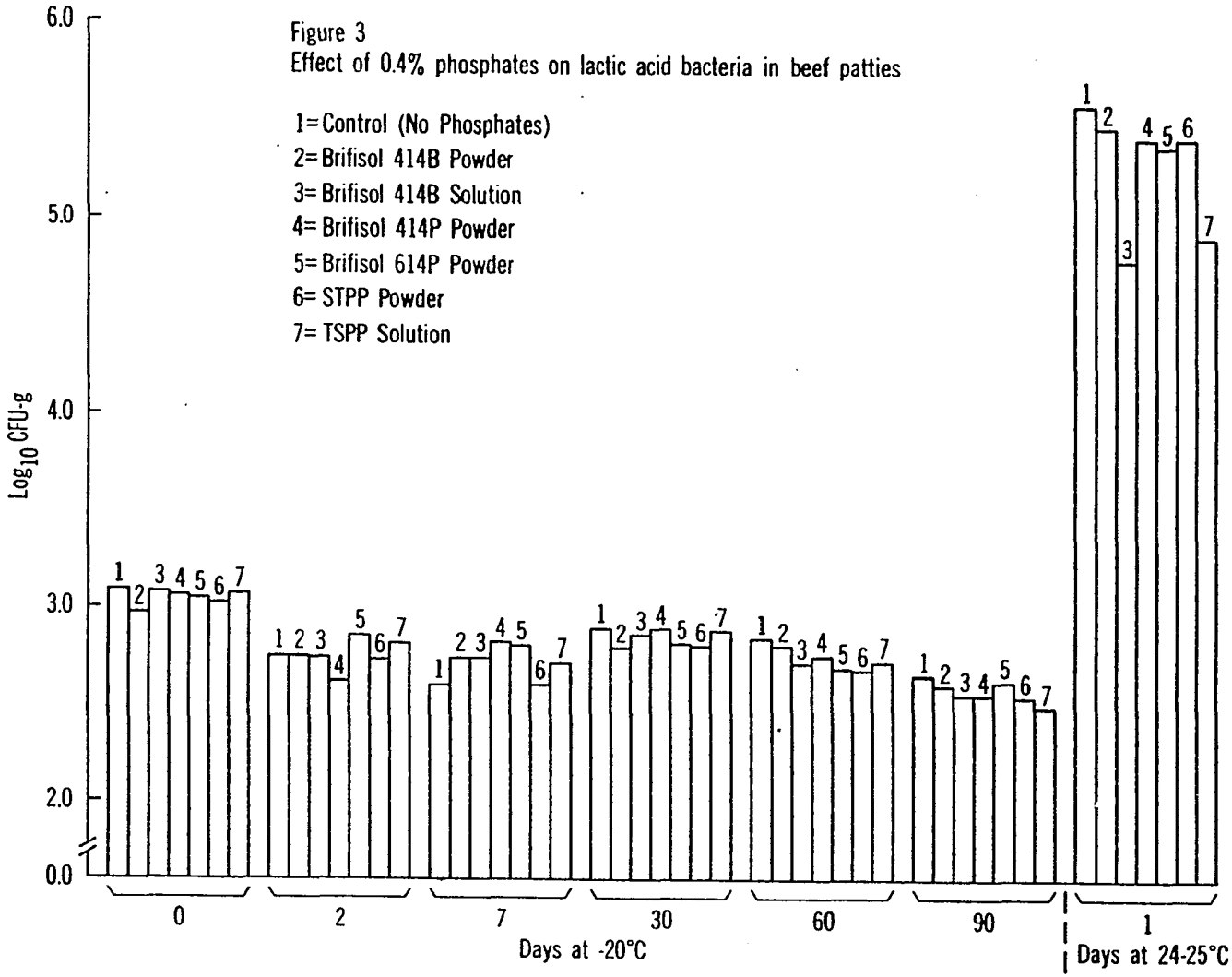

Figure 3. Effect of 0.4% phosphates on lactic acid bacteria in beef patties

Figure 4
Effect of 0.4% phosphates on presumptive Staphylococcus aureus

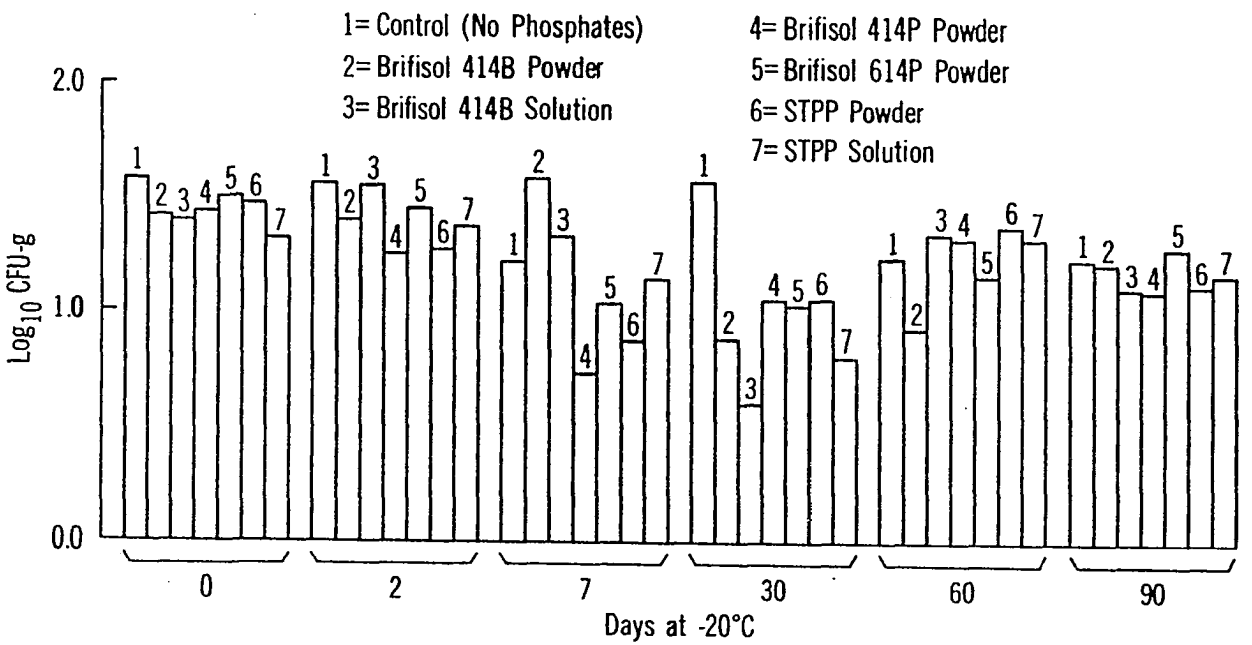

Figure 4. Effect of 0.4% phosphates on presumptive Staphylococcus aureus counts in beef patties

Table 1. Average pH values of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
0	5.95	6.10	6.20	6.17	6.31	6.23	6.50
2	5.97	6.10	6.19	6.15	6.30	6.22	6.45
7	5.96	6.09	6.20	6.12	6.30	6.21	6.47
30	5.94	6.09	6.20	6.14	6.28	6.21	6.43
60	6.02	6.12	6.21	6.16	6.31	6.24	6.47
90	5.99	6.08	6.19	6.15	6.30	6.25	6.46
DAYS AT 24-25°C							
1	5.86	5.99	6.17	6.06	6.25	6.12	6.41

[a]Standard error: 0.01.

Table 2. Average soluble orthophosphate content of beef patties[a] ($\mu g/g$ of meat)

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
0	817	904	836	952	1075	1186	1010
2	922	1084	1060	1090	1151	1292	1183
7	837	1042	1106	1037	1185	1307	1126
30	809	1017	1043	1109	1082	1206	1148
60	891	1167	1157	1065	1211	1250	1232
90	775	1152	1220	1094	1179	1301	1226
