

การลดการปนเปื้อนเชื้อซัลโมเนลล่าในเนื้อสุกรโดยการฉีดพ่นสารละลายต่างไตรโซเดียมฟอสเฟต

Decontamination of *Salmonella* on Pork Belly Using Trisodium Phosphate (TSP) Spraying

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บทคัดย่อ

สารละลายต่างไตรโซเดียมฟอสเฟต (TSP) เป็นวัตถุเจือปนอาหาร (INS 339 (iii)) ที่ใช้ในกระบวนการผลิตอาหาร วัตถุประสงค์ของการศึกษาในครั้งนี้เพื่อทดสอบประสิทธิภาพของวิธีการฉีดพ่นสารละลายต่างไตรโซเดียมฟอสเฟตที่แตกต่างกันในการลดการปนเปื้อนของแบคทีเรียซัลโมเนลล่า ลักษณะทางประสาทสัมผัสของสีเนื้อและผิวหนังสุกรและการตกค้างของฟอสเฟต แบ่งตัวอย่างเนื้อสุกรส่วนท้องขนาด 15x15 ตารางเซนติเมตร ออกเป็น 8 กลุ่ม เริ่มจากการปนเปื้อนด้วยเชื้อ nalidixic acid resistant *Salmonella enterica* serotype Enteritidis (SE) โดยปริมาณเชื้อ SE ที่ติดบนเนื้อเฉลี่ย $6.07 \pm 0.86 \log_{10}$ MPN/g ของเนื้อสุกรส่วนท้อง ใช้แรงดันในการฉีดพ่น 20 บาร์/นาที่ ภายใต้อุณหภูมิ 25 ± 2 องศาเซลเซียส เพื่อลดการปนเปื้อนด้วยวิธีการดังนี้ กลุ่มปนเปื้อนเชื้อแบคทีเรียที่ไม่ผ่านสารใดๆเลย (n=10) กลุ่มที่ฉีดพ่นด้วยน้ำปลอดเชื้อ 120 วินาที (n=10) สองกลุ่มตัวอย่างทำการฉีดพ่นด้วย 10%w/v TSP นาน 15 และ 30 วินาทีล้างออกทันทีด้วยน้ำปลอดเชื่อนาน 15 และ 30 วินาทีตามลำดับ (n=3) สามกลุ่มตัวอย่างทำการฉีดพ่นด้วย 12% w/v TSP นาน 15, 30 และ 60 วินาที ล้างออกทันทีด้วยน้ำปลอดเชื่อนาน 15, 30 และ 60 วินาที ตามลำดับ (n=10) และกลุ่มสุดท้ายคือ 12% w/v TSP นาน 15 วินาที ล้างออกด้วยน้ำปลอดเชื่อนาน 15 วินาที หลังจากเก็บรักษา 24 ชั่วโมง (n=6) ผลการทดลองพบว่า สภาวะที่สามารถลดเชื้อ SE ได้มากที่สุด คือ 12%w/v TSP นาน 15 วินาที ล้างออกด้วยน้ำปลอดเชื่อนาน 15 วินาที หลังจากเก็บรักษา 24 ชั่วโมง เท่ากับ $2.33 \pm 1 \log_{10}$ MPN/g ของเนื้อสุกรส่วนท้อง โดยลักษณะทางประสาทสัมผัสของสีเนื้อสุกรไม่เปลี่ยนแปลง การวิเคราะห์เปรียบเทียบฟอสฟอรัสซึ่งเป็นตัวแทนของฟอสเฟตที่พบในเนื้อสุกรพบว่าไม่แตกต่างกันทางสถิติกับกลุ่มเนื้อสุกรที่ไม่ผ่านสารใดๆเลย ($p=0.06$) วิธีการฉีดพ่น

12%w/v TSP นาน 15 วินาทีล้างออกด้วยน้ำปลอดเชื้อนาน 15 วินาทีหลังจากเก็บรักษา 24 ชั่วโมง มีประสิทธิภาพในการลดเชื้อซัลโมเนลล่าที่ปนเปื้อนเนื้อสุกรและผลิตภัณฑ์จากเนื้อสุกรในกระบวนการเชือดและชำแหละสุกร

