Quality Evaluations of Refrigerated Korean Beef Loins Treated with Trisodium Phosphate and Chitosan

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Trisodium Phosphate와 키토산으로 처리한 냉장 한우 쇠고기등심의 품질평가

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Abstract

The effect of trisodium phosphate and chitosan on aerobic plate counts, generation time, pH, shear force, and sensory evaluations in Korean beef loins stored at 4 or 10°C was assessed. The beef loins were treated with $5 \sim 7.5\%$ (w/v) trisodium phosphate and 1% (w/v) chitosan at exposure times of 10 min. The generation time of aerobic microorganisms on the beef loins increased with higher trisodium phosphate levels during storage at 4°C . During storage at 10°C , treatments of 5% trisodium phosphate and 1% chitosan were the most effective for preventing the growth of aerobic spoilage microorganisms. Shear values of the beef loins treated with trisodium phosphate and chitosan or chitosan alone were lower than those of trisodium phosphate during storage at 4°C . The results of sensory evaluation indicated that the beef loins treated with trisodium phosphate and chitosan were in the "liked less to typical" category for odor and appearance scores compared to the controls during storage at 4°C .

Key words: Korean beef, trisodium phosphate, chitosan, aerobic plate counts, sensory evaluation.

Introduction

Phosphates as antimicrobial surface treatments in refrigerated meat and its products have been used for retarding the growth of undesirable microorganisms under extended storage without suitable preservatives. A current study regarding retail distribution of meat and its product involves the storage at refrigerated temperatures because of the possibility of the growth of food spoilage and pathogenic microorganisms (1~5).

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In general, antimicrobial of trisodium phosphate works by removing a thin layer of fat on the surface of poultry skin allowing for removal of the bacteria from the surface of carcass⁽⁶⁾. According to Rathgeber and Waldroup (4), the use of Brifisol KTM (a commercial blend of sodium acid pyrophosphate and orthophosphoric acid) significantly reduced Escherichia coli, coliforms and aerobic plate counts on broiler carcass and increased shelf -life by 1 to 2 days when stored at 4.4°C. Researchers (2,3,7~9) suggested that decontamination of refrigerated meat and its product with undesirable microorganisms was highly dependent on the concentration, type, and exposure times of preservatives used. Molins et al. (10) reported that 0.4% sodium tripolyphosphate, tetrasodium phosphate and three commercial phosphate blends inhibited the growth of undesirable microorganisms in frozen beef patties that were subsequently held at abused temperatures. Marcy et al. (9) noted that the cooked pork sausage treated with 0.4% sodium acid pyrophosphate at 5° C caused significantly lower counts of mesophilic and facultative anaerobic organisms after 48 hr of temperature abuse $(20 \sim 22^{\circ}\text{C})$.

Phosphates have remarkable antimicrobial properties in fresh poultry, possibly because membrane-bound phosphatases in meat products would not be released by extensive cell disruption from cutting and mincing (20). Also, Kim and Marshall noted that trisodium phosphate was strongly inhibitory against the growth of aerobes in chicken legs compared to monopotassium phosphate, monosodium phosphate, and sodium pyrophosphate during storage at 4°C. Lower bacterial counts for 16 days at 4°C resulted from the use of 7.5 or 10% trisodium phosphate. Additionally, phosphates have been suggested as improving of beef and pork quality including the color, flavor, tenderness, and water holding capacity by inhibiting oxidative changes and increasing water holding capacity (11,12). Mendonca et al. (11) reported that the combination of potassium sorbate and phosphates preserved the red color in pork chops.

Chitosan is available in large quantities as waste products of the shellfish industry, which is completely solubilized in acid solvents (13,14). It has been applied as an effective agent for the preservation of fruits, the antagonistic action against fungal plant pathogens, and meat preservatives in the food industry (13-15). Because of these benefits, chitosan could be an effective agent for improving microbiological and physical qualities of meat during refrigerated storage.

Although phosphates and chitosan have been used for improving the quality and the safety of refrigerated meat and its product (2,3,8,13,16), there were no studies on the com-

bined effects of phosphate and chitosan on Korean cattle beef refrigerated. The purpose of this study was to evaluate the combined effects of trisodium phosphate and chitosan on the microbiological and physical qualities of the beef during storage at 4 and 10°C.

