

## Enhance antibacterial effects of combination sodium diacetate and surfactant agent on *Staphylococcus aureus* and *Escherichia coli* and quality of fresh chilled ground pork

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### Abstract

*Staphylococcus aureus* and *Escherichia coli* are important foodborne pathogenic bacteria and serious intimidations to human health worldwide. This study was to reduce and control growth of *S. aureus* and *E. coli* and the spoilage bacteria in fresh ground pork stored at 5 and 15°C using sodium diacetate (SD) alone and in combination with polyethylene glycol (PEG). Microbial and physical qualities of ground pork (inoculated with *S. aureus* and *E. coli* 4log cfu/g) added 0.25%SD, 1.25%SD and 0.16%SD in combination with 0.16%PEG were investigated. The results showed that the adding 1.25%SD and 0.16%SD in combination with 0.16%PEG could significantly ( $p < 0.05$ ) reduce *S. aureus*, *E. coli*, aerobic plate count (APC) and psychrotrophic bacteria population in ground pork. The both treatments could control growth of both pathogenic bacteria in ground pork stored at 5°C for 14 days. In addition, these treatments could control growth of *S. aureus* and *E. coli* stored at 15°C for 4 and 6 days, respectively. The adding both treatments could control growth of APC and extend shelf life of ground pork stored at 5 and 15°C for 12 and 8 days, respectively. However, the low pH of high concentration SD alone caused the highest weight loss and discoloration. No adverse effect of low concentration SD in combination with PEG in ground pork was observed. Its weight loss and color was not significantly different from those of non-treated at both storage temperatures ( $p > 0.05$ ). Hence, this study suggests that 0.16%SD in combination with 0.16%PEG has great potential to be used as a good preservative for fresh chilled ground pork.

### Keywords

Salt of organic acid  
Polyethylene glycol  
Pathogenic bacteria  
Antibacterial activity

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### Introduction

*Staphylococcus aureus* and *Escherichia coli* are the pathogenic foodborne bacteria. *S. aureus*, which is commonly found on the skin and mucous membranes of animals and humans, is involved in a wide variety of infections (Loir *et al.*, 2003). Several foods have been implicated in food poisoning incidents attributed to *S. aureus* and *E. coli*, with meat and meat products being the most frequent vehicles of intoxication (Smith *et al.*, 1983). In pork carcasses, a prevalence of *S. aureus* and *E. coli* as high as 50.00 and 83.33%, respectively, from floor slaughtering process and 16.67 and 33.33%, respectively, from hanging slaughtering process in abattoirs of Southern Thailand (Tangwatcharin and Wattanachant, 2009). In addition, Ganyarat (2007) also found *S. aureus* and coliform bacteria contaminated in 43.3 and 96.7% of 30 raw pork samples sold in Bangkok, respectively.

The problem of safe preservation in the meat industry has grown to be more complex as today's products require more safety and greater assurance

of protection from pathogens. Many attempts have been made to control the growth of pathogens on the surface of meat and meat products with the use of chemical antimicrobials. Sodium diacetate (SD) was shown to be effective in limiting growth of *S. aureus* and *E. coli* (Shelef and Addala, 1994). However, in developed countries, maximum level of sodium diacetate use in meat products is 0.25% of final product (Health Canada, 2016; Food and Drug Administration, 2016). Surfactants constitute the most important group of detergent products. Generally, these are water-soluble surface-active agents comprised of a hydrophobic portion, usually along alkyl chain, attached to hydrophilic or water solubility enhancing functional groups (McDonnell and Russell, 1999). The bactericidal activity of a variety of glycols has been studied by Robertson *et al.* (1948). They investigated the bactericidal action in vitro of a number of glycols for pneumococci, hemolytic streptococci, and staphylococci. Plitman *et al.* (1973) investigated the bacteriostatic and

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bactericidal activity of several diols, employing *S. aureus* as test organism. Moreover, Vaamonde *et al.* (1982) showed that polyethylene glycol 400 (PEG) appeared to have a significant inhibitory effect on one strain of *S. aureus*. The present study describes investigations that were carried out to explore the bactericidal effect of concentrated PEG against various pathogenic bacteria relevant to infected wounds and other superficial lesions. The bacterial species studied included *S. aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *E. coli*. Mattia (2016) reported that PEG is a “Generally Recognized as Safe” (GRAS, 21 CFR 170.36) cost-effective food additive.

