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Antibacterial activity of sodium phytate, sodium pyrophosphate, and sodium tripolyphosphate against *Salmonella typhimurium* in meats

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Abstract : The approval of use of certain food-grade phosphates as food additives in a wide variety of meat products greatly stimulated research on the applications of phosphates in foods. Although phosphates have never been classified as antimicrobial agents, a number of investigators have reported that phosphates have antimicrobial activities. Phytic acid is a natural plant inositol hexaphosphate constituting 1-5% of most cereals, nuts, legumes, oil seeds, pollen, and spores. In this study, we investigated antibacterial activities of sodium phytate (SPT), sodium pyrophosphate (SPP), sodium tripolyphosphate (STPP) on *Salmonella typhimurium* in tryptic soy broth and in row meat media including chicken, pork and beef. SPT, SPP and STPP at the concentrations of 0.5 and 1% dose-dependently inhibited the growth of *S. typhimurium* in tryptic soy broth at various pHs. The antibacterial activities of SPT and STPP were the stronger than that of SPP. In chicken, pork, and beef, SPT, SPP and STPP at the concentrations of 0.1, 0.5 and 1.0% significantly inhibited the bacterial growth in a dose-dependant manner ($p < 0.05$). The antibacterial activities of SPT, SPP, and STPP were more effective in chicken than beef. SPT and STPP at the concentration of 1% reduced the bacterial count by about 2 log units. The addition of SPT, SPP and STPP at the concentration of 0.5% in meats increased the meat pHs by 0.28-0.48 units in chicken, pork, and beef. These results suggest that SPT and STPP were equally effective for the inhibition of bacterial growth both in TSB and meat media and that SPT can be used as an animal food additive for increasing shelf-life and functions of meats.

Key words : antibacterial activity, food additives, *Salmonella typhimurium*, sodium phosphate, sodium phytate

Introduction

Microbial control in animal foods is very important for preventing food-borne diseases [1]. The meat industry has greatly expanded its use of phosphates in meat processing. Phosphates have been approved for use in meat products to protect flavor, to increase yields and to compensate for the reduced levels of sodium chloride [12-17]. In addition, phosphates are also known to reduce bacterial population in culture media and in meats [4, 5, 9. 20-22, 25]. Although the antibacterial mechanism was not fully understood, the presence of phosphates causes change in membrane permeability, thereby resulting in the lysis of bacterial cells [11, 19].

However, there are still only a few reports on antimicrobial activity of phosphates including sodium tripolyphosphate (STPP), sodium pyrophosphate (SPP), sodium acid pyrophosphate (SAPP), sodium polyphosphate glassy (SPG), and a commercial blend of phosphates in meat products [20-22, 24. 25]. In addition, their effects on meat systems are still controversial [4, 22]. Especially in raw meats, the phosphates had no or little antibacterial effect because of the presence of

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poly- or pyrophosphatases in meats, which can hydrolyze the phosphates into orthophosphates [22].

Phytic acid is a natural plant inositol hexaphosphate constituting 1-5% of most cereals, nuts, legumes, oil seeds, pollen, and spores [6, 10]. Phytate is very stable polyphosphorylated carbohydrate the majority of which is not degraded during transit through the human gastrointestinal tract [6, 7]. One phytate molecule can bind up to 6 divalent cations and the metal could possibly bridge at least 2 phytate molecules depending on the redox state [8]. Metal phytate complex has been known to be highly insoluble over a wide pH range [8]. Therefore, phytate is believed to interfere with mineral bioavailability in human and animals [2]. Also, phytic acid is powerful inhibitor of iron-driven hydroxyl radical formation because of its ability to form a unique iron chelate that become catalytically inactive [7, 8, 13].

Phytic acid is heat- and acid-stable for hydrolysis [15]. Sodium phytate is not hydrolyzed into orthophosphates in raw and cooked beef, while tetrasodium pyrophosphate and sodium tripolyphosphate are rapidly hydrolyzed into orthophosphates [15]. The properties of sodium phytate may be applied for antimicrobial activity in raw meats. So far, very few data are available for antibacterial effect of sodium phytate in an *in vitro* system or in the meat system [9, 20].