Materials and Methods

Beef samples and treatments

Korean cattle beef loins were obtained from commercial processing plant, packed with the ice and used within 24 hr postmortem. For each treatment, one kg of the beef loins (average weight 50g per loin) were submerged in 2L of 1% (w/v) chitosan (Showa Chemical Inc., Japan) for 10 min, 5% (w/v) trisodium phosphate (Spectrum Quality Product Inc., Gardena, CA, USA) after dipping in 1% chitosan solution for 10 min, 7.5% trisodium phosphate after dipping in 1% chitosan, or either 5% or 7.5% (w/v) trisodium phosphate for 10 min, and drained on a sanitized stainless-steel grill for 2 min. Controls were dipped in 2L tap water only for 10 min to compensate for possible physical removal of bacteria and for moisture uptake. Treated beef loins were packed in 6 groups in large Whirl-Pak bags (Fisher Chemical Co., USA), respectively. The bags were closed and stored at 4°C. Samples were analyzed at 4 days intervals,

Microbiological Analysis

Individual beef loins were aseptically transfered to Whirl-Pak bags, weighed, and diluted 1:1 with 0.1% (w/v) sterile peptone water. Samples were shaken for 50 times using standard rinse method⁽²⁾. The liquid from each sample was diluted and plated in volumes of 0.1 ml on standard plate count agar (Difco Laboratories, Detroit, MI) for aerobic plate counts. The plates were incubate for 48 hr at 37 °C before colonies were counted. The number of bacteria was expressed as

mean Log₁₀ CFU/g for the triplicate treatments

Calculation of Generation Times

The growth rates of aerobic microorganisms were determined using the following to calculate generation times⁽¹⁷⁾. Two points on the logarithmic growth phase of each curve were used in the calculation.

Generation time =
$$(0.301 (T_2 - T_1))/(\log P_2 - P_1)$$

Where : T_1 = time of P_1 , T_2 = time of P_2 , P_1 = CFU/g at T_1 , and P_2 = CFU/g at T_2

Shear Force Values

Upon reaching each aging period, the beef loins were gently dried with absorbent tissue paper, weighed, packed, and sealed with a semi-vaccum in Kapak pouches (Kapak Corporation, Minneapolis, MN, USA). These packages were heated in water bath at 75°C for one hour and cooled at room temperature for one hour. The pouches were unwrapped. gently dried, and weighed again. Cooking loss was the difference in weight after heating. For the measurement of tenderness, three to four core samples (1.8 cm diameter) were taken from the slice after cooking. Each core sample was cut with a Warner-Bratzler blade attached to TA.XT2 Texture Analyzer (Texture Technologies Group, Scarsdale, NY, USA) at the speed of 180 mm/min. The shear force requirement was the mean of the maximum forces required to shear each set of core samples.

pH Values

The pH was measured from homogenates of 2.5g of muscle in 10ml of 5mM Iodoacetate -150mM KCl (adjusted to pH 7.0) according to Bendall⁽¹⁸⁾.

Sensory Evaluation

Sensory evaluations of samples was per-

formed by a panel of ten trained members. Odor and appearance of beef loins were evaluated during storage at 4 °C. Treated beef loins were judged against fresh control beef loins (fresh daily), which were assigned a score of 5. Samples liked less than the control were scored 1 to 4, where 1 = disliked most. Samples liked more than the control were scored 6 to 9, where 9 = liked most. Untreated control beef loins also was stored at 4°C for comparison against fresh control and treated beef loins.

Statistical Analysis

A completely randomized design with three replicate experiments was used. Each sampling point represented means calculated from 4 samples per replicate per sampling day. Aerobic plate counts, generation time, pH, shear force, and sensory data were analyzed using ANOVA, with means seperated by the least significant difference test at $P < 0.05^{(19)}$.

Results and Discussion

Microbiological Changes

Results have been transformed into generation times to facilitate the direct comparison of treatments on Korean beef loins. which were the effect of different levels and types of antimicrobial agents during storage at 4° or 10°C. When the beef loins were dipped in trisodium phosphate and/or chitosan solution alone for 10 min, treatments of 1% chitosan had significantly lower (P<0.05) generation times than those of 5% and 7.5% trisodium phosphate combined with 1% chitosan or trisodium phosphate alone during storage at 4°C (Fig. 1). The generation times of aerobic microorganisms on the beef loins treated with 5% or 7.5% trisodium phosphate combined with 1% chitosan or trisodium phosphate alone were significantly higher (P <0.05) than control. The generation times of control, 1% chitosan, 1% chitosan combined