Keywords: สารละลายต่างไตรโซเดียมฟอสเฟต เนื้อสุกร การลดการปนเปื้อนเชื้อซัลโมเนลล่า

Abstract

Trisodium Phosphate (TSP) is a food additive (INS 339 (iii)) used in food production. The objectives of this study were to determine the effects of different TSP solution spraying methods on the reduction number of *Salmonella* on pork bellies, the color of the meat and skin as well as the phosphate residue in the pork. Samples of pork bellies (15x15 cm²) were divided into 8 groups. The samples were inoculated with $2.64 \pm 0.75 \times 10^6$ MPN/g of nalidixic acid resistant *Salmonella enterica* serotype Enteritidis (SE). Then various decontamination methods were used which included spraying the samples with liquid at a pressure of 20 bar/min at a temperature of 25 ± 2 °C. An untreated control group (n=10), sterile distilled water spraying for 120 sec (n=10). Two other groups of samples were sprayed with a 10% w/v solution for 15 or 30 sec followed by immediate rinsing off with sterile distilled water for the same number of seconds (n=3). Three other groups were sprayed with 12% w/v TSP solution for 15, 30 or 60 seconds, then were rinsed off immediately with sterile distilled water for an equal period (n=10). Another group of samples was sprayed with 12% w/v TSP solution for 15 seconds; samples in this group were rinsed off after 24 hours with sterile distilled water for 15 sec (n=6). It was found that spraying 12% w/v TSP solution for 15 seconds and rinsing off after 24 hours with sterile distilled water for 15 seconds was able to reduce SE by $2.33 \pm 1 \log_{10}$ MPN/g in the pork samples. Moreover, there was negligible color change on surface of pork belly and skin and phosphate residues in the pork belly samples were expressed by the concentration of phosphorus. Concentrations in this group were not significantly different from the untreated sample ($p=0.06$). Spraying with 12% w/v TSP solution for 15 seconds and rinsing off after 24 h with sterile distilled water for 15 seconds appears to be a potentially effective method for reducing salmonella contamination of pig carcasses during pork production in slaughterhouses.

Keywords: Trisodium Phosphate, pork, decontamination of *Salmonella*

1. Introduction

Pork is widely recognized as one of the significant sources of *salmonella* food poisoning in humans along with eggs and poultry meat. *Salmonella* can be introduced vertically or horizontally from feed, during breeding, from the environment, on the farm, at

the slaughtering line, as well as during handling and storage both at the market and in the home. Several studies have identified carrier pigs as a predominant source of *Salmonella* contamination of pig carcasses during the slaughtering process at rates ranging from 6.2% to 69%. (Kasbohrer, 2000; Zweifel et al., 2005;

Heinz, 2008; Van Hoek et al., 2012; Schmidt et al., 2012). In Thailand, the prevalence of *Salmonella* in pork has been reported to be high, ranging from 29% to 65% (Padungtod and Kaneene, 2006; Sanguankiet et al., 2010).

Trisodium phosphate (Na_3PO_4 or TSP) is highly alkaline at concentrations used to reduce *salmonella* on raw poultry carcasses (Hinton, 1996). Its use is permitted by the Thai Food and Drug Administration and it has been approved by the U.S. Department of Agriculture as a food ingredient. Treatment of carcasses with TSP reduces levels of Gram-negative bacteria such as *Pseudomonas* spp., *E. coli*, *Campylobacter*, *Salmonella* and *Listeria* spp. TSP should preferably be applied during pre-chilling or chilling by dipping or spaying. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) allows adding TSP in foods at levels of less than 2,200 mg/kg of food. The Acceptable Daily Intake (ADI) of TSP is 70 mg/kg (Capita et al., 2000, 2002). The high alkalinity of TSP appears to remove fat films and bacteria as well as disrupting fatty molecules in the cell membrane which causes bacterial cells to leak intracellular fluid (Balamurugan et al., 2003 and Zweifel, 2009). There have been many studies of the application of TSP as a food decontaminant in the laboratory and in industrial experiments on chicken and beef processing lines, but few have been done on pork processing lines (Kanellos, 2004; Giese, 1993; Kim and et al., 1994).