In this study, we determined antibacterial activity of sodium phytate on *Salmonella typhimurium* using a culture medium at various pHs. In addition, we investigated the antibacterial effect of sodium phytate in raw meats including beef, pork, or chicken. We also compared the antibacterial effect of sodium phytate with that of traditional phosphates such as sodium pyrophosphate and sodium tripolyphosphate.

Materials and Methods

Chemicals

Sodium phytate (SPT), SPP, and STPP were obtained from Sigma (USA). The test compounds were prepared immediately prior to use and sterilized by filtration through a 22 Millipore membrane (Millipore, USA).

Bacterial cultures

Salmonella typhimurium (ATCC14028) was obtained from Department of Veterinary Infectious Disease, College of Veterinary Medicine at Seoul National University. The stock cultures were maintained at appropriate conditions. TSB (Tryptic soy broth; Difco, USA) and TSA (Tryptic soy agar; Difco, USA) were used for bacterial cultures.

Antibacterial effect in selective medium

In a 15 ml conical tube, 10 ml of medium containing sodium phytate (SPT), SPP, and STPP at the final concentrations of 0.5 and 1% was inoculated with 0.1 ml (7×10^8 cells/ml) of bacterial solution (*Salmonella typhumurium*). The bacteria were cultured in a shaking incubator for 1 h at 37°C then transferred to an incubator to observe the growth of bacteria for 240 min at 37°C. An aliquot of the bacterial solution was determined spectrophotometrically at 600 nm with an interval of 30 min for 240 min. The pHs of medium were adjusted with diluted HCl to 5.5, 6.0, 6.5, and 7.0.

Antibacterial effect in meat model system

We purchased the fresh beef, pork and chicken breast flesh meat from Cheongju Homeplus. The mixture containing 0.5 ml of 0.1% peptone water and 0.1 ml of different concentration of SPT, SPP, STPP and 0.3 g meats mixed by homogenizer. The portions of these solutions were incubated with 0.1 ml (7×10^8 cells/ml) of bacterial solution for 18 h at 37°C. After incubation, 0.5 ml of the mixture was vortexed with 0.5 ml of peptone water and then the suspension was centrifuged for 30 min at 14,000 rpm. The supernatant was serially diluted and 0.1 ml of the diluted solution was spread on TBA. The bacteria were incubated for 16 h at 37°C and the number of colony was counted.

Determination of meat pH

One gram of meat samples and 9 ml of distilled water were grinded by a homogenizer for 10 sec. The homogenates were centrifuged at 3,000 rpm. The supernatants were measured with pH meter.

Statistical analysis

All data are presented as means ± SD of three determinations, and groups of data were analyzed by ANOVA. The difference was considered significant at the level of $p < 0.05$.

