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# Increasing the Quality of Blood Tofu in an Industrial Slaughterhouse of Thailand

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## ABSTRACT

Blood tofu, or cooked duck blood curd, is a Chinese delicacy in East Asia. Its quality and shelf-life are low due to microorganism contamination during production. Therefore, the present study was performed to investigate the role of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol 400 (PEG) combinations in increasing the quality of blood tofu. A total of 45 cooked duck blood curd samples were randomly divided into 3 groups with 3 replicates per group. The first two groups were used to investigate the effect of SD, NaCl, and PEG combinations on microbiological and physical analyses for non-inoculated samples. Another group was used to determine the effect of antimicrobial combinations on *Lactobacillus plantarum*, *Pseudomonas fluorescens*, *Salmonella typhimurium*, *Escherichia coli*, and *Staphylococcus aureus* in inoculated samples that were inoculated with these bacteria. All groups were treated with control-sterilized water, 0.15% SD (w/v) + 1.25% NaCl (w/v), 0.30% SD (w/v) + 1.25% NaCl (w/v), 0.15% SD (w/v) + 0.15% PEG (w/v), and 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). The results indicated that soaking cooked duck blood curd samples in antimicrobial agent combinations could reduce mesophile and psychrophile bacteria counts in non-inoculated samples. Additionally, 0.15% SD + 1.25% NaCl + 0.15% PEG combination had a higher reduction in mesophile and psychrophile counts, compared to soaking the samples in 0.30% SD + 1.25% NaCl, 0.15% SD + 1.25% NaCl and 0.15% SD + 0.15% PEG combinations. Similarly, this combination showed a significant decrease in lactic acid bacteria, *Pseudomonas*, *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* counts in inoculated samples. Furthermore, soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination did not negatively affect the samples' physical quality. Soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination inhibited the growth of mesophile, psychrophile, and *Pseudomonas* in non-inoculated samples after storage for 10, 6, 10, and 8 days in a slaughter warehouse at 7℃, respectively, and extended shelf-life of samples for 16 days. Regarding physical quality changes, this treatment delayed the reduction of pH, hue, hardness, and chewiness of the samples after storage for 10, 8, 12, and 10 days, respectively. Thus, SD, NaCl, and PEG combination had a high preservative potential for cooked duck blood curd used in industrial slaughterhouses.

**Keywords:** Blood curd, Duck, Organic acid salt, Polyethylene glycol, Quality changes

## INTRODUCTION

Blood is the first by-product collected during the meat-producing system in industrial slaughter. Due to its valuable nutritional value and functional properties, blood can be considered a beneficial raw material in the feed and food industries (Toldrá et al., 2016). Blood comprises 75-82% water and 17-18% protein, like oxyhemoglobin and methemoglobin, which exist in red blood cells (Leoci, 2014). Nevertheless, blood can be contaminated by pathogens, such as lactic acid bacteria (LAB), *Pseudomonas*, *Salmonella*, *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*) during the slaughter process through contact with animals' skin, stomach, and intestines. These microbial can be brought into the blood and grow quickly (Dàvila et al., 2006).

Cooked blood curd or blood tofu is one of the famous Chinese foods in East Asia. Normally, it is served with noodles, congee, and soup. The cooked blood curd is prepared from fresh blood coagulating by setting fresh blood in a container. Then, it is cooked in heated water. After the product cooling, it is packed in a plastic bag containing water (Wang and Lin, 1994). The short shelf-life of the product may be due to the survival of contaminated microorganisms in the production process (Wang et al., 2010).

Sodium diacetate (SD) is derived from acetic acid. It acts as a bactericidal agent by expanding the lag phase of spoilage and pathogenic microbial and extending the shelf life of meat and meat products (FDA, 2016). Sodium diacetate is approved by the United States Food and Drug Administration and is generally recognized as a safe ingredient in food products (FDA, 2000). However, the maximum recommended level for SD is up to 0.25% due to off-flavor detection (FDA, 2016). At 0.20% concentration of SD, it has an unfavorable effect on the odor and taste of food products (Stekelenburg and Kant-Muermans, 2001). For sodium chloride (NaCl), a high salinity induces water efflux which is counterpoised by a rise of cooperative solutes (Krämer, 2010). However, the use of high concentrations of salt can adversely affect the consumers' perception of overall palatability (Omotoyinbo and Omotoyinbo, 2016). Polyethylene glycol (PEG) 400 is a surfactant for food additive Generally Recognized as Safe (GRAS, 21 CFR 178.3750, FDA, 2022). Increasing the level of PEG to 20% (w/v) could effectively inhibit the growth of various pathogenic bacteria, including *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa* (Chirife et al., 1983; Holcapkova et al., 2018).