Effects of a chemical reagent on meat color and other sensory properties are important for retail

display. The study by McElyea et al., 2002 found that TSP did not affect *Salmonella* Typhimurium; however, a combination of 1% cetylpyridinium chloride (CPC) and TSP lowered the number of *Salmonella* Typhimurium. Unfortunately, CPC treatment also caused ground pork to be lighter in color ($p < 0.05$), whereas TSP enhanced pork redness ($p < 0.05$). For that reason, TSP was chosen for this study.

2. Objective

To evaluate the efficacy of different TSP application procedures for decontamination of *Salmonella* and to evaluate concurrent changes in sensory characteristics in pork belly.

3. Materials and methods

This study involved a two-stage experiment. The first stage of the experiment consisted of evaluating the decontamination efficacy and the sensory characteristic impacts of a range of decontamination procedures. The best methods from the first stage, those which met predetermined criteria, were selected for inclusion in the second stage of the experiment. Those criteria included reduction of Nalidixic acid resistant *Salmonella enterica* serotype Enteritidis (SE) contamination by $\geq 2 \log_{10}$ MPN/g with no or minimal undesirable sensory effect. In the second stage of the experiment, the color of the pork and the amount of phosphate residue were evaluated.

3.1 Stage 1 of the experiment

3.1.1 Bacterial strain and media

Nalidixic acid resistant *Salmonella enterica* serotype Enteritidis (SE) which had been stored at -80 °C was subcultured on 5% sheep blood agar (Merck, Darmstadt, Germany) and incubated at 37 °C for 18 h. Two isolated colonies were then transferred into 5 mL of tryptic soy broth (TSB) (Merck, Darmstadt, Germany) and incubated at 37 °C for 18 h. Bacteria in the stationary phase of growth were used as inoculates. SE cultures in TSB broth contained approximately 10^8 CFU/ml or 0.5 McFarland (OD 640 nm, absorbance 0.15). The SE cultures were diluted by 0.85% NaCl solution. The final concentration of SE with which the pork belly with skin samples were inoculated for the decontamination experiment was $2.18 \pm 1.9 \times 10^6$ CFU/ml.

3.1.2 Porcine samples

Pork belly samples (15×15 cm²) consisting of red meat, fat and skin were obtained from a pig slaughter house. Samples were transported and stored under refrigerated conditions at 4 °C. The pork skin samples were inoculated with the SE suspension which was spread over the entire surface and left at room temperature for 15 minutes to allow the SE to attach to the surface of the pork belly.

3.1.3 Chemical treatment

Eight samples of pork belly were randomly assigned to each of the nine different treatment groups. Each of the samples in the eight treatment groups were individually sprayed using different treatment protocols: 1) Control group (n=10). 2)

Water spraying for 120s (n=10). 3) 10%w/v TSP solⁿ spraying for 15s following by water spraying for 15s 4) 10%w/v TSP solⁿ spraying for 30s following by water spraying for 30s (n=10). 5) 12%w/v TSP solⁿ spraying for 15s following by water spraying for 15s (n=10). 6) 12%w/v TSP solⁿ spraying for 30s following by water spraying for 30s (n=10). 7) 12%w/v TSP solⁿ spraying for 60s following by water spraying for 60s (n=10). 8) 12%w/v TSP solⁿ spraying for 15s, storage at 4°C for 24 h and water spraying for 15s (n=10). All treatments were applied at a water pressure of 20 bars at room temperature (25±2 °C). Some groups started from preliminary study before laboratory setting. So the initial numbers of sample were 3 samples per group. The total amount of water used for each for each treatment was measured.