Results

Antibacterial effects of sodium phytate inTSB

Salmonella typhimurium needed a lag period of 120-

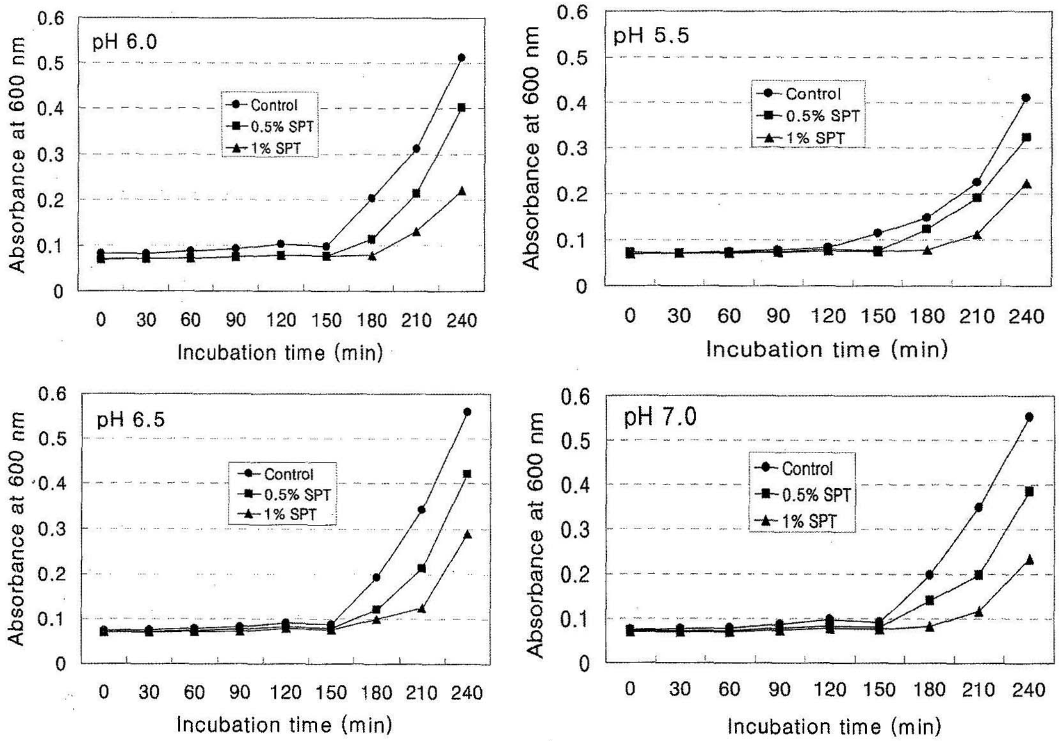

Fig. 1. Antibacterial effects of sodium phytate (SPT) at the final concentrations of 0.5 and 1% against *S. typhimurium* in tryptic soy broth at various pHs. The bacteria were incubated for 240 min at 37°C.

150 min to grow rapidly in TSB. The pHs of TBS used in this study varied with 5.5-7.0 that were generally ranged in animal foods. At pH 5.5, 1% SPT inhibited bacterial growth by about 57.5% after incubation for 240 min. Meanwhile, 0.5% SPT inhibited the bacterial growth by about 27.3% at the same pH of 5.5 (Fig. 1). At pH 6.0, 1% SPT inhibited bacterial growth by 67.4% after incubation for 240 min, whereas 0.5% SPT did by 25.6%. At pH 6.5 and 7.0, 1% SPT inhibited the bacterial growth by about 55% and 71%, respectively, com pared with the control. Regardless of pH of TSB, SPT effectively inhibited the bacterial growth in a dose-dependent manner (Fig. 1).

Antibacterial effects of sodium pyrophosphate in TSB

Similar to SPT, SPP also effectively inhibited bacterial growth in a dose-dependent manner (Fig. 2). At pH 5.5, 1% SPP inhibited bacterial growth by about 53.3% and 0.5% SPP did by 32.1%, compared with the control (Fig. 2). At PH 6.0, 1% SPP also inhibited the bacterial growth by about 61%. The inhibitory effects of 1% SPP at the pHs used in this study were ranged with about 49-61% (Fig. 2). The inhibitory effect of SPP was slightly lower than SPT at the same concentrations.

Antibacterial effects of sodium tripolyphosphate in TSB

One percent of STPP inhibited bacterial growth by about 78% at pH 5.5 and by about 67% at pH 6.0 (Fig. 3). STPP at the level of 0.5% also effectively inhibited the bacterial growth by about 37.2-55.9% at the pHs used in this study. The inhibitory effects of STPP were also dose-dependent regardless of pHs of growth medium (Fig. 3). The antibacterial effectiveness of STPP against *S. typhimurium* was similar to that of SPT and stronger than SPP.

Antibacterial effects of sodium phytate against *S. typhimurium* in meats

Antibacterial effect of SPT on *S. typhimurium* in raw meat media including chicken, pork and beef were investigated (Fig. 4). SPT at the concentration of 0.1,