As new ingredients are incorporated into meat products as antimicrobials (Ponrajan *et al.*, 2011), it is important to evaluate their impact on color, shelf-life and quality. Therefore, the objective of this research was to compare the antibacterial activity of SD and SD+PEG against *S. aureus* and *E. coli* and enhance shelf-life of ground pork.

## Materials and Methods

### Test strains

*S. aureus* TUSA1 and *E. coli* TUEC1 were used in the present study. These strains were previously isolated from pig carcasses in Southern Thailand abattoirs by the standard procedure (BAM, 2001a and 2002) and their identity was confirmed by the Department of Medical Sciences, Ministry of Public Health of Thailand. These organisms were maintained on Mueller Hinton agar (MHA) (Merck, Germany). The overnight cultures were prepared by inoculating approximately 2 ml Mueller Hinton broth (MHB) (Merck, Germany) with 2-3 colonies taken from MHA. Broths were incubated overnight at 35°C. Inocula were prepared by diluting overnight culture in saline to 10<sup>8</sup> cfu/ml (McFarland standard of 0.5).

### Antimicrobial agents

SD was supplied by Chemipan Corporation Co. Ltd. (Bangkok, Thailand). Polyethylene glycol 400 (PEG) was provided by Chemipan Corporation Co. Ltd. (Bangkok, Thailand). All antibacterial were food grade. The concentrations of SD and PEG were assessed as % (v/v).

### Meat model

Fresh pork ham and back fat were purchased from the hygienic slaughter house of Nakornpathom province, Thailand. Ground pork was prepared from 95% lean and 5% fat. After that, ground pork was

divided into 4 groups and 3,200 g/group/replication. Three groups were used to determine the effects of antimicrobials on aerobic plate count (APC) and psychrotropic bacteria (one group, experiment 2) and physical analyses (two groups, experiment 3), for which ground pork was not inoculated with the bacterial suspension. The another group was used to determine the effect of antimicrobials on *S. aureus* and *E. coli*, for which the ground pork was inoculated with *S. aureus* TUSA1 and *E. coli* TUEC1 suspension (experiment 1) as follows: the ground pork was individually submerged in 0.32 ml of the bacterial inoculum (*S. aureus* TUSA1 and *E. coli* TUEC1 containing approximately 10<sup>8</sup> cfu/ml, prepared in sterile 0.85% (w/v) saline solution) before adding the antimicrobials. The initial count of *S. aureus* TUSA1 and *E. coli* TUEC1 was approximately 10<sup>4</sup> cfu/g. The ground pork was randomly divided into four treatments and adding the antimicrobials as follows: (1) control - non treated; (2) added 0.25% (g/g) SD; (3) 1.25% (g/g) SD and (4) added 0.16% (g/g) SD + 0.16% (g/g) PEG. Each treated group was weighed 100 g and packed in the polyethylene plastic bag. Then, the packages were stored in the air-circulated refrigeration at 5 and 15°C for 0, 2, 4, 6, 8, 10, 12 and 14 days for storage times. The microbiological and physical chemistry analyses were determined. The sample meats were submitted to count for *S. aureus* TUSA1 (BAM, 2001a) and *E. coli* TUEC1 (BAM, 2002), APC (BAM, 2001b) and psychrotropic bacteria (ISO 17410:2001) according to standard procedures. The results were transformed to log cfu per gram of meat (log cfu/g).

### Microbiological analyses

The sample meats were submitted to count for *S. aureus* (BAM, 2001a) and *E. coli* (BAM, 2002) aerobic plate count (APC) (BAM, 2001b) and psychrotropic bacteria (ISO, 2001) according to standard procedures. The results were transformed to log cfu/g. The plates were incubated at 35 ± 2°C for 24-48 hr before colonies were counted. *S. aureus* and *E. coli* were enumerated on Baird Parker agar (Merck, Germany) to which egg-yolk tellurite emulsion 20% (Merck, Germany) was added and violet red bile agar (Merck, Germany), respectively. Then, coagulase test for *S. aureus* and IMViC test for *E. coli* were determined. Enumeration of APC was done on plate count agar (Merck, Germany).