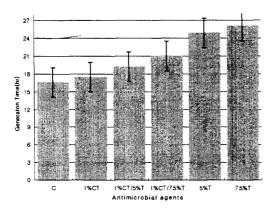


Fig. 1. Generation times of aerobic microorganisms on refrigerated (${}^{\circ}$ C) Korean beef loins dipped in trisodium phosphate (TSP) and chitosan (CT), either alone or combined for 10 min. Key: 1% CT/5% T = treatment of 5% trisodium phosphate after dipping in 1% chitosan for 10 min: 1% CT/7.5% T = treatment of 7.5% trisodium phosphate after dipping in 1% chitosan for 10 min.

with 5% trisodium phosphate, 1% chitosan combined with 7.5% trisodium phosphate, 5% trisodium phosphate, and 7.5% trisodium phosphate were 16.6, 17.4, 19.2, 21.2, 24.9, and 26.1 hr, respectively. There were no significant differences (P>0.05) between 1% chitosan and 2% of chitosan at 10 min dipping (data not shown). The generation times of beef loins treated with 7.5% trisodium phosphate for 10 min increased by 9.5 hr compared to the controls. During storage at 1 0°C, the generation times of beef loins treated with trisodium phosphate combined with chitosan or trisodium phos phate alone did not increase (P>0.05) between treatments at dipping times of 10 min (data not shown). It is postulated that phosphates may cause a decline in water activity of refrigerated meat and have indirect antimicrobial effects due to chelation of metal ions essential for bacterial metabolism and cell integrity (20)

Table 1 showed that trisodium phosphate combined with 1% chitosan or alone had a

Table 1. Number of days to reach spoilage levels of 10⁷ CFU/g of Korean beef loins dipped for 10 min in trisodium phosphate (TSP) and chitosan (CT), either alone or combined during storage at 4 or 16°C

Treatment	Storage days at 4°C	Storage days at 10°C		
Control	4ª	2ª		
1% CT	8^{b}	4^{b}		
1% CT/5% TSP	8^{b}	8^{d}		
1% CT/7.5% TSP	$12^{\rm c}$	6°		
5% TSP	12^{c}	6 ^c		
7.5% TSP	16^{d}	6°		

 $^{\rm a^{-d}}$ Means within the same column with different superscrips are significantly different (P < 0.05). Means of 3 replications, Key: 1% CT/5% T = treatment of 5% trisodium phosphate after dipping in 1% chitosan for 10 min: 1% CT/7.5% T = treatment of 7.5% trisodium phosphate after dipping in 1% chitosan for 10 min.

significantly inhibitory effects to aerobic spoilage microorganisms compared to control for storage at 4 and 10°C. In this trials, the amount of trisodium phosphate became important only with dipping times of 10 min during storage at 4°C. During storage at 4°C, microbialogical shelf-life of the beef loins treated with 5% or 7.5% trisodium phosphate containing 1% chitosan or 5% trisodium phosphate alone was extended by 12 days compared to the control. The levels of 7.5% trisodium phosphate increased (P<0.05) the shelf-life of beef for 16 days at 4°C. However, during storage at 10°C, treatment of 5% trisodium phosphate containing 1% chitosan was the most effective for preventing the growth of aerobic spoilage microorganisms, which could be extended microbiological shelf-life of the beef loins for 8 days. Kim and Marshall suggested that spoilage of meat generally occured when microbial counts reach to 10^7 CFU/g. Thus, shelf-life of the beef loins treated with trisodium phosphate

containing 1% chitosan for 10 min could be 4~12 days longer than the controls. Darmadji and Izumimoto (13) have found that the minced beef mixed with 0.5~1.0% chitosan inhibited the growth of spoilage bacteria during incubation at 30°C for 48 hr or storage at 4°C for 10 days. They noted that the mode of inhibition by chitosan on the growth of some spoilage bacteria and some meat starter cultures might be due to the interaction of chitosan with membranes or cell wall components. We have shown that chicken legs treated with 5% trisodium phosphate for 10 min had aerobic plate counts nearly 3 logs lower than controls after storage of 12 days at 4°C (2). Shelf-life of chicken legs could be extended to 16 days with 7.5 or 10% trisodium phosphate treatment. Hwang and Beuchat (21) and Marshall and Jindal have found that trisodium phosphate is more active in reducing spoilage bacterial levels on meat surface and extending shelf-life than other phosphates.

Changes in pH

The pH values of Korean beef loins showed that chitosan treatments or controls significantly(P<0.05) decreased for 12 days compared to other treatments (Fig. 2). Treatments of 7.5% trisodium phosphate containing 1% chitosan or 7.5% trisodium phosphate alone after its dip had significantly (P < 0.05) increased pH values compared to other treatments, but treatment of 7.5% trisodium phosphate gradually drop in pH beyond 4 to 12 days. The beef loins treated with a combination of 1% chitosan and 7.5% trisodium phosphate were from pH 6.76 immediately after treatment to 6.81 after 16 days, which were no significantly difference (P> 0.05) in pH values during storage at 4°C.