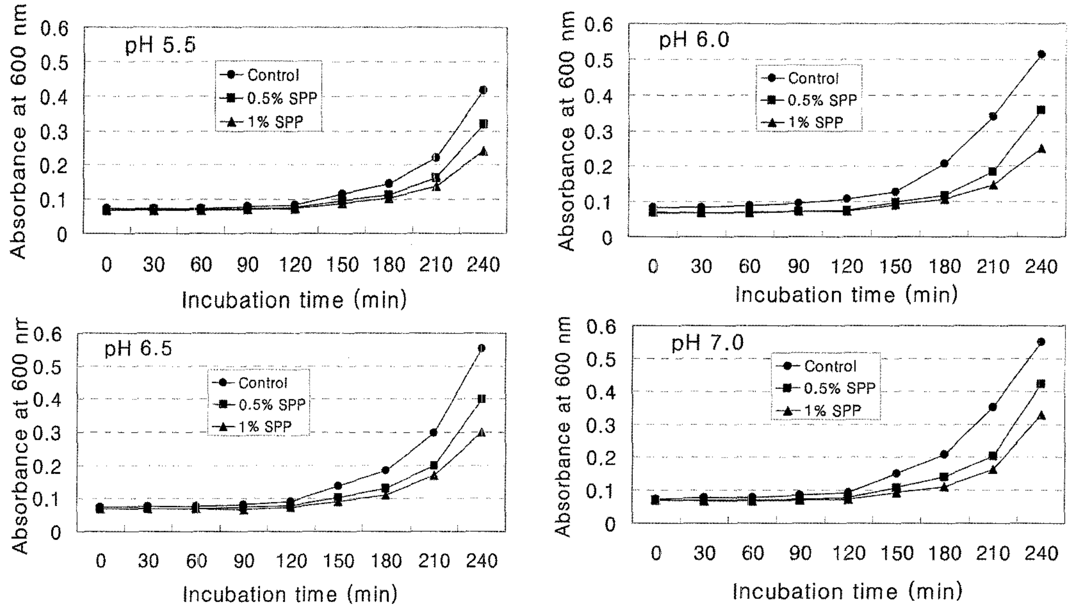

Fig. 2. Antibacterial effects of sodium pyrophosphate (SPP) at the final concentrations of 0.5 and 1% against *S. typhimurium* in tryptic soy broth at various pHs. The bacteria were incubated for 240 min at 37°C.

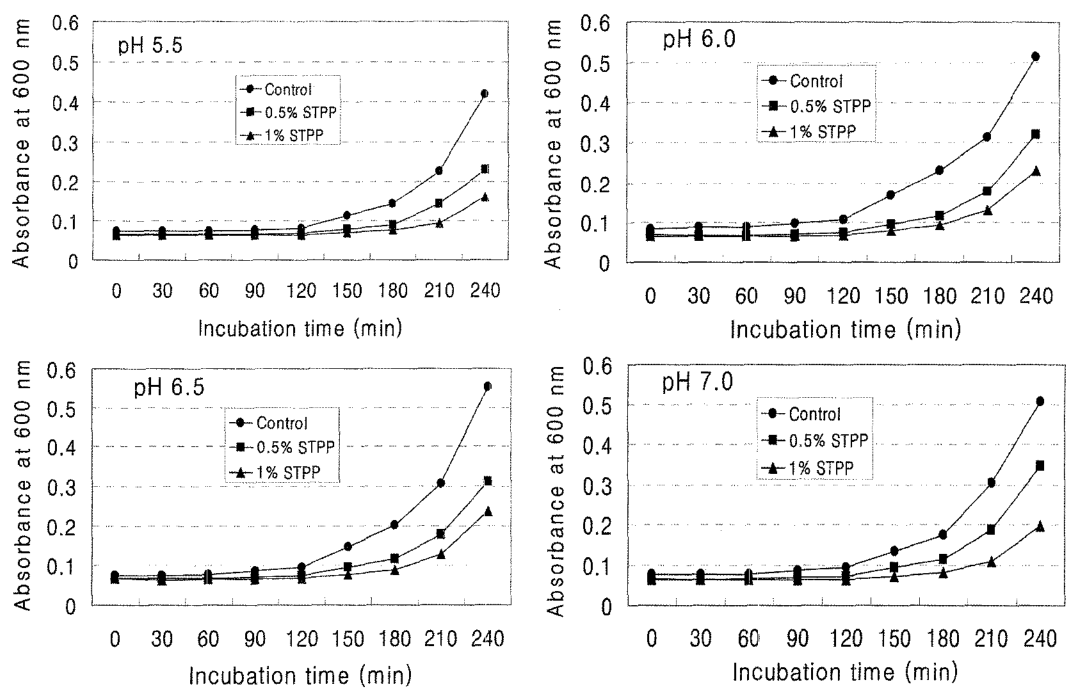

Fig. 3. Antibacterial effects of sodium tripolyphosphate (STPP) at the final concentrations of 0.5 and 1% against *S. typhimurium* in tryptic soy broth at various pHs. The bacteria were incubated for 240 min at 37°C.