3.1.4 Microbiological analysis

3.1.4.1 Quantitative detection method for Nalidixic acid resistant *Salmonella enterica* serotype Enteritidis (SE)

SE was detected using the three tube MPN technique. Samples of 25 g of pork belly were shaken for 1 min in 225 mL of buffered peptone water. The mixture was then homogenized using a stomacher for 60s, after which the homogenate was incubated for 24 h at 37°C. Following incubation, 0.1 mL of the rinse solution was transferred to Rappaport-Vassiliadis (RV) broth (Merck, Darmstadt, Germany) and incubated for 24 h at 42°C. Broth samples were then streaked onto Xylose Lysine Tergitol-4 (XLT4) Agar and Brilliant-green Phenol-red Lactose Sucrose (BPLS) agar (Merck, Darmstadt, Germany) and

incubated for 24 h at 35°C. Suspect colonies were identified and subjected to biochemical testing.

3.1.4.2 Qualitative detection method for Nalidixic acid resistant *Salmonella enterica* serotype Enteritidis (SE) in 25 g of pork belly ; ISO 6579: 2002

After incubation for 24 h at 37°C, 0.1 mL of the homogenate was transferred to Rappaport-Vassiliadis (RV) Broth (Merck, Darmstadt, Germany) and 0.5 mL of the rinse solution was transferred to Muller-Kauffmann Tetrathionate (TT) Broth (Merck, Darmstadt, Germany) and incubated for 24 h at 42°C. The broths were then streaked onto Xylose Lysine Tergitol-4 (XLT4) Agar and Brilliant-green Phenol-red Lactose Sucrose (BPLS) Agar (Merck, Darmstadt, Germany) and incubated for 24 h at 35°C. Suspect colonies were identified and subjected to biochemical testing.

3.1.5 pH measurement of meat

Ten gram samples of meat were weighed and placed in a sterile stomacher bag containing 10 ml of sterile 0.85% NaCl then homogenized in a stomacher for 2 minutes. The pH value was measured using an electronic pH-meter (Cyberscan® pH 310 series.). For calibration, the pH meter was standardized at pH 4 and 7 prior to the measurement.

3.2 Stage 2 Experiment

3.2.1 Sensory Analysis

The TSP treatment condition in the laboratory experiment was able to reduce the bacterial population by ≥ 2 log reductions. The next step was sensory analysis. Samples of pork bellies were divided

into 2 groups. 1) Untreated control group (n=9) and 2) Effective TSP condition group (n=9).

3.2.2 Color measurement

The color of skin, fat and meat at 10 randomly selected areas were determined with 10 replications of each samples using a CR-400 Chroma meter (Minolta, Osaka, Japan). Results were recorded following the Complete International Commission on Illumination (CIE) system color profile of Lightness (L*), redness (a*), and yellowness (b*).

3.2.3 Water - holding capacity determination

To determine water - holding capacity, fifty 200 g samples of surface belly skin (15x15 cm x 10 mm) were placed in plastic bags, approximately 2 cm above the bottom of the bags. The bags were hung in a refrigerator at 4°C for 48 hours. The percent water loss (drip loss) was calculated as: weight before chilling - weight after chilling / weight before chilling) X 100.

3.2.4 Phosphate residue: Spectrophotometric Method

Phosphate residues were measured following the AOAC official method 986.24 (AOAC International, 2010). Phosphorus (P) standard was accurately weighted amount of test portion to contain ca 4.0 mg P into an ashing dish and evaporated to dryness on a hot plate. Then the reagent was ignited in a muffle furnace at a maximum temperature of 600 °C until all reagents were eliminated. Forty mL of hydrochloric acid solution with several drops of HNO₃ were brought to a boil on a hotplate. One hundred mL of the solution were transferred to a volumetric flask and diluted to volume with H₂O.

Best-fit linear calibration was accomplished using phosphorus standard solutions. The series of standard solutions were 0.0, 0.5, 0.8, 1.0, and 1.5% P. Twenty grams of ammonium molybdate was pipetted into 200 mL of hot water and allowed to cool. One gram of ammonium molybdovanadate was dissolved in 125 mL hot water and cooled. Then, 160 mL HCl were added. Molybdate solution was gradually added to the vanadate solution with H₂O to 1 L. The flasks were allowed to stand for 10 min to complete color development. The absorbance of the standard and the test solutions in 1 cm cells at maximum near 400 nm was determined using 0.0 mg standard absorbance vs mg P of standards to determine mg P for each tested portion.