In a previous study by Tangwatcharin and Teemeesuk (2019), the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of SD and NaCl to inhibit the growth of *E. coli*, *S. aureus*, *S. typhimurium*, and *Pseudomonas fluorescens* (*P. fluorescens*) in medium broth were determined. The fractional inhibitory concentration index (FBCI) indicated that the utilization of 0.15% (w/v) SD + 1.25% (w/v) NaCl resulted in improved inhibition of these pathogenic bacteria. Moreover, this study achieved *in vitro* antimicrobial efficacy of this combination to control the growth of pathogenic bacteria. Additionally, the 0.16%(w/v) SD + 0.16%(w/v) PEG combination appeared to have a significant inhibitory effect on *S. aureus* and *E. coli* and extended the shelf life of fresh ground pork for 12 and 8 days of storage at 5℃ and 15℃, respectively (Tangwatcharin et al., 2018). With this in mind, the current study aimed to evaluate the effects of SD, NaCl, and PEG combinations on microbiological and physical qualities of cooked duck blood curd and their application in slaughter warehouses.

## MATERIALS AND METHODS

### Bacterial strains and inocula

*Lactiplantibacillus plantarum* (*L. plantarum*) TISIR543, *P. fluorescens* DMST20076, *S. Typhimurium* DMST22842, *E. coli* DMST4212, and *S. aureus* DMST4745 were obtained from the culture collection at Department of Medical Sciences, Ministry of Public Health, Thailand. Each strain was cross-linked on Mueller Hinton agar (MHA, Merck, Germany) and incubated at 35℃ for 18 hours. These cultures were prepared by inoculating 10 ml of 0.90% (w/v) sodium chloride (NaCl, normal saline) with 2-3 colonies taken from MHA. Inocula were prepared by diluting in 10 ml of normal saline to $10^8$ CFU/ml (McFarland standard of 0.5). As required, these suspensions were further diluted with 99 ml of normal saline (1:100 dilution). The initial concentrations were adopted at approximately $1\times10^6$ CFU/ml (Tangwatcharin et al., 2018).

### Experiment design

Cooked duck blood curd samples (*Cherry valley* crossbred ducks at 47 days) were collected in three batches on different days in a cooked duck blood curd line in an industrial slaughterhouse in East Thailand. All samples were weighed 500 g/sample, packed in a polyethylene bag filled with slaughterhouse water consumption, kept in a polystyrene box containing ice, and transported to a laboratory room within 3 hours for further analysis (Tangwatcharin and Teemeesuk, 2019). Then, 45 samples were divided into 3 groups. Two groups were used to investigate the effect of SD, NaCl, and PEG combinations on microbiological (one group) and physical analyses (one group), for which the samples were not inoculated with the inocula following Tangwatcharin et al. (2019a). Another group was used to determine the effect of antimicrobial combinations on *L. plantarum*, *P. fluorescens*, *S. typhimurium*, *E. coli*, and *S. aureus*. The cooked duck blood curd samples were inoculated with all 5 bacteria suspensions. The samples were individually soaked in 300 ml of each bacterial inoculum ($10^6$ CFU/ml) for 20 minutes and dried in laminar airflow. The initial count of each bacterium was $10^4$ CFU/g. After that, all samples were weighed. For all groups, the cooked duck blood curd samples were randomly divided into 5 treatments and soaked in 300 ml of antimicrobial, including T1 for control in which sterilized water was used, T2 for 0.15% SD (w/v) + 1.25% NaCl (w/v), T3 for 0.30% SD (w/v) + 1.25% NaCl (w/v), T4 for 0.15% SD (w/v) + 0.15% PEG (w/v), and T5 for 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). Each group was packed in the polyethylene bag, stored at 7℃ for a day, and weighed before analysis. Then, microbiological and physical qualities were estimated. This experiment was replicated three times.

Later, changes in microbiological and physical qualities of cooked duck blood curd soaked in SD, NaCl, and PEG combination during cold storage were determined. Non-inoculated samples were prepared according to the method reported by Tangwatcharin et al. (2018). Samples were randomly divided into two treatments as soaking in slaughterhouse water consumption (control) and 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). The quality of slaughterhouse water consumption was checked monthly, and the lower limit of drinking water standard no. 257-2549 for the food industry of Thailand was considered (Thai Industrial Standard Institute, 2006). Samples were stored in the slaughter warehouse at 7℃ for 18 days, and collected on days 1, 2, 4, 6, 8, 10, 12, 14, 16, and 18. The collected samples were transported and analyzed within 3 hours.