In general, changes in pH induced by phosphate addition may play an important role in the ability of these compounds to chelate metal ions essential in bacterial meta-

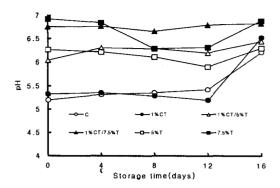


Fig. 2. Mean pH values of refrigerated (4°C) Korean beef loins dipped in trisodium phosphate (TSP) and chitosan (CT), either alone or combined for 10 min. Key: 1% CT/5% T = treatment of 5% trisodium phosphate after dipping in 1% chitosan for 10 min: 1% CT/7.5% T = treatment of 7.5% trisodium phosphate after dipping in 1% chitosan for 10 min.

bolism and/or cell integrity (20). He noted that withdrawl of metal cations through chelation by phosphates may be determinant for the activity enzymes that require metal ions as co-factors. Young et al. (24) have found that the use of STPP in chicken meat increased pH and the addition of NaCl in the presence of STPP slightly decreased this effect. Furthermore, they noted that the effect of polyphosphates on meat quality increased pH and ionic strength, which could be effective in improving water holding capacity. Yu and Lee⁽²⁵⁾ reported that the shear values of beef in a high pH (> 6.3) were significantly (P 0.05) more tender than those of low pH (< 5.8) or intermediate pH from 5.8 to 6.3 after 24 hr chilling in a 4°C cooler. There was no difference between the latter two groups.

Changes in Shear Values

The Warner-Bratzler shear values were due to chitosan, which was most effective when it was combined with increasing levels of trisodium phosphate by 7.5% (Fig. 3). No

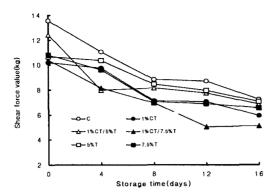


Fig. 3. Mean shear force values on refrigerated (4°C) Korean beef loins dipped in trisodium phosphate (TSP) and chitosan (CT), either alone or combined for 10 min. Key: 1% CT/5% T = treatment of 5% trisodium phosphate after dipping in 1% chitosan for 10 min: 1% CT/7.5% T = treatment of 7.5% trisodium phosphate after dipping in 1% chitosan for 10 min.

significant difference (P>0.05) was observed in the shear values between 1% chitosan and 7.5% trisodium phosphate for 12 days. The shear values were the lowest levels for the beef loins treated with a combination of 1% chitosan and 7.5% trisodium phosphate beyond 8 to 16 days.

It has been suggested that the mechannism of cold-toughening in microstructure of beef may include increased overlap, shorter sarcomeres, and thus opportunity for increased actin-myosin lateral cross-linking of contractile filaments in rigor mortis (23). Beltran et al. (26) noted that Warner-Bratzler shear values were significantly lower when pH values of beef were over 6.3. Additionally, enzyme such as m- and μ - calpain were known to have a pH optimum close to 7.0 and high ultimate pH would lead to an improvement of tenderness due to increased proteolytic activity. Nielsen et al. (16) reported that sodium tripolyphosphate below and above 0.2% in restructured meat resulted in decreasing hardness values. However, the highest hardness values from phosphate was observed at 0.2% trisodium pyrophosphate, particularly in the presence of transglutaminase. Otherwise, although the beef loins treated with 1% chitosan only had a lower (P<0.05) the shear values than those of 5% trisodium phosphate combined with 1% chitosan or either 5% trisodium phosphate alone and the controls after storage of 8 days, it was resulted in the decline of generation time due to microbiological spoilage. Since there is a relationship in the shear values between chitosan and trisodium phosphate, further studies should be needed.

Sensory Evaluation

Sensory data of beef loins dipped in both trisodium phosphate and chitosan or either alone for 10 min are shown in Table 2. Sensory scores indicated that treatments of trisodium phosphate and chitosan or either trisodium phosphate alone were in the "liked less to typical" category in odor and appearance compared to the fresh controls. Untreated controls or 1% chitosan treatments were significantly lower (P<0.05) in appearance scores compared to the other treatments associated with spoiling meat after storage of 8 days. For odor and appearance scores, untreated control or 1% chitosan treatment had significantly (P<0.05) lower compared to those fresh controls and other treatments after storage of 12 days. Darmadji and Izumimoto (13) reported that minced beef mixed with 0.5% chitosan was more acceptable (p< 0.05) on the development of red color and was significantly different from controls during storage of 10 days at 4°C. Previous research (2) have suggested that chicken legs treated with 10 and 15% trisodium phosphate solution for 10 min were resulted in lower (P<0.05) appearances compared to other treatments due to its brownish color. Hathcox et al. (1995) reported that chicken breast and thighs treated with 12% trisodium phosphate did not affect consumer acceptance of raw and fried