### Physical analysis

Three randomly selected areas from an exterior color of ground pork samples were measured in the *L\*a\*b\** model of CIE. For pH measurement, pH

values were determined with a pH meter (Model 320, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) after blending 5 g of sample with 20 ml of distilled water for 60 s in a homogenizer (Ultra-Turrax T25, Janke & Kunkel, Staufen, Germany).

Ground pork samples were weighed before and after storage and weight loss for each meat was calculated as below:

$$\text{weight loss} = \frac{(\text{before stored weight} - \text{stored weight})}{\text{before stored weight}} \times 100$$

### Statistical analysis

All experiments were carried out in triplicate and the results were expressed as mean  $\pm$  standard deviations. All statistical computations were performed to determine significant differences ( $p < 0.05$ ) by ANOVA followed by Duncan's new multiple range test (Steel and Torrie, 1980).

## Results and Discussion

### Effects of sodium diacetate in combination with polyethylene glycol on microbiological quality of fresh chilled ground pork

The minimal inhibitory concentration of SD and PEG against *S. aureus* TUSA1 and *E. coli* TUEC1 were 0.31 and 2.50%(w/v), respectively. The minimal bactericidal concentrations (MBC) of SD against both pathogenic bacteria were 1.25 and 2.50%(w/v), respectively. MBC of PEG against *S. aureus* TUSA1 was 2.50%(w/v). For synergistic effects, fractional bactericidal concentration indexes of the combined action of SD with PEG were 0.31 (0.31%(w/v) SD + 0.16%(w/v) PEG) for against *S. aureus* TUSA1 and 0.52 (1.25%(w/v) SD + 0.04%(w/v) PEG) for *E. coli* TUEC1, suggesting synergy and partial synergy, respectively (data not shown).

The results revealed that the use of SD alone and in combinations with PEG reduced *S. aureus* and *E. coli* count on ground pork stored at 5 and 15°C (Figure 1). *S. aureus* and *E. coli* in ground pork added 1.25%SD and 0.16%SD in combination with 0.16%PEG was decreased by 0.99 to 1.25 log cfu/g before storage and growth was retarded throughout the 14 and 4 days of storage time at 5 and 15°C, respectively. At the end of the 14 days of storage time at 5 and 15°C, *S. aureus* and *E. coli* in ground pork non-treated (control) and added 0.25%SD were in the range of 1.48-2.89 and 0.79-3.64 log cfu/g higher compared to *S. aureus* and *E. coli* counts in ground pork added 1.25%SD and 0.16%SD in combination with 0.16%PEG (Figure 1). SD exerts an antimicrobial effect due to its hyper-acidification via proton donation at the