**Microbiological analyses**

Twenty-five g of the sample was diluted in 225 ml of 0.85% NaCl (w/v, saline solution) and homogenized in a stomacher bag mixer (Interscience, France). The homogenate was serially diluted (1:10) with saline solution. For non-inoculated samples, each dilution was grown in plate count agar (PCA, Merck, Germany). The samples were then incubated at 35 ± 2℃ for 24-48 hours for mesophile count (BAM, 2001a) and at 7 ± 2℃ for 10 days for psychrophile count (ISO, 2001). Furthermore, yeast and mold counts were analyzed by plating in potato dextrose agar (PDA, Merck, Germany) and then incubated at 25 ± 2℃ for 5 days (BAM, 2001b).

For inoculated samples, the following media and incubated conditions were employed. For *Pseudomonas* spp. count, *Pseudomonas* CFC agar (Oxoid, United Kingdom) was used with incubation of samples at 25 ± 2℃ for 24-48 hours (Rajmohan et al., 2002). For *Samonella* spp. count, xylose lysine deoxycholate (Merck, Germany) was utilized with incubation of samples at 37 ± 2℃ for 24-48 hours (Van der Zee, 2003). *Escherichia coli* count was measured using violet red bile agar (Merck, Germany) and incubation of samples at 37 ± 2℃ for 24-48 hours (Tangwatcharin et al., 2018). *Staphylococcus aureus* count was gauged using Baird Parker agar (Merck, Germany) by adding 5% (v/v) egg-yolk tellurite emulsion 20% (Merck, Germany) and then incubation of samples at 37 ± 2℃ for 24-48 hours (BAM, 2001c). The LAB was counted by de Man, Rogosa, and Sharpe agar and anaerobic incubation of samples at 30℃ for 24-48 hours (Bover-Cid and Holzapfel, 1999). Finally, oxidase, aerobics, and ability to ferment glucose for *Pseudomonas* spp., triple sugar iron agar test, lysine iron agar test for *Salmonella* spp., Indole, Methyl Red, Voges Proskauer and Citrate (IMViC) tests for *E. coli*, and coagulase test for *S. aureus* were performed to confirm the findings. The results were transformed to log cfu/gram of sample (log cfu/g).

Mesophile, psychrophile, yeast and mold, LAB, *Pseudomonas* spp., *S. aureus* counts were analyzed using previous methods to indicate the changes in the microbiological quality of cooked duck blood curd soaked in SD, NaCl, and PEG combination during cold storage in slaughter warehouse. Additionally, *Bacillus* spp. counts were determined using the methods proposed by Turner et al. (1996). Later, the enrichment and the most probable number (MPN) method was used for *Salmonella* (ISO, 2017), *Listeria monocytogenes* (BAM, 2017), *E. coli* (BAM, 2020), and *Clostridium* spp. (Turchi et al., 2016; Tangwatcharin et al., 2019a).

**pH**

For pH measurement, the non-inoculated sample was directly determined at five different locations using a portable pH meter (Mettler Toledo SevenGo SG2, Mettler Toledo, Switzerland).

**Weight loss**

Cooked duck blood curd samples were first weighed before being soaked in sterilized water or antimicrobial and second weighed after cold storage. The sample weights were applied to compute the weight loss (%)**.**

**Color**

The color was measured on the cut surface of non-inoculated samples. The International Commission on Illumination (CIE) L* (lightness), CIE a* (redness), and CIE b* (yellowness) values were estimated by using a HunterLab MiniScan EZ 4000L (Hunter Associates Laboratory, USA). For each sample, the means of the readings were obtained at five locations. Hue angle (h°) was calculated according to the equation:

$$\text{Hue angle (h°)} = \text{arctg (CIE b*/ CIE a*)}$$

**Texture profile analysis**

Three pieces of each sample were cut into cubes (15×15×15 mm). Texture profile analysis (TPA) was estimated using an Instron universal testing (model 3344, USA) by a cylindrical aluminum probe, 55 mm inner diameter at room temperature (25℃). Texture profile analysis textural variables were estimated with crosshead

speed 1 mm/sec, holding time 1 second, working distance 40% strain (Bourne, 2002). The collection and processing of data were performed by the Blue-hill 2 software (Instron Engineering Corporation, USA). Among the textural parameters analyzed during a TPA test, only hardness, cohesiveness, gumminess, springiness, and chewiness were estimated for the force-time curves generated for each sample.

**Statistical analyses**

Each experiment was replicated three times. The SPSS (version 28) was used for the statistical analyses. The data were analyzed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine the mean comparison ($p < 0.05$). Pearson's correlation coefficients were carried out to determine the relationship among variables.