3.3 Statistical analysis

The *Salmonella* counts were transformed using logarithm to the base 10. The reduction in bacterial populations attributable to spraying was calculated by subtracting the logarithm to the base 10 of sprayed samples from untreated control samples. The bacterial reduction, pH levels, percent drip loss levels and color values were evaluated by ANOVA and t-test using the program R (R Core Team, 2014).

4. Result and Discussion

4.1 Stage 1 Experiment

4.1.1 Effect of antimicrobial treatment combinations on microbial populations

The natural contamination of *Salmonella* spp observed in this study was $-0.82 \log_{10}$ MPN/g.

There was no *Salmonella* spp found in 25 g of pork belly samples. The initial concentration of *Salmonella* in the untreated control pork belly samples (n=10) was approximate $6.42 \pm 1.9 \log_{10}$ MPN/g. Although the population of SE decreased at all concentrations, the reduction with 10% w/v and 12% w/v TSP was not statistically significantly. Figure 1 shows the mean log reductions of the *Salmonella* counts following each of seven treatments. 12% w/v TSP solution spraying for 15s, storage at 4°C for 24 h and water spraying for 15s decreased the population of SE on pork surfaces by $2.33 \pm 1 \log_{10}$ MPN/g, a significant difference from the control group. Moreover, the mean values were significantly different with water spraying for 120s, 12% w/v TSP solution spraying for 60s followed by water spraying for 60s, 10% w/v TSP solution spraying for 15s followed by water spraying for 15s, 10% w/v TSP solution spraying for 30s following by water spraying for 30s, 12% w/v TSP solution spraying for 15s followed by water spraying for 15s, 12% w/v TSP solution spraying for 30s followed by water spraying for 30s (p<0.05). The results indicate that the duration of spraying was an important factor in determining the efficacy of the TSP treatment. In this experiment, water spraying for 120s did not significantly decrease the bacterial contamination compared to either 12% w/v TSP solution spraying for 60s followed by water spraying for 60s or 12% w/v TSP solution spraying for 15s, storage at 4°C for 24 h and then water spraying for 15s. The TSP spraying treatment results also showed a consistent pattern of effect on the level of the *Salmonella* counts. Multi-

factor analysis of variance on the log₁₀ transformed values of the various counts revealed a significant difference ($p>0.05$) between distilled water treatment and TSP treatment. Distilled water treatment was used as a physical parameter control in order to determine whether microflora was removed by a merely mechanical effect (Del Rio et al., 2007). TSP

treatment provided not only a mechanical effect but also an antimicrobial effect from chemical solution. TSP treated samples had higher pH values after spraying. The pH of the TSP spray was between 11.3-11.6. The average pH values of treated pork belly after using TSP spray are shown in Tables 1 and 2.

Table1 Summary of *Salmonella* log reduction, pH and volume of water-using on pork bellies by all treatments

Decontamination method	Number samples	Volume of water (L)	pH	The mean of log ₁₀ MPN/g <i>Salmonella</i> reduction
Water spraying for 120s	10	16	5.89±0.2	-0.17±0.7
12%w/v TSP sol ⁿ spraying for 60s following by water spraying for 60s	10	8	6.24±0.2	0.81±0.57
10%w/v TSP sol ⁿ spraying for 15s following by water spraying for 15s	3	2	6.07±0.2	0.56±0.3
10%w/v TSP sol ⁿ spraying for 30s following by water spraying for 30s	3	4	6.19±0.3	0.44±0.5
12%w/v TSP sol ⁿ spraying for 15s following by water spraying for 15s	3	2	6.14±0.2	0.86±0.5
12%w/v TSP sol ⁿ spraying for 30s following by water spraying for 30s	3	4	5.9±0.2	0.76±0.4
12%w/v TSP sol ⁿ spraying for 15s, storage at 4°C for 24 h and water spraying for 15s	6	4	6.7±0.2	2.33±1*

Remarks: temperature of water was ambient (approximately . 30°C)