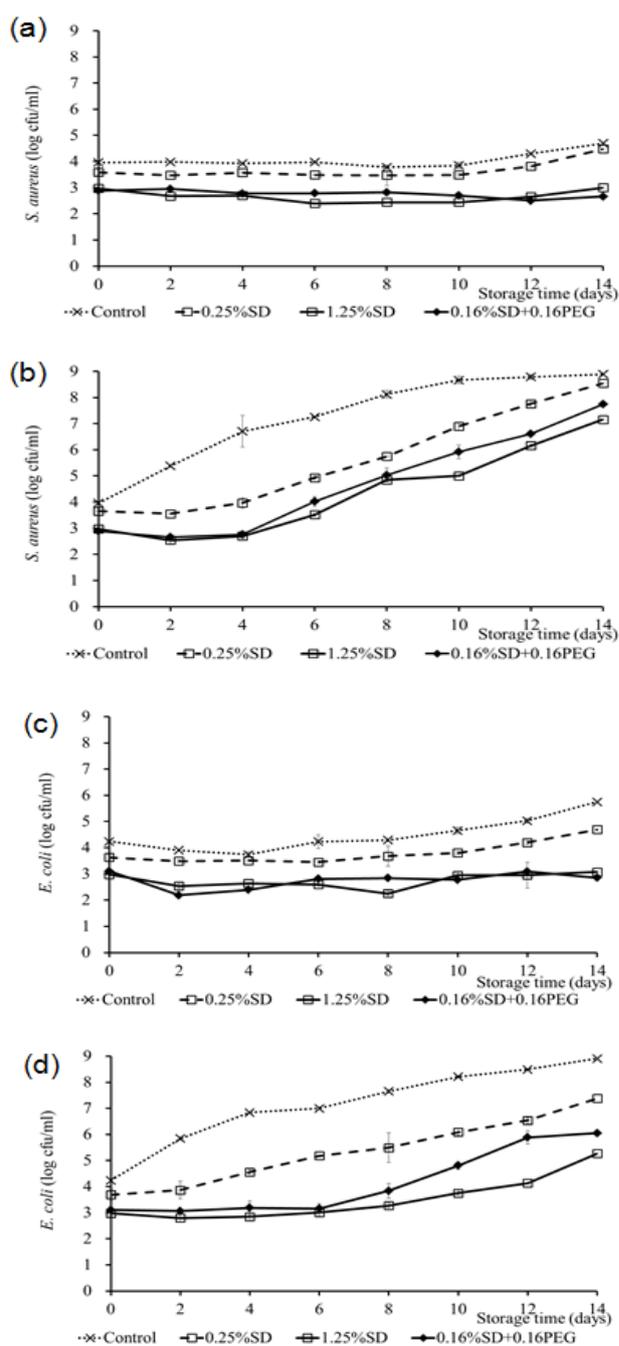


Figure 1. Numbers of *S. aureus* (a and b) and *E. coli* (c and d) in ground pork adding 0.25% sodium diacetate, 1.25% sodium diacetate and 0.16% sodium diacetate + 0.16% polyethylene glycol 400 stored at 5 (a and c) and 15°C (b and d) for 14 days

plasma membrane interface of the microorganism and intracellular cytosolic acidification, an excess of which can disrupt the H<sup>+</sup>-ATPase enzyme, which is required for ATP synthesis (Silva *et al.*, 2012). Lag phase extension and growth rate reduction for *L. monocytogenes* were also observed in ground ham added 0.25%SD (Hwang and Tamplin, 2007). Furthermore, 0.16%SD in combinations with 0.16%PGE was not active to against *S. aureus* and *E. coli* in ground pork after storage at 15°C for 4 and 6

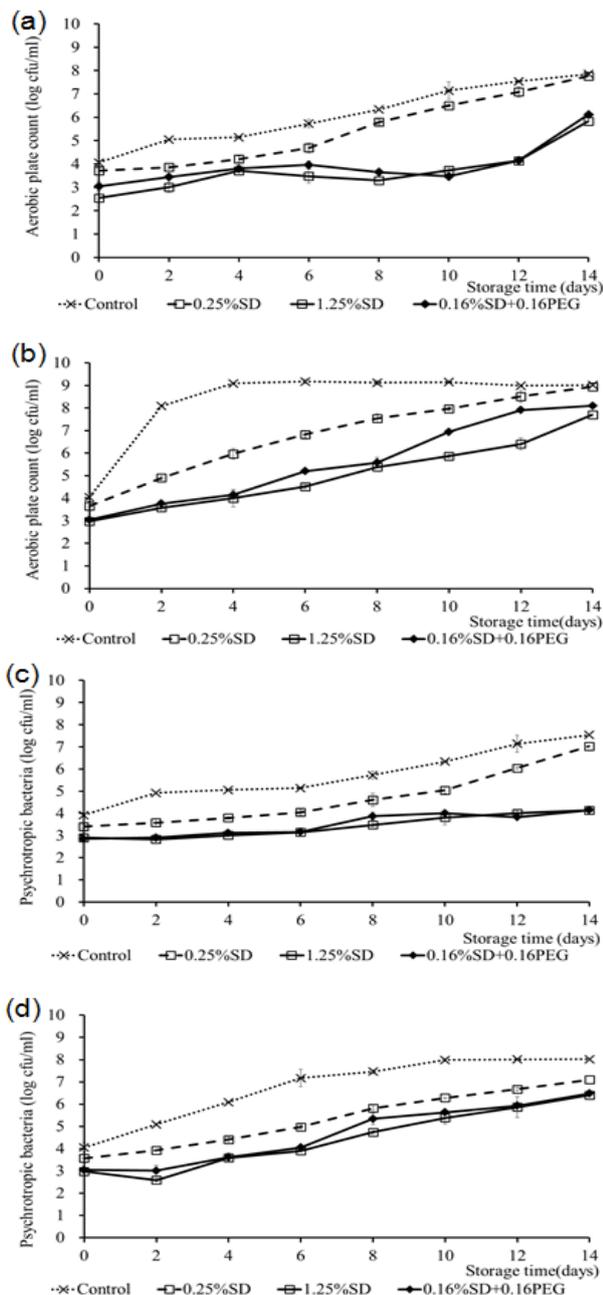


Figure 2. Numbers of aerobic plate count (a and b) and psychrotrophic bacteria (c and d) in ground pork adding 0.25% sodium diacetate, 1.25% sodium diacetate and 0.16% sodium diacetate + 0.16% polyethylene glycol 400 stored at 5 (a and c) and 15°C (b and d) for 14 days