0.5 and 1% significantly inhibited the bacterial growth in a dose-dependent manner ($p < 0.05$). 1% SPT reduced the bacterial count by 2.3, 2.0, and 2.1 log units in chicken, pork, and beef, respectively (Fig. 4). The antibacterial effect of SPT was stronger in chicken than pork or beef.

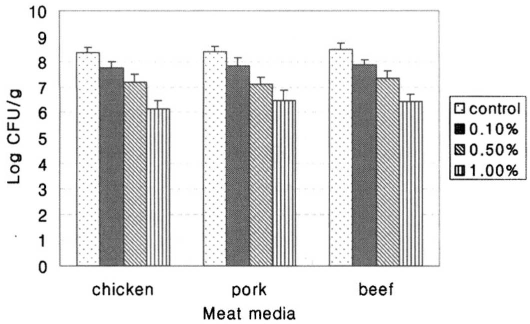

Fig. 4. Antibacterial effect of sodium phytate (SPT) at the final concentrations of 0.1, 0.5, and 1% on *Salmonella typhimurium* in fresh raw meat medium. The bacteria were incubated in raw meat broth for 18 h at 37°C. Data represent mean ± SD of three determinations.

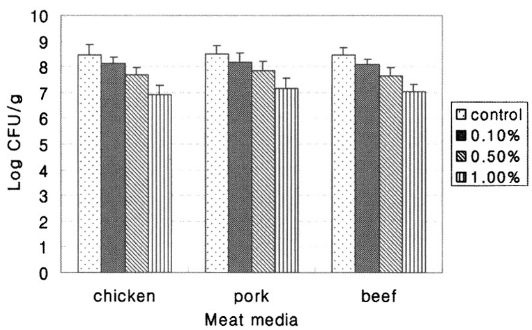

Fig. 5. Antibacterial effect of sodium pyrophosphate (SPP) at the final concentrations of 0.1, 0.5, and 1% on *Salmonella typhimurium* in fresh raw meat medium. The bacteria were incubated in raw meat broth for 18 h at 37°C. Data represent mean ± SD of three determinations.

Antibacterial effects of sodium pyrophosphate against *S. typhimurium* in meats

Antibacterial effect of SPP against *S. typhimurium* in raw meats including beef, pork and chicken were also investigated. (Fig. 5). At the concentration of 1%, SPP significantly inhibited the bacterial growth by 1.6, 1.4, and 1.5 log units in chicken, pork, and beef, respectively ($p < 0.05$). The antibacterial effects of SPP at the concentrations of 0.1, 0.5, and 1% were dose-dependent in the meat media. As compared with SPT, the antibacterial effectiveness of SPP against *S. typhimurium* was the weaker.

Antibacterial effects of sodium tripolyphosphate against *S. typhimurium* in meats

STPP effectively inhibited bacterial growth in meat media including chicken, pork, and beef (Fig. 6). The antibacterial activities of STPP at the concentrations of 0.1, 0.5, and 1% were dose-dependent. One percent of STPP significantly reduced the bacterial growth by 2.0, 2.2, and 1.8 log units in chicken, pork, and beef, respectively ($p < 0.05$). The antibacterial effectiveness of STPP was almost equal to that of SPT.

