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Research Article

Effect of organic acid and salt mixture on shelf-life extension and growth inhibition of *Staphylococcus aureus* and *Escherichia coli* O157:H7 in *moo yor*

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Abstract

This research investigated the effects of organic acids and salts solution on shelf-life extension and growth inhibition of *Staphylococcus aureus* and *Escherichia coli* O157:H7 in *moo yor* (pork bologna). A mixture of lactic acid, acetic acid, citric acid and their sodium salts was added to *moo yor* at various concentrations of 0 (control), 0.5, 0.75 and 1% (w/v) then stored at 4°C for 31 days. Total microbial counts and sample qualities were determined during storage. Total microbial counts significantly decreased with increasing mixture concentration and storage time ($p \leq 0.05$). The 0.75 % solution was selected for further study due to its inhibitory effects against bacteria and some economic aspects. Throughout storage, treated samples had less % yield loss with slightly pale colour and less firmness than the untreated samples, whereas pH values were not different. For all attributes, no significant difference in sample acceptances between treatments was found and overall difference was not observed ($p > 0.05$). When compared to untreated and commercial-preservative-added controls, the 0.75 % solution showed promising inhibitory effects toward *S. aureus* and significantly suppressed both *S. aureus* and *E. coli* O157:H7 growths throughout the experiment ($p \leq 0.05$). In summary, the mixture of organic acids and their salts shows great potential in extending shelf-life and inhibiting growth of *S. aureus* and *E. coli* O157:H7 in refrigerated *moo yor* while maintaining acceptable product qualities.

Keywords: food, spoilage inhibition, organic acids, organic salts, *moo yor*, pork bologna, *S. aureus*, *E. coli* O157:H7, Thailand

Introduction

Moo yor (pork bologna) is a ready-to-eat (RTE) processed meat product. Owing to its convenience for consumption, *moo yor* is a popular meat product in Thailand. Equivalent products are also popular in Vietnam and Laos. Similar to other RTE meat products, *moo yor* is a perishable food susceptible to growth of spoilage and pathogenic microorganisms. As a result, the meat industry has to prolong the product shelf-life and maintain consumer safety. In addition, according to Thai community product standards, commercial *moo yor* must be free from pathogens such as *Staphylococcus aureus*, *Salmonella, sp.*, *Clostridium perfringens* and *Escherichia coli* O157:H7, therefore the control of these pathogens in this product is crucial [1, 2]. Meat manufacturers usually use food preservatives along with good manufacturing practice (GMP) and proper storage conditions to prolong the product's shelf-life [3, 4]. Thus, commercial *moo yor* is generally sold under refrigerated storage temperature in addition to the use of food preservatives such as sodium benzoate to prolong the shelf-life [1, 2].

However, the use of food preservatives has a drawback on consumer perception, so several other methods are explored to preserve processed meat. Organic acids and their salts have benefits in shelf-life extension of refrigerated meat, poultry and fish products by inhibiting spoilage microbial growths and pathogens [3, 4, 5, 6]. There are reports of decontamination of carcasses by lactic acid and acetic acid [7, 8], susceptibility of *E. coli* O157:H7 to lactate [9], and reduction of spoilage microorganisms in ground beef patties by organic-acid salts [5]. Despite these results, using a mixture of organic acid and organic salts is reported to be more efficient in extending product shelf-life and inhibiting pathogen growths [3, 4]. A mixture of organic acid salts was successfully used to reduce spoilage flora in cured-cooked meat products [2] whereas "Tomax®", a commercial mixture of lactic acid, acetic acid, citric acid and their sodium salts, showed promising results in prolonging shelf-life of refrigerated pork and poultry meat in the pilot experiments [10, 11]. However, the study of using the mixture of organic acid and organic salts in meat product formulation is still limited [2]. The efficacy of Tomax® on inhibiting growth of *Escherichia coli* and *Salmonella typhimurium* were only tested in medium and on the surface of refrigerated meat [10, 11]. No one has yet added the mixture of organic acid and their salts in *moo yor* to determine its microbial growth inhibition potential for *S. aureus* and *E. coli* O157:H7, which is stated in *moo yor* product standards [1]. In addition, the impact of using an organic acid and salts mixture on the organoleptic quality of the product is unclear. Thus, this research investigated the effects of organic acids and salts solution on shelf-life extension, growth inhibition of *S. aureus* and *E. coli* O157:H7, as well as subsequent product acceptance of treated *moo yor*.