## RESULTS AND DISCUSSION

**Qualities of cooked duck blood curd**

Soaking the samples in antimicrobials significantly affected mesophiles and psychrophiles in non-inoculated cooked duck blood curd ($p < 0.05$, Table 1). The samples soaked in 0.15% SD + 1.25% NaCl + 0.15% PEG were the lowest these microbial counts ($p < 0.05$). Moreover, the soaking samples in 0.15% SD + 1.25% NaCl + 0.15% PEG combination exposed higher reduction of mesophile and psychrophile counts than those of 0.30% SD + 1.25% NaCl, 0.15% SD + 1.25% NaCl, and 0.15% SD + 0.15% PEG ($p < 0.05$). The reduction of spoilage microorganism count were included 1.82 ± 0.07, 1.63 ± 0.11, 1.59 ± 0.09, and 1.47 ± 0.13 log cfu/g, respectively for mesophile and 1.40 ± 0.12, 1.17 ± 0.09, 1.08 ± 0.08, and 0.96 ± 0.13 log cfu/g (means ± standard deviation), respectively, for psychrophile when compared with control ($p < 0.05$). Moreover, yeast and mold were not detected in all samples (<1 log cfu/g).

Similarly, soaking the samples in antimicrobials reduced LAB, *Pseudomonas* spp. *Salmonella* spp., *E. coli*, and *S. aureus* counts in inoculated cooked duck blood curd ($p < 0.05$, Table 1). The samples soaked in 0.15% SD + 1.25% NaCl + 0.15% PEG combinations showed the lowest counts of the investigated bacteria ($p < 0.05$). In a previous study, the FBCI of 0.15% SD + 1.25% NaCl was 0.25 against *E. coli* and 0.62 against *S. Typhimurium* and *P. fluorescens* (Tangwatcharin and Teemeesuk, 2019). Sodium diacetate is a weak organic acid salt that effectively inhibits most tested bacteria and connects with the outer surface of bacterial cells, resulting in the disorder of cell membrane integrity and leakage of the intracellular lysate and dissolution of the cytoplasmic membrane (Tangwatcharin et al., 2018). The physical stress in the cellular structure of *E. coli* and *Salmonella* causes cell damage, or shrinkage due to a low salinity, which leads to an immediate influx of small solutes (Hajmeer et al., 2006; Krämer, 2010; Omotoyinbo and Omotoyinbo, 2016). Adding 0.16% SD + 0.16% PEG was a potential antimicrobial for reducing *E. coli* and *S. aureus* in fresh ground pork (Tangwatcharin et al., 2018). This could be due to the synergistic antibacterial efficacy of PEG. Lipopolysaccharide (LPS) in bacterial cells, as well as the cell phospholipid, is a nonpolar surface, especially in Gram-negative bacteria (Rosen, 2004). These nonpolars allow the non-ionic PEG surfactant linked by SD + NaCl combination to associate with the outer surface of the target bacterial cells, leading to disorders in cell membrane integrity and finally the intracellular lysate leakage and cytoplasmic membrane dissolution (Tangwatcharin et al., 2018).

Table 2 shows the color measured by the instrument. There was no significant difference between samples soaked in water (control) and 0.15% SD + 1.25% NaCl ($p > 0.05$). However, a high concentration of SD (0.30% SD) had a significantly negative impact, especially on the CIE a* and hue ($p < 0.05$). The color of blood is due to the demeanor of hemoglobin molecules in the red blood cells (Leoci, 2014). After cooking, this globin is denatured and reduced heme. Hemochromogen and hemichromogen are formed from oxyhemoglobin and methemoglobin, which are dull red and brown pigments, respectively. However, reductions of hemichromogen appear after using reducing agents, such as SD. The porphyrin ring may be opened, forming a green verdohem. In the intense condition, the iron will be lost from the porphyrin, split from the protein moiety, and open out, forming the chair of pyrroles characterizing colorless bile pigments (Toldrá et al., 2016). In accordance with the present study, samples soaked in 0.30% SD combination with NaCl exhibited higher CIE a* and lower hue than those in other antimicrobials and control ($p < 0.05$). There were significant negative correlations between CIE a* and hue ($r = -0.861$, $p < 0.05$). Due to hemichromogen reduction, abiding red hemochromogen in cooked blood curd was more apparent. Nevertheless, the pH values, weight loss, CIE b*, and texture analysis profiles were not affected by soaking in antimicrobials ($p > 0.05$).