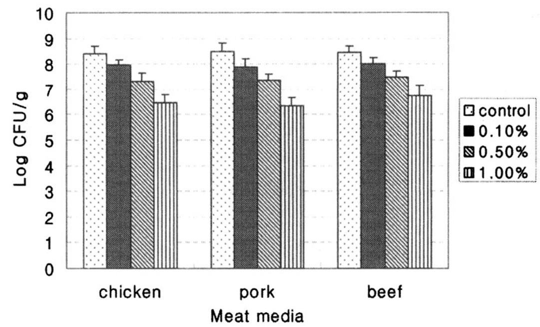

Fig. 6. Antibacterial effect of sodium tripolyphosphate (STPP) at the final concentrations of 0.1, 0.5, and 1% on *Salmonella typhimurium* in fresh raw meat medium. The bacteria were incubated in raw meat broth for 18 h at 37°C. Data represent mean ± SD of three determinations.

Table 1. Levels of pH in meats

Treatments	Meats		
	Chicken	Pork	Beef
Control	5.56 ± 0.05^{a}	5.51 ± 0.06^{a}	5.47 ± 0.06^{a}
SPT	6.04 ± 0.04^{b}	5.97 ± 0.04^{b}	5.92 ± 0.04^{b}
SPP	5.96 ± 0.05^{bc}	5.88 ± 0.07^{c}	5.81 ± 0.04^{c}
STPP	5.94 ± 0.05^{c}	5.79 ± 0.06^{c}	5.78 ± 0.06^{c}

Data represent mean ± SD of three determinations.
$^{a-c}$ Means within columns sharing the same superscript letter were not significantly different at $p < 0.05$.
SPT: sodium phytate, SPP: sodium pyrophosphate, STPP: sodium tripolyphosphate.

Effect of sodium phytate, sodium pyrophosphate, and sodium tripolyphosphate on pHs in meats

The final pHs in animal foods are generally ranged with 5.3-7.0, although the pHs are dependent on animal nutrition status, transportation stress to slaughter, refrigeration after slaughter, autolysis of muscle, etc. The addition of SPT, SPP, and STPP at the final concentration of 0.5% in meats increased meat pHs (Table 1). SPT increased the meat pHs by 0.45-0.48 units, SPP by 0.34-0.40 units, and STPP by 0.28-0.31

units. The pH of chicken was mostly increased with addition of SPT, SPP, and STPP. The 1% solution of SPT, SPP and STPP were 10.5, 10.2, and 9.8, respectively.

Discussion

The approval of the use of certain food-grade phosphates as food additives in a wide variety of meat products greatly stimulated research on the applications of phosphates in foods. Phosphates are used as additives to enhance the major four functional properties in meats: 1) increasing water holding capacity, 2) enhancing emulsification, 3) retarding oxidative rancidity and color deterioration, and 4) enhancing cured color development. At present, phosphate addition to meat products can not exceed 0.5% of final product weight [3]. That limit is based on improvement of functional properties of meat, but not on antimicrobial effect. Although phosphates have never been classified as antimicrobial agents, a number of investigators have reported that phosphates have antimicrobial activities.

In the present study, sodium phosphates including sodium pyrophosphate and sodium tripolyphosphate effectively inhibited the growth of *Salmonella typhimurium* both in media and meats such as chicken, pork, and beef. In addition, phytate, which contains 6 phosphates, also significantly reduced the bacterial growth in media and meats. Early work has related to poultry processing. Spencer and Smith [34] showed that chilling chicken flyers in a solution of polyphosphates for 6 h increased shelf-life and decreased the rate of microbial spoilage. Steinhauer and Banwart [25] and Chen *et al.* [1] also reported that dipping chicken parts or carcasses in phosphate-containing chilling water resulted in decreased bacterial counts and prolonged shelf-life. A process using tetrasodium phosphate (TSP) to reduce viable *Salmonella* spp. on chicken carcasses has been approved by USDA [5]. The process has been reported to reduce populations of *Salmonella* spp., *E. coli,* Camphylobacter and *S. aureus* on chicken carcasses, but it has less effect on total aerobic microorganisms. The mechanism through which TSP kills *Salmonella* spp. is still not fully understood.