DAYS AT 24-25°C							
1	829	1816	2255	1708	1681	1842	2325

[a]Standard error: 16.

Table 3. Average TBA numbers of beef patties[a]
(mg malonaldehyde / 100 g tissue)

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
0	0.960	0.281	0.295	0.337	0.301	0.297	0.247
1	1.190	0.230	0.282	0.261	0.261	0.269	0.259
4	1.115	0.210	0.250	0.296	0.263	0.266	0.239
8	1.198	0.286	0.325	0.329	0.338	0.354	0.330
12	1.288	0.299	0.369	0.394	0.323	0.349	0.305

[a]Standard error: 0.006.

Table 4. Average HUNTER L values of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
0	39.1	39.0	39.7	39.7	40.4	39.9	40.3
1	41.1	40.6	40.2	39.8	40.4	40.6	41.0
4	41.0	41.1	40.5	39.4	40.9	41.1	41.0
8	40.6	41.7	40.1	40.1	39.9	41.2	40.9
12	39.3	37.9	38.2	37.6	39.5	38.3	38.3

[a]Standard error: 0.2.

Table 5. Average HUNTER a values of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
0	13.8	15.0	16.0	17.0	16.8	16.6	15.7
1	11.7	11.6	13.2	12.4	12.5	12.6	12.2
4	11.3	11.6	12.5	12.0	13.1	13.2	12.4
8	10.4	10.5	10.3	11.0	11.6	10.5	10.9
12	10.4	10.8	11.3	11.8	11.8	11.1	10.6

[a]Standard error: 0.1.