*: The decontamination method could reduce *Salmonella* $\geq 2 \log_{10}$ MPN/g

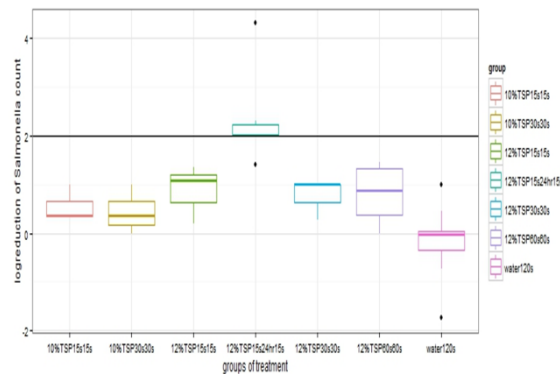


Figure 1 Summary of *Salmonella* reductions (\log_{10} MPN/g) on pork belly by all treatments.

Results of treatment with 12% w/v TSP solution spraying for 15s, storage at 4°C for 24 h, then water spraying for 15s differed significantly ($p>0.05$) from the control group (distilled water spraying for 120 sec) and the 10% w/v TSP treated group (except 12% w/v TSP treated group). The TSP solution lead to an increase in pH to 5.9-7, While the pH of the untreated control samples were 5.8-6.3. The pH values obtained in this study are consistent with those of Mehvar et al., (2005) which found that of all the chemicals tested, TSP caused the largest initial increase in skin pH after treatment, while the organic acids group had the opposite effect.

Table 1 also shows the volume of water used in each treatment. The results indicate that spraying time and water pressure were important factors in defining the volume of water used in spraying. The highest volume of water used was with water sprayed for 120 sec and 12 %w/v TSP solution for 60s following by water sprayed for 60s with water pressure 20 bar per min.

4.2 Stage 2 Experiment

4.2.1 Effect of antimicrobial treatment combinations on instrumental color and sensory characteristics

The external surface of pork belly consists of skin, fat and red meat. Red meat has a red color while both skin and fat have a white and/or yellow color. The present study found that the meat color, the L^* value, of the untreated control group and the 12% w/v TSP solution spraying for 15s, stored at 4°C for 24 h, then water spraying for 15s, were 42.84 ± 3.3 and 42.44 ± 0.7 , respectively. The redness (a^*) values were 13.14 ± 0.3 and 13.12 ± 0.2 , respectively. and the yellowness (b^*) values were 0.84 ± 0.5 and 0.27 ± 0.4 , respectively. As to skin color, the study found the L^* values of the TSP treatment samples were higher than the untreated control group, but the difference was not statistically significant. The colorimeter showed that surface lightness L^* increased after spraying with TSP. At the same time, redness a^* and yellowness b^* were slightly reduced after decontamination treatment. The results were consistent with the study of Morris, 1997).

Rodriguez de Ledesma et al. (1996) observed that TSP treatment resulted in organoleptic changes in the poultry skin surface and exposed muscle tissue, producing a darker brownish color; consumers preferred the untreated controls. The study by Hathcox et al. (1995) of chicken breasts and thighs reported that treatment with 12% w/v TSP did not affect consumer acceptance of either raw or fried pieces. McElyea et al. (2002) found that TSP significantly enhanced pork redness a^* compared to the control. However, the color of samples treated with TSP was lower than the untreated controls

because of an instrumental color which occurred immediately after rinsing in this study.

The drip loss from fresh pork is the result of shrinkage of muscle proteins and subsequent expressing of fluids from the meat. The standard meat quality, measured as percent drip loss (adapted from PIC, 1997) was 3 to 6%. The present study found that the percent drip loss after 48 h of 12% w/v TSP solution spraying for 15s, storage at 4°C for 24 h and water spraying for 15s was 0.12%. Phosphate increased moisture retention through a combination of phosphate salt and ATP in the meat tissue (Ellinger, 1972). The present study showed percent drip loss found that percent drip loss after 24 h with 12% w/v TSP treatment was 0.27%. That was too less reduction when compared with control group (0.95%). Because this condition had to rinse by distilled water after storage at 4°C for 24 h. So that was not the real percent drip loss after 24 h of this condition.