days, respectively. However, 1.25%SD was not active to against these pathogens in ground pork after storage at both temperatures for 2 days. This could be due to antibacterial efficacy of synergistic is more than that of SD only. This causes a lipopolysaccharide (LPS) in bacterial cells, as well as the cell phospholipid is nonpolar surface, especially gram-negative bacteria. These nonpolar allow the nonionic PEG surfactant linked by SD to associate with the outer surface of the target bacterial cells, resulting in disruption of cell membrane integrity and eventually leading to leakage of the intracellular lysate and dissolution of

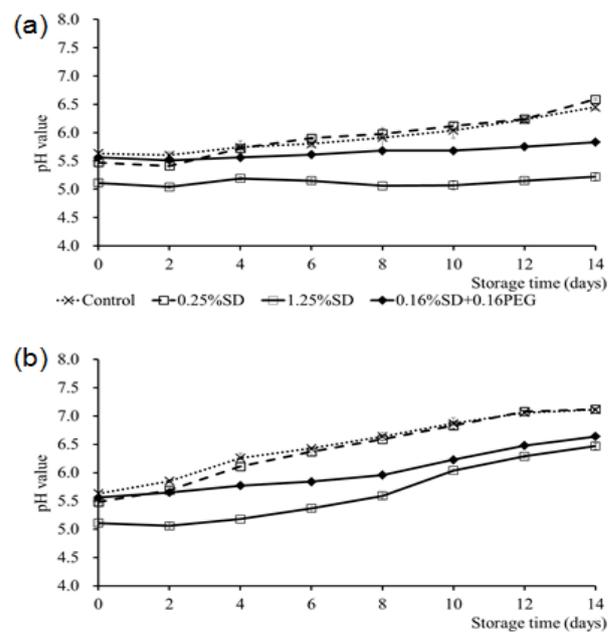


Figure 3. The pH value of ground pork adding 0.25% sodium diacetate, 1.25% sodium diacetate and 0.16% sodium diacetate + 0.16% polyethylene glycol 400 stored at 5 (a) and 15°C (b) for 14 days

the cytoplasmic membrane (Rosen, 1989). According to this study, SD in combination with PGE could control growth of *E. coli* greater than *S. aureus* in ground pork stored at 15°C. Consequently, this surfactant behaved as efficient bacteriostatic and bactericidal agent.

APC is an important indicator for meat quality and shelf-life (Rahman *et al.*, 2013). As shown in Figure 2, the adding of 1.25%SD and 0.16%SD in combination with 0.16%PEG could cumulatively inhibit these microorganisms and extend shelf life of ground pork after storage at 5 and 15°C for 12 and 8 days, respectively. Their aerobic bacterial loadings were lower limit of Thai agricultural commodity and food standard for pork (TACFS 6000-2004) which its limit is not higher 5.70 log cfu/g (National Bureau of Agricultural Commodity and Food Standard, 2004). On the contrary, the shelf life of ground pork added 0.25%SD was 6 and 2 days after storage at 5 and 15°C, respectively. Similarly, adding of 1.25%SD and 0.16%SD in combination with 0.16%PEG in ground pork can inhibit the growth of psychrophilic bacteria after storage at 5 and 15°C for 6 and 2 days, respectively (Figure 2). The spoilage and foodborne pathogenic bacteria are much easier to grow on fresh meat and often cause spoilage of meat (Zhou *et al.*, 2010). Some bacteria such as *Pseudomonas* spp. and *Listeria* spp. could grow well after storage at low temperature that limited the shelf life of fresh pork (Zhang *et al.*, 2010).