Material and Methods

Moo yor preparation

Ground pork was blended throughout with garlic and pork lard. Then ice, along with a mixture of organic acids and their sodium salts, salt, sugar and pepper were added during blending to form the emulsion of *moo yor*. A commercial mixture of organic acids and their salts consisting of lactic acid, acetic acid, citric acid, sodium lactate, sodium acetate and sodium citrate (Tomax®, Sensate Pty Limited, Australia) were added with ice at various concentrations of 0 (control), 0.5, 0.75 and 1% (w/v). After mixing, the *moo yor* samples were packed in a stainless steel mold and steamed (using boiling water) for 30 min then cooled to room temperature in an ice bath. Subsequently, samples were stored at 4°C for further study.

Determination of a mixture solution of organic acid and organic salts

Treated *moo yor* samples with the addition of a mixture solution in various concentrations were stored at 4°C for 31 days for the storage study. Total microbial counts of each treatment were determined using plate count agar (PCA) during storage. In addition, sample qualities including pH using a pH meter (Horiba model F-21, USA), colour in CIE system (L^* , a^* , b^* and hue angle ($\tan^{-1} a^*/b^*$) using a colourimeter (Hunterlab, USA), texture using the universal testing machine model 441 (Instron, Ltd., England) and weight using a balance (Adam model AFP 2001, Australia) were determined. The percentage of weight loss was calculated by the weight difference between the initial weight and storage weight divided by the initial weight. Data in triplicate from duplicate sets of samples was analyzed to select the optimum concentration of an organic acids and salts mixture added to the product formulation.

The sensory evaluation of *moo yor* samples with the addition of a mixture solution at an optimum concentration was compared with untreated (control). All samples were freshly prepared and kept at 4°C for 1-2 days prior to sensory test. There were 45 panelists assessing samples using a 9-point hedonic scale and triangle test. All statistical analysis was performed at 95% confidential level.

The optimum concentration of a mixture solution was used to further investigate their effects on inhibiting growths of *Staphylococcus aureus* and *Escherichia coli* O157:H7. Samples treated with mixture solution were compared to untreated and preservative-added (0.1% sodium benzoate) samples as negative and positive controls, respectively.

Preparation of bacteria strains and microbiological analysis

Microorganisms used in this study were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* O157:H7 12743 (Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand).

S. aureus and *E. coli* O157:H7 cultures were prepared in tryptic soy broth (TSB) (Merck, Germany) at 37°C for 24 hr prior to use in the experiment. The active culture was diluted in 0.1% sterile peptone water to ensure the initial counts of 10^6 cfu/ml on all tested samples. Each 100-g *moo yor* sample was treated under UV light at 253 nm in the biological safety cabinet (Clean Air model CA/REV 4, Clean Air Techiek B.V., Woerden, Netherland), dipped in the culture solution for 5 minutes and drained on the sterile screen for 10 minutes before being stored at 4°C. Viable populations were determined during storage at 0 and 30 minutes, 1, 2, 4, 7, 13, 19, 25 and 31 days. Treated samples were randomly cut for 25 g and serially diluted before plating on selective media. Selective media used for *S. aureus* and *E. coli* O157:H7 were Mannitol Salt (Phenol Red) Agar (Merck, Germany) and Sorbitol MacConkey (SMAC) agar (Merck, Germany), respectively [12, 13]. The plates were incubated at 37°C for 48 hr and the microbial results were reported as log cfu/g and log reduction ($\log(N_i/N_0)$). The log reduction was calculated by comparing the current microbial count at a given storage day ($\log N_i$) to the initial microbial count ($\log N_0$) of each treatment. Thus, a negative value of log reduction as a result of the microbial count at a given storage day being lower than the initial count indicates the microbial growth inhibition in the treatments. Data was reported from duplicate sample sets.

Results and Discussion

Effect of organic acids and organic salts mixture on qualities of yor (pork Bologna).