**Table 1.** Effect of sodium diacetate, sodium chloride, and polyethylene glycol in combinations on bacteria on non-inoculated and inoculated cooked duck blood curds

| Bacteria | T1 | T2 | T3 | T4 | T5 | p-values |
|---|---|---|---|---|---|---|
| **Non-inoculated cooked duck blood curd (log cfu/g)** | | | | | | |
| Mesophile | 3.62 ± 0.09$^{a}$ | 2.03 ± 0.13$^{b}$ | 1.99 ± 0.14$^{bc}$ | 2.15 ± 0.10$^{b}$ | 1.80 ± 0.05$^{c}$ | < 0.05 |
| Psychrophile | 2.74 ± 0.12$^{a}$ | 1.66 ± 0.10$^{b}$ | 1.57 ± 0.08$^{b}$ | 1.78 ± 0.16$^{b}$ | 1.34 ± 0.07$^{c}$ | < 0.05 |
| Yeast and Mold | <1 | <1 | <1 | <1 | <1 | |
| **Inoculated cooked duck blood curd (log cfu/g)** | | | | | | |
| *Pseudomonas* spp. | 3.91 ± 0.07$^{a}$ | 2.89 ± 0.10$^{bc}$ | 2.84 ± 0.16$^{c}$ | 3.11 ± 0.12$^{b}$ | 2.54 ± 0.15$^{d}$ | < 0.05 |
| *Salmonella* spp. | 4.15 ± 0.04$^{a}$ | 2.64 ± 0.18$^{c}$ | 2.58 ± 0.09$^{c}$ | 3.15 ± 0.13$^{b}$ | 2.32 ± 0.06$^{d}$ | < 0.05 |
| *Escherichia coli* | 4.16 ± 0.05$^{a}$ | 2.54 ± 0.14$^{c}$ | 2.47 ± 0.10$^{c}$ | 3.04 ± 0.08$^{b}$ | 2.27 ± 0.12$^{d}$ | < 0.05 |
| *Staphylococcus aureus* | 4.09 ± 0.13$^{a}$ | 2.85 ± 0.08$^{b}$ | 2.80 ± 0.10$^{b}$ | 3.09 ± 0.14$^{b}$ | 2.51 ± 0.03$^{c}$ | < 0.05 |
| Lactic acid bacteria | 4.02 ± 0.11$^{a}$ | 2.99 ± 0.05$^{c}$ | 2.91 ± 0.07$^{c}$ | 3.17 ± 0.06$^{b}$ | 2.71 ± 0.21$^{c}$ | < 0.05 |

T1: Control, T2: 0.15% SD (w/v) + 1.25% NaCl (w/v), T3: 0.30% SD (w/v) + 1.25% NaCl (w/v), T4: 0.15% SD (w/v) + 0.15% PEG (w/v), T5: 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). Values are given as means ± standard deviation from triplicate determinations. $^{a,b,c,d}$ Different superscript letters within the same row indicate significant differences ($p < 0.05$).

**Table 2.** Effect of sodium diacetate, sodium chloride, and polyethylene glycol combinations on the physical quality of non-inoculated cooked duck blood curd.

| Parameters | T1 | T2 | T3 | T4 | T5 | p-values |
|---|---|---|---|---|---|---|
| pH | 7.54 ± 0.19 | 7.55 ± 0.15 | 7.34 ± 0.41 | 7.52 ± 0.24 | 7.57 ± 0.21 | 0.7832 |
| Weight loss (%) | 2.17 ±0.24 | 2.35 ± 0.31 | 2.51 ± 0.22 | 2.31 ± 0.29 | 2.29 ± 0.18 | 0.0825 |
| CIE L* | 32.98 ± 0.68 | 32.76 ± 0.39 | 32.21 ± 0.15 | 32.67 ± 0.41 | 32.70 ± 0.25 | 0.4492 |
| CIE a* | 11.12 ± 0.33$^{b}$ | 11.57 ± 0.37$^{b}$ | 12.55 ± 0.23$^{a}$ | 11.52 ± 0.24$^{b}$ | 11.50 ± 0.37$^{b}$ | 0.0001 |
| CIE b* | 20.65 ± 0.68 | 20.52 ± 0.61 | 21.24 ± 0.52 | 20.60 ± 0.22 | 20.55 ± 0.43 | 0.3061 |
| Hue | 61.70 ± 0.43$^{a}$ | 60.58 ± 0.65$^{b}$ | 59.42 ± 0.47$^{c}$ | 60.64 ± 0.31$^{b}$ | 60.87 ± 0.51$^{ab}$ | 0.0030 |
| Hardness (N) | 2.28 ± 0.29 | 2.60 ± 0.19 | 2.80 ± 0.22 | 2.52 ± 0.48 | 2.47 ± 0.31 | 0.7345 |
| Cohesiveness (ratio) | 0.60 ± 0.02 | 0.61 ± 0.03 | 0.58 ± 0.02 | 0.61 ± 0.03 | 0.60 ± 0.02 | 0.2826 |
| Gumminess (N) | 1.43 ± 0.16 | 1.56 ± 0.17 | 1.63 ± 0.12 | 1.58 ± 0.28 | 1.50 ± 0.15 | 0.4958 |
| Springiness (ratio) | 0.89 ± 0.01 | 0.86 ± 0.01 | 0.86 ± 0.01 | 0.86 ± 0.02 | 0.86 ± 0.01 | 0.3660 |
| Chewiness (N) | 1.29 ± 0.14 | 1.41 ± 0.15 | 1.54 ± 0.20 | 1.46 ± 0.21 | 1.32 ± 0.18 | 0.7212 |