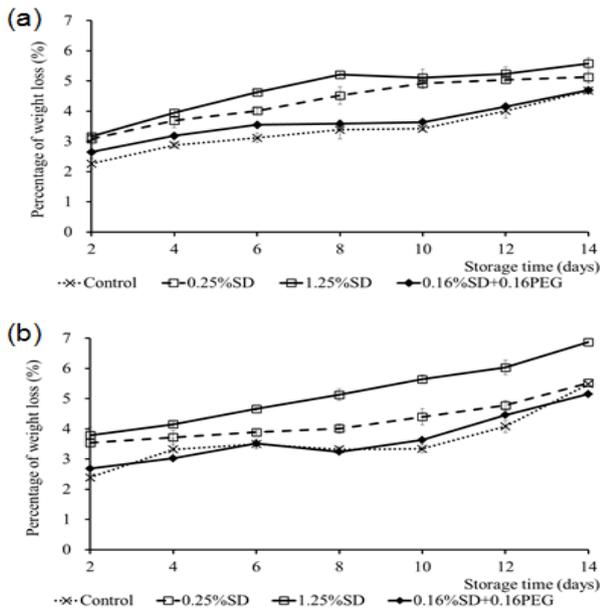


Figure 4. Percentage of weight loss (%) of ground pork adding 0.25% sodium diacetate, 1.25% sodium diacetate and 0.16% sodium diacetate + 0.16% polyethylene glycol 400 stored at 5 (a) and 15°C (b) for 14 days

#### Effects of sodium diacetate in combination with polyethylene glycol on physical quality of fresh chilled ground pork

The pH values of non-treated ground pork use for control samples were 5.67. The pH values of ground pork added SD alone and in combination with PEG stored at 5 and 15°C for 14 days are presented in Figure 3. The pH values of ground pork added 1.25%SD and non-treated were significant difference ( $p < 0.05$ ) while their ground pork added other antimicrobial and non-treated were not significant difference ( $p > 0.05$ ) before storage at both temperatures. The pH of all the ground pork increased gradually with increase of storage time at both storage temperature, but the different antibacterial resulted in varying pH of the samples. The adding of 1.25%SD and 0.16%SD in combination with 0.16%PEG retarded the increase of pH during 14 days at both storage temperatures. The reason should attribute to both treatments effectively inhibiting the growth of microorganisms. The higher APC may account for the higher pH of ground pork added 0.25%SD and non-treated, compared to 1.25%SD and 0.16%SD in combination with 0.16%PEG after 14 days storage. The elevating pH is mainly caused by degradation of proteins and production of amines in pork, and the higher pH and longer aging periods will result in increased microbial proliferation and decreased shelf-life (Tan and Shelef's, 2002; Holmer *et al.*, 2009; Rahman, 2013). However, the pH of all ground pork remained below pH 7.0 and 7.5 after 14 days at 5 and 15°C,