Table 2 Sensory evaluation and Phosphate residue of the pork belly from pig carcasses after treatment with Trisodium phosphate (TSP)

Method	Experiment (n=9/treatment)	
	Untreated sample	12%w/v TSP15s24hr15s
Skin		
L*	69.45±0.6	70.95±0.7
a*	6.26±0.3	5.23±0.2
b*	7.8±0.3	7.49±0.2
Meat		
L*	43.64±0.7	42.44±0.7
a*	13.14±0.3	13.12±0.2
b*	0.84±0.5	0.27±0.4

Method	Experiment (n=9/treatment)	
	Untreated sample	12%w/v TSP15s24hr15s
Fat		
L*	75.55±0.4	75.22±0.2
a*	4.39±0.2	4.09±0.09
b*	5.6±0.1	6.07±0.1
Ultimate pH at skin		
Ultimate pH at	6.7±0.1	6.73±0.1
Ultimate pH at meat		
Ultimate pH at	6.23±0.1	6.42±0.1
Ultimate pH at fat		
Ultimate pH at	5.87±0.04	6.29±0.1
% drip loss (24hr)		
% drip loss	0.95%	0.77%
% drip loss (48hr)		
% drip loss	0.19%	0.12%w/v
Phosphorus in pork (mg/g)		
Phosphorus in	1.17	1.52
Phosphate residue (mg/kg)		
Phosphate	2679.5	3483.5

Stage 1 of the experiment found that 12% w/v TSP solution spraying for 15s, storage at 4°C for 24 h, and then water spraying for 15s resulted in a ≥ 2 log reduction in the bacterial population. The analysis of residual amounts of chemical reagent in the pork is important for control of the quality of the products for consumer. Measurements of residual phosphate were made using wavelengths available on a spectrophotometer and reagents that are all stable at room temperature. The results are shown in Table 2. Amounts of phosphate (P_2O_5) in the untreated control group and the TSP group were significantly different ($p=0.0495$). There are no living organisms known

that can synthesize the phosphate anion. Very few natural compounds in living organisms contain phosphorus in any form other than phosphate anions. Phosphorus in the form of phosphate anions is expressed in every type of food consumed by living organisms. The study of Sherman, (1947) published a complication of the phosphorus content of beef, pork and eggs: 2.05 ± 2.5 , 1.66 ± 2.0 and 2.24 ± 1.4 mg/g, respectively. As shown in Table 2, amounts of phosphorus in the untreated samples were within the natural range for pork. Amounts of phosphorus in the TSP group were higher than in untreated control group, but the difference was not significant ($p=0.06$). Many investigators presented their published reports at the seventh meeting of the FAO/WHO Expert Committee on Food Additives held in 1963 on toxicity of phosphates in food processing. Excess ingestion of any inorganic salt may upset the mineral balance in the body, adversely affecting the osmotic pressure of body fluids and preventing absorption or utilization of necessary mineral nutrients. A literature review was published supporting the Committee's recommendation for acceptable daily intakes of phosphates in human diets (Ellinger, 1972). Those levels are as follows: unconditional acceptance at levels < 30 mg/kg body weight and conditional acceptance at levels of 30-70 mg/kg body weight. The 12% w/v TSP solution spraying for 15s, storage at 4°C for 24 h and water spraying for 15s reduced SE to $\geq 2 \log_{10}$ MPN/g and did not significantly change the sensory characteristics or phosphate residue; however, the rinsing off after 24 h caused the carcass and trimming

line to become wet which could result in cross contamination and which also increased the water-holding capacity of the carcass and raised the pH, a situation that warrants further study.

5. Conclusion

12% w/v TSP solution spraying for 15 sec and rinsing off after 24 h with sterile distilled water for 15 sec reduced SE by $\geq 2 \log_{10}$ MPN/g in the pork samples. That was accomplished with negligible effect on the color of the surface of the pork belly with skin. The pattern of SE reduction observed in this study suggests that TSP could be applied in slaughterhouses as an effective decontaminant which does not result in organoleptic changes to the pork belly and which results in safer phosphate residual levels. Therefore, TSP decontamination should not be carried out during the post chilling process result in interference with other activities of the slaughtering line.

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