T1: Control, T2: 0.15% SD (w/v) + 1.25% NaCl (w/v), T3: 0.30% SD (w/v) + 1.25% NaCl (w/v), T4: 0.15% SD (w/v) + 0.15% PEG (w/v), T5: 0.15% SD (w/v) + 1.25% NaCl (w/v) + 0.15% PEG (w/v). CIE L*: lightness, CIE a*: redness, CIE b*: yellowness, N: Newton unit. Values are given as means ± standard deviation from triplicate determinations. $^{a,b,c}$ Different letters within the same row indicate significant differences ($p < 0.05$).

**Shelf life of cooked duck blood curd**

For storage in the warehouse at 7°C, soaking the samples in 0.15% SD + 1.25% NaCl + 0.15% PEG could control the growth of mesophile and psychrophile in samples stored for 10 and 6 days, respectively, compared to the control before storage ($p < 0.05$). Additionally, it could extend the shelf-life of the sample to 16 days (Figure 1A). These microbial are a considerable indicator of cooked food quality and shelf-life in cold storage (Ercolini et al., 2009). Mesophile count was the lower limit of Thailand's food and container microbiology standard (Department of Medical Sciences, 2017), which was not higher than 6 log cfu/g for cooked food and stored at cold temperature. Soaking the samples in SD, NaCl, and PEG combination could control the growth of *Pseudomonas* spp. and LAB in samples stored for 8 and 10 days, respectively (Figure 1B). Normally, their contaminations in blood products were found during the bleeding process. Due to these bacteria growth at low temperatures, they restricted the shelf-life of blood products (Dàvila et al., 2006). In the current study, all samples found <1 log cfu/g for yeast and mold, *S. aureus* and *Bacillus* spp., and < 3 MPN/g for coliforms, *E. coli*, *L.*

*monocytogenus*, and *Salmonella* spp. and *Clostridium* spp. throughout 18-day storage.

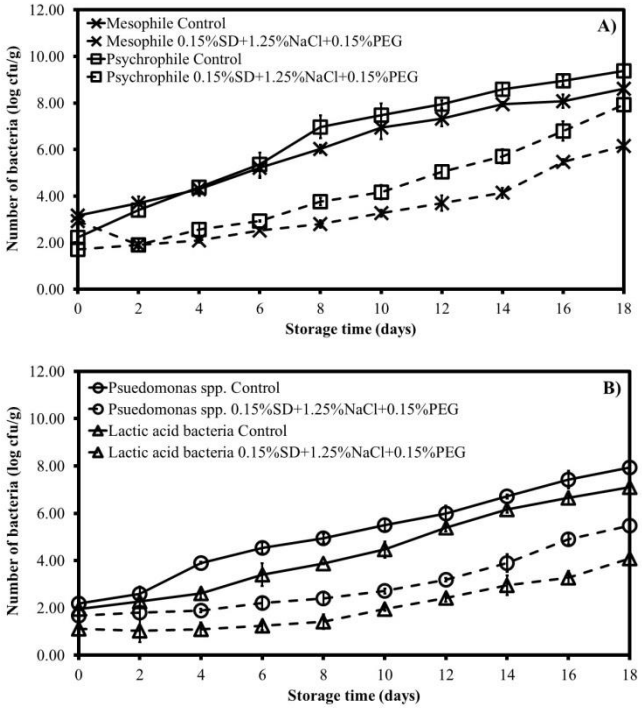


**Figure 1.** Effect of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol (PEG) combination on number of mesophile and psychrophile **(A)** and lactic acid bacteria and *Pseudomonas* spp. **(B)** of cooked duck blood curd stored at 7°C for 18 days in a slaughter warehouse.

The changes in physical quality of all samples stored in a warehouse at 7°C are demonstrated in Figure 2. Soaking the samples in an antimicrobial combination restrained the decrease of sample pH during storage for 14 days, then the pH decreased gradually throughout storage time. In contrast, the pH of control samples continuously reduced with an increase in storage time ($p < 0.05$, Figure 2A). The reason should ascribe to SD, NaCl, and PEG combination against LAB growth. Lactic acid bacteria generate lactic acid as a main metabolic end-product of carbohydrate fermentations, and then this increase in lactic acid is accompanied by a decrease in pH (Tangwatcharin et al., 2019b). In the present study, LAB count had a negative correlation with pH value ($r = -0.847$, $p < 0.05$).