The *moo yor* samples were subjected to different concentrations of the organic acid and organic salts mixture before being stored under 4°C. An absence of organic acids and salts mixture allowed a drastic increase of total microbial count in the control sample to be 2.12 log₁₀ CFU/g in 2 days, whereas those of treated samples gradually increased during storage (Fig. 1). Throughout storage time, treated samples had microbial counts significantly lower than control by 0.5-2 log₁₀ CFU/g ($p \leq 0.05$). This confirmed the results previously reported that a mixture of organic acids and salts inhibited microbial growth [2, 3, 10, 11], and a reduction of 1.5 log cycles in carcass surface decontaminant by organic acids can be expected [8]. According to the standard [1], the total microbial count in *moo yor* must not exceed 3 log₁₀ CFU/g, thus addition of an organic acid and salt mixture extended the shelf-life of *moo yor* samples for at least 31 days, whereas shelf-life of the control was only 26 days. Amongst treatments, the mixture solution with active ingredients of 0.75 % and 1% were more efficient in decreasing microbial load than 0.5% and control. This was because the microbial inhibition increased with increasing concentration of the organic acids and salts mixture [2]. Thus 0.75% solution was selected for further study due to the inhibitory effects against bacteria and economic aspects.

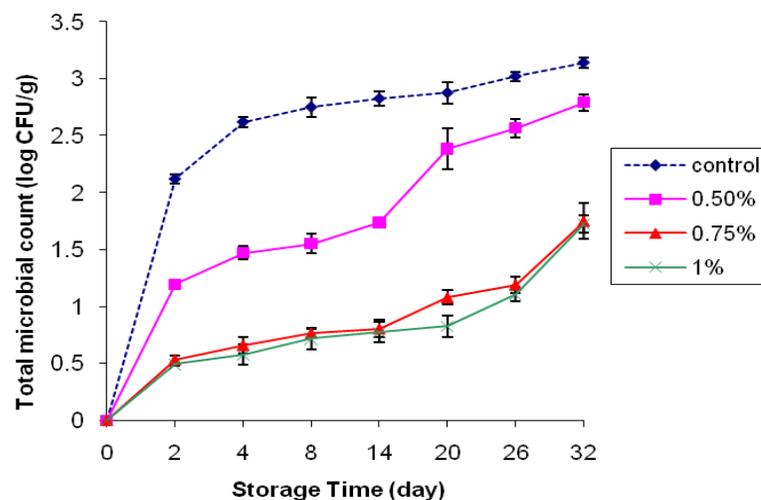


Figure 1. Effects of the mixture of organic acids and their salts on total microbial counts in treated samples and control during storage ($p \leq 0.05$).

Physical and chemical properties of *moo yor* samples were also determined during storage. Throughout storage, treated samples showed less % weight loss than control (Fig. 2), indicating that their total yields were higher. The pH values of all samples were not different throughout storage ($p > 0.05$) (Fig. 3). A similar trend was found when using sodium salts of acetic acid, lactic acid and citric acid in frankfurters [14], but opposite results were reported when using organic acids and salts in pork bologna [15]. The mixture of organic acid and salt affected *moo yor* colour since treated samples were slightly lighter than control as indicated by lightness value (L^*) (Figs. 4-5). An increase in a mixture concentration significantly decreased redness (a^*), data not shown, which resulted in an overall paler colour of treated

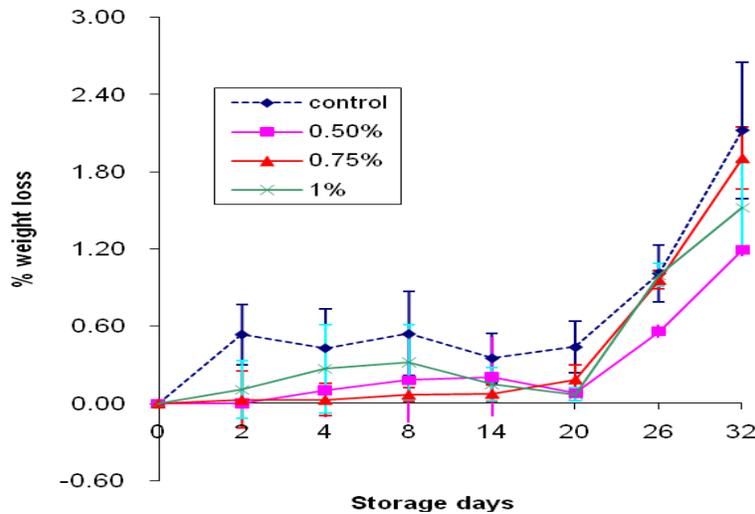


Figure 2. Effects of the mixture of organic acids and their salts on weight loss (%) in treated samples and control during storage ($p \leq 0.05$).