Table 6. Average HUNTER b values of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
0	9.9	10.1	10.4	10.5	10.4	10.5	10.0
1	9.6	6.9	7.4	9.5	9.5	9.5	9.4
4	9.7	10.0	9.8	9.6	9.8	10.3	10.2
8	8.6	9.0	8.6	8.7	8.9	8.4	9.0
12	9.3	9.7	9.5	9.5	9.7	9.8	9.6

[a]Standard error: 0.1.

Table 7. Average cook loss of beef patties[a]

(%)

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
1	21.9	13.7	18.4	16.0	18.4	18.9	15.4
4	26.5	19.8	19.4	21.9	20.2	24.3	21.6
8	25.2	16.4	18.0	21.1	19.9	20.9	18.1
12	25.0	16.1	18.7	19.4	20.0	23.1	18.2

[a]Standard error: 0.3.

Table 8. Average juiciness scores of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
1	4.0	4.7	4.5	4.4	4.5	4.3	4.7
4	4.0	4.8	4.6	4.6	4.7	4.1	4.8
8	3.7	5.0	4.8	4.2	4.4	4.3	4.6
12	3.9	5.0	4.6	4.5	4.6	4.5	5.1

[a]Standard error: 0.04.

Table 9. Average texture scores of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
1	4.2	4.3	4.4	4.1	4.7	4.4	4.6
4	4.2	4.4	4.6	4.4	4.5	4.3	4.6
8	3.9	4.3	4.7	3.9	4.5	4.2	4.1
12	4.1	4.6	4.5	4.5	4.5	4.5	4.8

[a]Standard error: 0.03.

Table 10. Average flavor scores of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
1	4.1	4.4	4.5	4.0	4.9	4.6	4.3
4	4.2	4.5	4.6	4.6	4.6	4.6	4.6
8	4.1	4.5	4.7	4.1	4.4	4.3	4.2
12	4.2	4.7	4.6	4.4	4.4	4.7	4.8

[a]Standard error: 0.03.

Table 11. Average overall acceptability scores of beef patties[a]

WEEKS AT -20°C	Control	414B powder	414B soln.	414P powder	614P powder	STPP powder	TSPP soln.
1	3.9	4.2	4.2	4.0	4.6	4.4	4.4
4	4.1	4.4	4.4	4.5	4.5	4.3	4.4
8	3.8	4.5	4.7	4.0	4.2	4.2	4.2
12	3.9	4.6	4.5	4.4	4.5	4.5	4.7

[a]Standard error: 0.03.

SUMMARY

The antimicrobial activity of poly- and pyrophosphates was demonstrated throughout this study, although the effectiveness of the various phosphates tested varied widely depending on the medium or system in which the phosphates were tested. In the first phase of this work, under laboratory conditions, 0.5% unheated tetrasodium pyrophosphate (TSPP), sodium tripolyphosphate (STPP) and sodium polyphosphate glassy (SPG) added to bacterial growth media were highly inhibitory to Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus Z88 and two commercial lactic starter cultures. "Petite" colony formation in phosphate-containing media was consistently observed. Younger cultures (3 h) and gram-positive organisms were more affected by phosphates than older cultures (24 h) and gram-negative bacteria. With the exception of TSPP, alkaline phosphates lost all or most antibacterial properties when they were incorporated into the media and subsequently heated to sterilizing or boiling temperatures. That finding suggested that heat-induced hydrolysis of the active (i.e., unhydrolyzed) species, reactions with, or absorption by, media components neutralized the activity of phosphates upon heating. The acid phosphate, sodium acid pyrophosphate (SAPP), heated or unheated, was ineffective as a bacterial growth inhibitor in laboratory media.

In the second part of this investigation, similar addition of 0.5% phosphates to uncooked, refrigerated (5°C) bratwurst did not result in any significant ($P>0.05$) inhibition of the natural mesophilic and

psychrotrophic bacterial populations, or affect the survival of inoculated Staphylococcus aureus Z88. Further, in contrast with the results obtained in laboratory media, 0.5% SAPP repeatedly produced lower bacterial counts in the sausage than any other phosphate tested or in control sausage with no phosphate addition. As a result, it was theorized that natural meat phosphatases might have hydrolyzed alkaline phosphates, but not SAPP, to produce non-inhibitory phosphate residual levels. The concept was also advanced that cooking the sausage shortly after adding the phosphates, in order to inactivate meat hydrolytic enzymes, could provide a means of retaining some antimicrobial properties of the phosphates.