On the contrary, the samples soaked in an antimicrobial combination delayed the increase of weight loss during storage for 10 days. After that, the weight loss increased with increasing storage time although the weight loss of control samples increased throughout storage time ($p < 0.05$, Figure 2B). Álvarez et al. (2009) reported that hemoglobin solubility varied in the 75-95% range, with the lowest value corresponding to pH 5.0 due to the protein precipitation. The solubility of duck blood powder decreased with decreasing pH values between 5 and 8 (Sorapukdee and Narunatsopanon, 2017). For NaCl soaking, the refolding of the protein was hampered by the presence of ions. An increase in levels of $Cl^-$ ions causes a lower pH value, and proteins form progressively more stable, hydrophobic, and molten globular forms (Kristinsson and Hultin, 2004). The solubility in water influences other functional properties, especially the formation and stability of gels, resulting in changes in weight loss and texture characteristics during storage time (Álvarez et al., 2009; Sorapukdee and Narunatsopanon, 2017). In the present study, there was a significant negative correlation between pH and weight loss ($r = -0.798$, $p < 0.05$).

Regarding the color, the CIE a* and hue of all samples increased and decreased, respectively, with increasing storage time ($p < 0.05$, Figures 2C and 2D). However, soaking the samples in an antimicrobial combination decelerated the change of the hue of samples during the 8-day storage. Due to the microbial growth, they reduced hemichromogen and formed green choleglobin (Toldrá et al., 2016). Thus, cooked blood curd was a lighter shade of red with available red hemochromogen. In this study, mesophile and LAB counts were negatively correlated with hue value ($r = -0.839$ and $-0.831$, respectively, $p < 0.05$).

The changes in textural characteristics showed that the samples soaked in an antimicrobial combination delayed the increase of hardness and chewiness during storage for 10 and 12 days, respectively. The hardness increased faster throughout storage time. For the control sample, the hardness and chewiness of samples increased with increasing storage time ($p < 0.05$, Figures 3A and 3B). Due to the efficiency of SD, NaCl, and PEG combination against microbial, pH value and weight loss of the sample were reduced, and then they affected the decrease of hardness and chewiness. Mesophile, psychrophile, LAB counts and weight loss had a negative correlation with hardness ($r = -0.735$, $-0.816$, $-0.817$ and $-0.861$, respectively, $p < 0.05$) and with chewiness ($r = -0.722$, $-07.95$, $-0.805$ and $-0.726$, respectively, $p < 0.05$). Additionally, pH value had positive correlations with hardness and chewiness ($r = 0.874$ and $0.860$, respectively, $p < 0.05$). Considering other textural characteristic, there were no significant differences in

cohesiveness, gumminess, and springiness of all samples ($p > 0.05$).