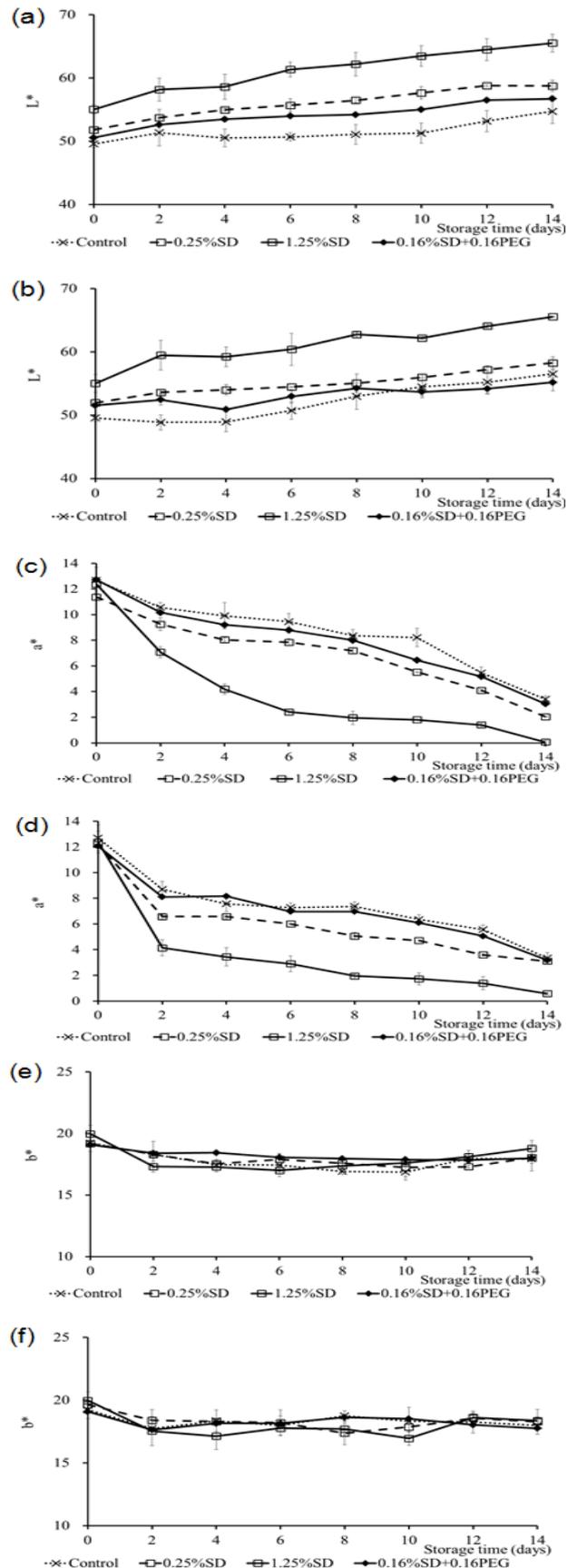


Figure 5.  $L^*$  (a and b),  $a^*$  (c and d) and  $b^*$  (e and f) of ground pork adding 0.25% sodium diacetate, 1.25% sodium diacetate and 0.16% sodium diacetate + 0.16% polyethylene glycol 400 stored at 5 (a, c and e) and 15°C (b, d and f) for 14 days

respectively.

The changes in weight loss of ground pork added in antimicrobials and stored at 4 and 15°C for 14 days are shown in Figure 4. After storage at 4 and 15°C, weight loss of all ground pork increased with the increasing storage period ( $P < 0.05$ ). However, the weight loss of ground pork added SD in combination with PEG at low concentration and non-treated was lighter both high concentrations of SD alone ( $P < 0.05$ ). The lighter weight loss may be due to the decrease pH value (Figure 3), which results in the denaturation of many proteins, including those involved in binding water (Savage *et al.*, 1990).

The changes in color of ground pork added antimicrobials and stored at 4 and 15°C for 14 days are shown in Figure 5. The initial  $L^*$ ,  $a^*$  and  $b^*$  values of non-treated ground pork were  $49.56 \pm 0.54$ ,  $12.69 \pm 1.01$  and  $19.23 \pm 0.71$ , respectively. The color of ground pork added both high concentrations of SD alone were much lighter ( $P < 0.05$ ) those of ground pork added SD in combination with PEG at low concentration and non-treated. The lighter color may be due to the decrease pH value (Figure 3), which results in increasing in weight loss (Figure 4) and higher reflecting property. Even though  $L^*$  value of all pork increased during storage (Figure 5a and 5b). The  $a^*$  values (redness) of ground pork added 1.25%SD were significantly lower ( $> 1.5$  units) compared to those of added 0.16%SD in combination with 0.16%PEG and non-treated ( $p < 0.05$ ). The  $a^*$  values of all ground pork decreased rapidly during storage at 5 and 15°C. However, the decrease rate of ground pork added 1.25%SD was higher those of other ground pork (Figure 5c and 5d). The decrease in  $a^*$  value after treat is reported to be associated with the effect of pH on the myoglobin proportion. Whereas, the decrease in  $a^*$  value during storage is attributed to the oxidation of oxymyoglobin to metmyoglobin. The discoloration of fresh meat was mainly caused by the increase of the amount of metmyoglobin (Ozer and Sarioçban, 2010; Zhang *et al.*, 2016). For  $b^*$  value (yellowness), all pork was constant till the end of the 14 days of storage time (Figure 5e and 5f).

## Conclusion

In conclusion, the combined treatment of SD and PEG at low concentration in fresh chilled ground pork was able to significantly reduce *S. aureus* and *E. coli* counts, inhibit bacterial growth and extend the shelf life of ground pork up to 12 and 8 days stored at 5 and 15°C. This result could meet the demand of transportation, distribution and storage of fresh chilled ground pork in trade. This study indicated that

SD in combination with PEG at low concentration has potential to be used as a good preservative on fresh chilled ground pork at both storage temperatures. However, further studies are necessitated to estimate the changes of the nutritional components of fresh chilled ground pork added SD in combination with PEG.

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