Storage tim		Odor score			Appearance score			
Treatment	0	4	8	12	0	4	8	12
Fresh control	5.0±0.00 ^{ab}	5.0 ± 0.00^{a}	5.0±0.00 ^a	5.0±0.00 ^a	5.0±0.00 ^{ab}	5.0 ± 0.00^{a}	5,0±0,00°	5.0 ± 0.00^{a}
Untreated control	5.1 ± 0.12^{ab}	$4.3 \pm 0.25^{\circ}$	$3.0 \pm 0.58^{\circ}$	1.1 ± 0.12^{c}	4.7 ± 0.00^{cd}	3.7 ± 0.40^{d}	2.3 ± 0.47^{d}	$1.3 \pm 0.23^{\circ}$
1% CT	5.1 ± 0.16^{ab}	4.5 ± 0.20^{bc}	$3.0 \pm 0.57^{\circ}$	2.4 ± 0.30^{b}	4.8 ± 0.06^{cd}	4.0 ± 0.00^{cd}	2.6 ± 0.00^{cd}	2.4 ± 0.06
1% CT/5% TSP	4.8 ± 0.29^{b}	$4.3 \pm 0.23^{\circ}$	3.5 ± 0.36^{bc}	2.6 ± 0.25^{b}	4.8 ± 0.12^{cd}	4.4 ± 0.23^{bc}	3.5 ± 0.76^{6}	2.5 ± 0.70^{t}
1% CT/7.5% TSP	4.8 ± 0.10^{b}	$4.3 \pm 0.25^{\circ}$	3.3 ± 0.35^{bc}	2.7 ± 0.25^{b}	5.0 ± 0.06^{ab}	4.6 ± 0.31^{ab}	$3.4 \pm 0.35^{\circ}$	2.9 ± 0.42^{h}
5% TSP	4.9±0.17 ^{ab}	4.9 ± 0.21^{ab}	3.4 ± 0.37^{bc}	2.9 ± 0.50^{b}	5.1 ± 0.21^a	5.0 ± 0.15^{a}	$3.5 \pm 0.17^{\circ}$	2.6 ± 0.40^{t}
7.5% TSP	5.0 ± 0.12^{ab}	4.9 ± 0.20^{ab}	3.8 ± 0.20^{b}	2.7 ± 0.72^{b}	4.5 ± 0.17^{e}	4.3 ± 0.25^{bc}	3.1 ± 0.46^{bc}	2.3±0.61 ^b

Table 2. Mean sensory evaluation scores of refrigerated (4°C) Korean beef loins dipped in trisodium phosphate (TSP) and chitosan (CT), either alone or combined for 10 min

^{a*e}Means (\pm standard errors) within the same column with different superscripts are significantly different (P<0.05). 1Means of 3 replications. Key: 1% CT/5% T = treatment of 5% trisodium phosphate after dipping in 1% chitosan for 10 min: 1% CT/7.5% T = treatment of 7.5% trisodium phosphate after dipping in 1% chitosan for 10 min.

pieces. The sensory data indicate that treating beef loins with 5 and 7.5% trisodium phosphate and 1% chitosan or either alone could improve consumer satisfaction during storage of 12 days at 4°C.

요 약

제3인산나트륨과 키토산으로 10분 동안 침 지한 한우 쇠고기 등심은 4℃와 10℃ 냉장동안 호기성 부패세균수의 증식 억제에 효과적이었 다. 7.5% (w/v) 농도까지 증가한 제3인산나트 륨용액으로 처리한 한우 쇠고기 등심은 4℃ 저 장 동안 항 미생물 효과를 증진하였다. 그러나 10℃ 저장 조건에서는 5% 제3인산나트륨과 1% (w/v) 키토산 용액으로 처리한 처리구에 서 호기성 부패세균수의 증식억제에 가장 효과 적이었다. Warner-Bratzler 전단력 측정결과 1% 키토산과 7.5% 까지 증가된 제3인산나트 륨용액으로 처리한 처리구가 가장 효과적 이었 다. 제3인산나트륨과 키토산 처리구의 냄새 및 외관에 대한 관능평가 결과는 4°C 저장 동안 신선한 한우 쇠고기등심 보다 낮게 좋은 것으 로 등급되었다.

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