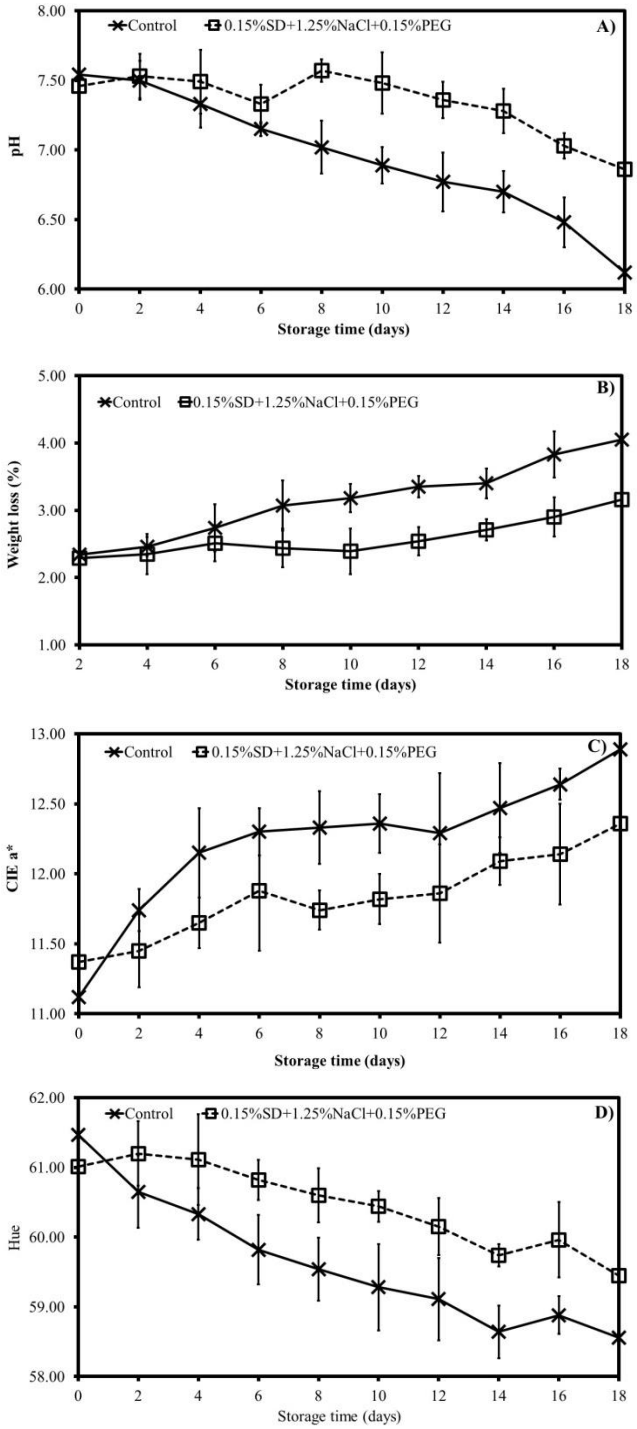


**Figure 2**. Effect of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol (PEG) combination on pH value **(A)**, weight loss **(B)**, CIE a*: redness **(C)**, and hue of cooked duck blood curd **(D)** stored at 7°C for 18 days in slaughter warehouse. a*: redness

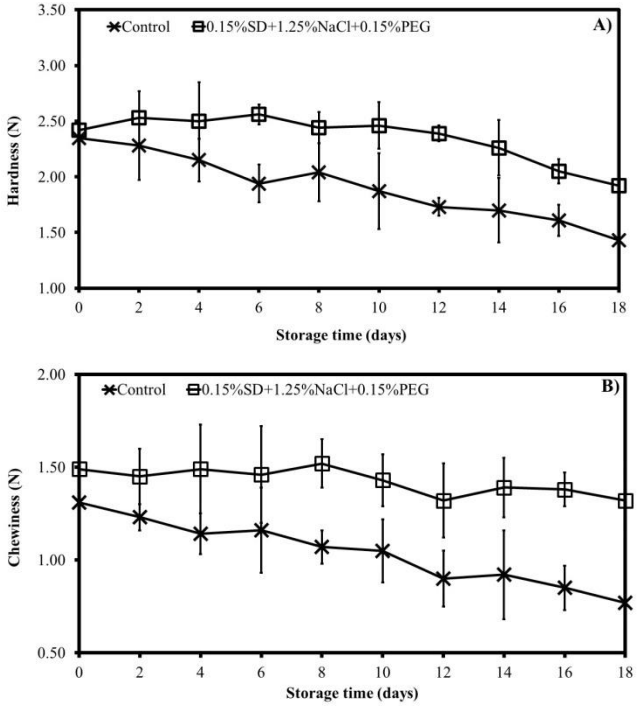


**Figure 3.** Effect of sodium diacetate (SD), sodium chloride (NaCl), and polyethylene glycol (PEG) combination on hardness **(A)** and chewiness **(B)** of cooked duck blood curd stored at 7°C for 18 days in slaughter warehouse.

## CONCLUSION

This study indicated that the combined treatment of SD, NaCl, and PEG could significantly decrease spoilage and restrict the growth of pathogenic microorganisms in cooked duck blood curd. This antimicrobial action could decelerate the changes in physical quality and extend the shelf life of cooked duck blood curd. The current results showed the potential use of SD, NaCl, and PEG combination as an appropriate preservative in cooked duck blood curd, and its application in slaughter warehouses and distribution. For future research, the direct addition of SD, NaCl, and PEG combination in raw blood before the cooking process can be studied to reduce the concentration of combined antimicrobials.

## DECLARATIONS

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**Authors' contribution**

Pussadee Tangwatcharin and Supaluk Sorapukdee created the idea and designed the study. Warraluk Teemeesuk collected data. Pussadee Tangwatcharin performed the statistical analysis and drafted and approved the final manuscript. All authors checked and confirmed the final analysis data and the final version of the manuscript before publication in the journal.

**Competing interests**

The authors have declared that there are no competing interests.

**Data availability statement**

The data presented in this study are available on request from the corresponding author.

**Consent to publish**

All authors informed their consent before inclusion in the study.

**Ethical consideration**

All the authors checked for ethical issues such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy.

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