The third part of the study examined the microbiological effects of 0.5% SAPP, STPP, TSPP and SPG in cooked, vacuum packaged bratwurst stored at 5°C for 7 days, followed by 2 days of elevated temperature holding (24-25°C). No significant ($P>0.05$) bactericidal activity could be attributed to phosphates during the low temperature storage period. However, significant ($P<0.05$) inhibition of naturally occurring aerobic (mesophilic and psychrotrophic) bacteria, as well as of inoculated Clostridium sporogenes PA 3679, by SAPP, STPP and TSPP was observed when the sausage was subjected to "abuse" temperatures of 24-25°C for 24 hours after the 7-day refrigerated holding period. At that time, SAPP and TSPP also significantly ($P<0.05$) inhibited the growth of the natural anaerobic bacterial flora present in the sausage. When the elevated temperature testing period was prolonged to 48 hours, only SAPP continued to exhibit significant ($P<0.05$) bacteriostatic properties on

all types of microorganisms examined, while TSPP remained effective only against C. sporogenes. These results confirmed that 0.5% phosphates did not affect the heat sensitivity of bacteria (including C. sporogenes spores), or microbial survival and growth in bratwurst held at 5°C. Also confirmed was that cooking the sausage to an internal temperature of 65.5°C shortly after incorporating 0.5% SAPP, STPP or TSPP was helpful in retaining some of the bacteriostatic activity of the phosphates. The results obtained might be of great importance in providing a safety margin against clostridia in cooked, vacuum packaged bratwurst, which is presently marketed without added sodium nitrite.

The microbiological effects of 0.5% SAPP or STPP in combination with 50 and 100 ppm sodium nitrite were examined for bratwurst-type sausage prepared, inoculated with C. sporogenes PA 3679 spores, cooked, packaged and stored similarly to that in Part III of the study. Under refrigerated storage conditions (5°C for 7 days), no phosphate or phosphate/nitrite combination was significantly ($P<0.05$) inhibitory to any bacteria examined in the bratwurst. However, this part of the study indicated that longer refrigerated storage periods might provide different results and would be worthy of future research. After 24 hours of temperature abuse, 0.5% STPP with 50 ppm $NaNO_2$ in the bratwurst effectively inhibited the growth of anaerobic bacteria (including C. sporogenes), but not of aerobic microorganisms. A combination of 0.5% SAPP and 100 ppm nitrite was highly and significantly ($P<0.05$) inhibitory to all bacteria tested in sausage after 24 and 48 hours of elevated temperature storage, followed in decreasing order of

effectiveness by SAPP alone, STPP with 50 ppm $NaNO_2$ and SAPP with 50 ppm $NaNO_2$. These results indicated that SAPP and STPP may provide an effective means of reducing nitrite levels in cooked, vacuumm packaged meat products while retaining critically needed anticlostridial protection.

The possibility that enzymatic breakdown of poly- and pyrophosphates in meats could render phosphates ineffective made it necessary to find a simple, rapid analytical method suited to measuring the hydrolysis. The need for such method was more pressing in the case of SAPP, which had proved to be the most effective bactericidal or bacteriostatic phosphate tested in cooked sausage. A method for the analysis of soluble orthophosphates in soils was successfully adapted to the measurement of those compounds, the common end product of pyrophosphate breakdown, in cooked and uncooked pork. Measurement of soluble orthophosphate levels in sausage containing SAPP or STPP indicated that a possible relationship existed between ortho-P concentration and bacterial inhibition by SAPP. However, a similar relationship could not be clearly demonstrated for STPP. This suggested that the antibacterial mode of action of the acid phosphate (SAPP) in cooked meats, might differ from that of alkaline phosphates. Bacterial enzymatic repression and/or inhibition by high levels of SAPP-generated orthophosphate might be involved. The study of that mechanism in the future seems justified, as does the examination of possible bacterial inhibition by combinations of SAPP and alkaline phosphates and of alkaline phosphates and orthophosphates.

The study was concluded with an examination of the microbiological and some physical, chemical and organoleptic effects of 0.4% pure phosphates (STPP, TSPP) or phosphate blends on the characteristics of frozen beef patties. This part of the study was carried out jointly between the Iowa State University Food Technology and Animal Science Departments. It was concluded that phosphates significantly ($P<0.05$) improved juiciness, cook yields and overall acceptability scores of the patties. No significant ($P>0.05$) microbiological quality improvements derived from phosphate addition although, similar to uncooked bratwurst, phosphate-treated patties had consistently lower bacterial counts than control patties. Soluble orthophosphate determinations indicated that phosphate hydrolysis in ground beef occurred even at -20°C. Of possible importance to meat processing was the finding that addition of phosphates in solution resulted in significantly ($P<0.05$) higher quality in frozen beef patties than adding the phosphates in powder form.

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