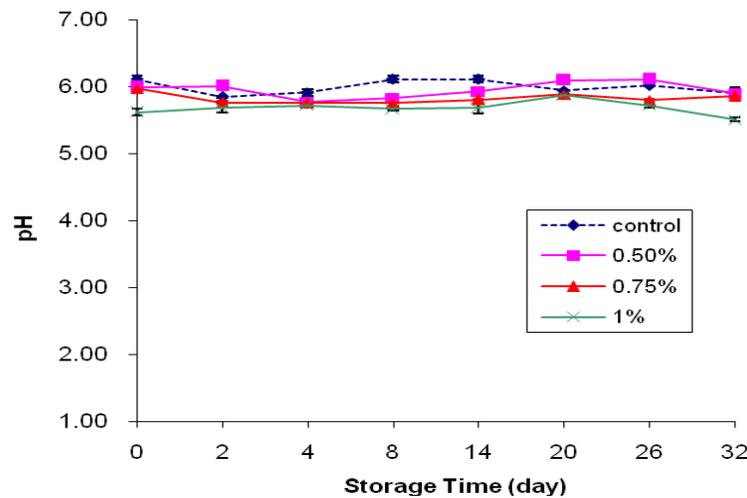


Figure 3. Effects of the mixture of organic acids and their salts on pH of treated samples and control during storage ($p \leq 0.05$).

samples shown by hue angle value in Fig. 5 ($p \leq 0.05$). The texture of samples measured by an instrument showed that the peak force was affected by storage time and treated samples, overall, were slightly less firm than control (Fig. 6). This was due to the fact that increases in concentration of organic acids and salts and storage time may cause paler and less-firm samples [7, 8, 14, 16]. Therefore, using the appropriate concentration of organic acids and salts mixture is crucial to prevent a decrease in pH value and adverse effects on product qualities [14, 15, 17, 18].

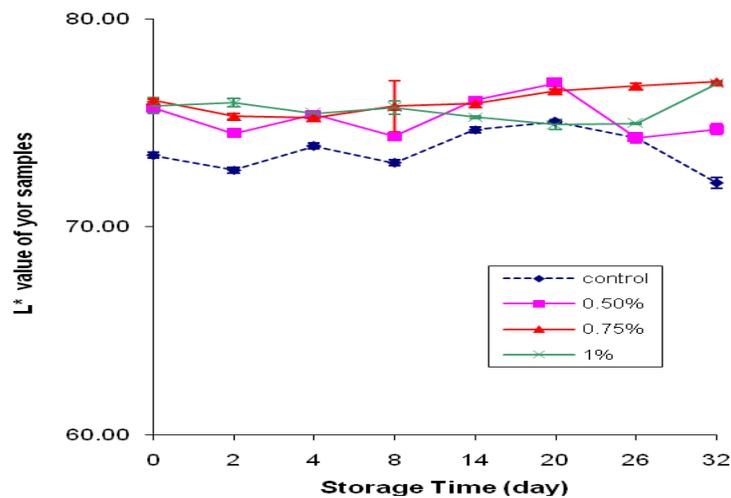


Figure 4. Effects of the mixture of organic acids and their salts on brightness (L^*) of treated samples and control during storage ($p \leq 0.05$).

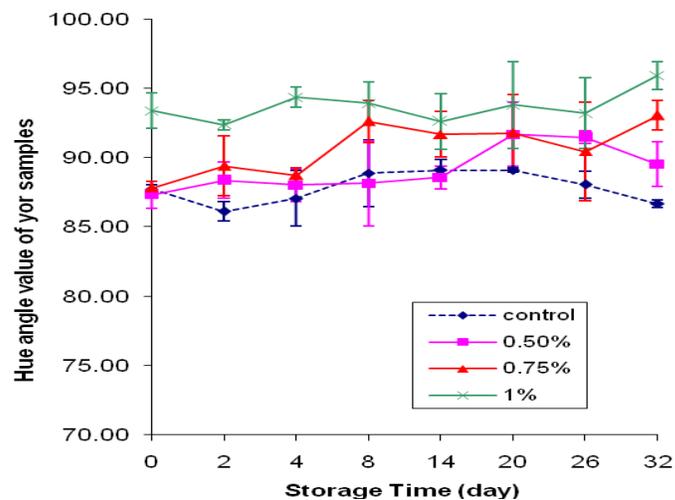


Figure 5. Effects of the mixture of organic acids and their salts on colour of treated samples and control (indicated by hue angle value) during storage ($p \leq 0.05$).