In laboratory media, 0.5% tetrasodium pyrophosphate (TSPP) was lethal or highly inhibitory to *Staphylococcus aureus, Psedomonas aeruginosa, Salmonella typhimurium* and two commercial lactic starter cultures [21]. Untreated STPP and SPG were also inhibitory to these bacterial species, while unheated sodium SAPP was not inhibitory or enhanced the bacterial recoveries. Heating reduced the effectiveness of phosphates as inhibitors [21]. Lee *et al.* [18] reported that 0.1% sodium ultraphosphate (SUP) and SPG, and 0.5% SAPP, STPP, and TSPP were inhibitory on growth of early-exponential-phase cells of *S. aureus* ISP40 in a culture medium. The inhibitory effect of phosphates was weak in a meat medium. The inhibitory activity of phosphates might be due to cellular lysis of the *S. aureus*, as indicated by leakage of intracellular nucleotides. EDTA (3.4 mM) also induced the great amount of bacterial leakage at 0.5 h of incubation of *S. aureus*. Bacterial leakage caused by EDTA, SPG (0.1%) and SUP (0.1%) was comparable at 1 h of incubation. However, Ca (II) and Mg (II), at the concentration of 0.01 M, reversed the bacteriolytic effects of SUP and SPG to *S. aureus* [19]. The growth inhibition of TSPP was not reversed by Mg (II) or Ca (II), but it was reversed by Fe (III) addition. They concluded that the antibacterial effects of phosphates can be altered substantially by the metal-ion content of the environment. Molins *et al.* [22] also reported that SAPP was inhibitory to psychrotrophic bacteria when added at 1.0% level to fresh ground pork stored at 5°C. Addition of SAPP at 1.0% resulted in 50% shelf-life extension compared with that in untreated meat or in meat that received 0.5% and 1.0% orthophosphate. SAPP at 0.5% was also effective in reducing microbial growth in uncooked bratwurst stored at 5°C [20]. Recently, Hue *et al.* [9] reported that SPT effectively inhibited the growth of *E. coli* O157 : H7 in medium and raw and cooked meats. They also reported that SPP and STPP had an inhibitory activity against *E. coli* O157 : H7 in medium and meats [9]. At acidic pHs, phytic acid in the presence of Fe (II) effectively inhibited the growth of *E. coli* O157:H7. In the present study, the antibacterial activity of SPT against *S. typhimurium* was similar to that against *E. coli* O157 : H7. These results imply the possible use of SPT for controlling food-borne pathogens in meat industry.

Meanwhile, the antibacterial activity of phosphates has been still controversial. Flores *et al.* [4] investigated antibacterial effect of 0.5% phosphate blend in fresh and processed meat products. There was minimal or no effects of the phosphate blend on the growth of

E coli O157:H7 in ground beef and fresh pork sausage, *L. monocytogenes* or *S. typhimurium* in smoked sausage and boneless ham. Molins *et al.* [21, 22] reported that 0.5% STPP, TSPP, and SPG had no significant microbial inhibition on aerobic mesophilic and psychrotrophic bacteria in uncooked bratwurst stored at 5°C for 7 days. Molins *et al.* [22] also reported that 0.4% STPP, TSPP, and three commercial phosphate blends did not significantly reduced mesophilic, psychrotrophic, *S. aureus* and lactic acid bacterial numbers in frozen beef patties, although some of the phosphates inhibited the growth of bacterial in patties that were subsequently held at abuse temperatures. They accounted for the ineffectiveness of the phosphates in reducing microbial counts by the presence of poly- and pyrophosphatases naturally present in muscle cells. 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 interaction such as those between phosphates and meat proteins may also explain the failure of phosphates as bacterial inhibitors in uncooked, processed meats.

However, phytic acid was heat- and acid-stable for hydrolysis and it was not easily hydrolyzed to orthophosphates in raw meats [15]. The properties of sodium phytate would be applied as a good additive for antimicrobial activity in raw meats. In conclusion, Antibacterial effects of SPT, SPP, and STPP against *S. typhimurium* were investigated using medium at various pHs and meats. In the present study, SPT effectively inhibited the growth of *S. typhimurium* in media at various pHs (5.5-7.0) as well as meats including chicken, pork, and beef. In addition, the phosphates used in this study also had an equal antibacterial activity against *S. typhimurium* as compared with SPT. These results suggest that SPT were very effective for the inhibition of bacterial growth both in TSB and meat media and that SPT in substitution for phosphates can be used as an animal food additive for controlling food-borne pathogens.

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