Despite the above result, the colour and texture changes were not recognized by panelists because the liking scores in colour, texture, odor, taste and overall acceptance of treated samples were not significantly different from control (Fig. 7) ($p > 0.05$). The result agreed with research previously reported [4, 11, 19].

Effect of organic acids and organic salts mixture on pathogens inoculated in moo yor

The organic acids and their sodium salts mixture with an active ingredient of 0.75% was selected for further experiment. *Staphylococcus aureus* and *Escherichia coli* O157:H7 were selected in this study according to the moo yor standard [1]. Generally, commercial the moo yor product uses sodium benzoate as a preservative, thus the result of samples treated by

organic acids and salts mixture were compared with untreated (control -) and preservative-added (control+) samples. The results were reported as log cfu/g and log reduction ($\log(N_i/N_0)$). The log reduction compared the current microbial count at a given storage day ($\log N_i$) to the initial microbial count ($\log N_0$) of each treatment. Thus, a negative value of log reduction indicates the microbial growth inhibition in the particular treatments.

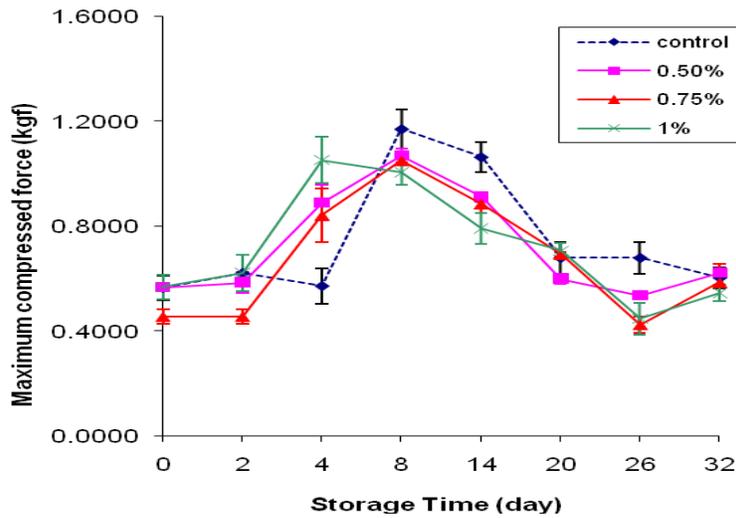


Figure 6. Effects of the mixture of organic acids and their salts on *moo yor* texture of treated samples and control during storage ($p \leq 0.05$).

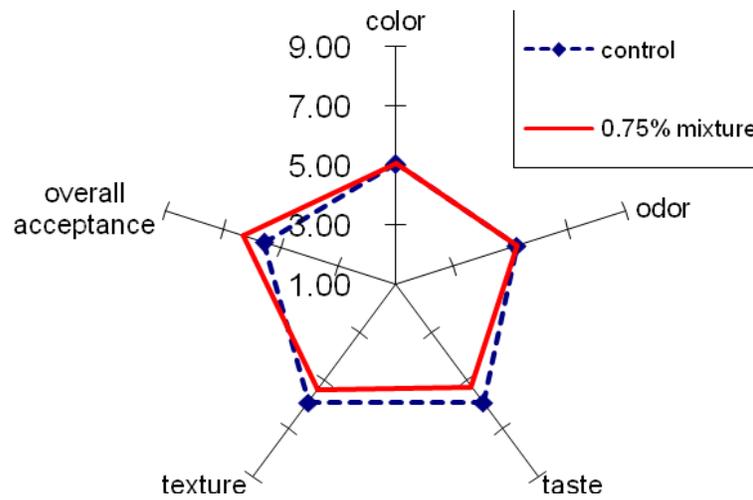


Figure 7. Effects of the mixture of organic acids and salts solution on product acceptance.

Figure 8 shows the susceptibility results of *S. aureus* to organic acids and salts mixture as compared to controls with and without preservative (sodium benzoate). Throughout storage, the highest count of *S. aureus* growth was observed in untreated control followed by preservative-treated control and 0.75%-mixture-treated samples (Fig. 8a). Addition of a 0.75% mixture solution showed about 1 log reduction of *S. aureus* in samples stored for 2 days (Fig. 8b) and suppressed the microbial growth at lower level than preservative-treated and untreated controls throughout 31 storage days (Figs. 8a-8b). The results agreed with other work [8, 12, 20] that this antimicrobial effect may be caused by a pH reduction below the growth range and metabolic inhibition by the un-dissociated molecules [20].

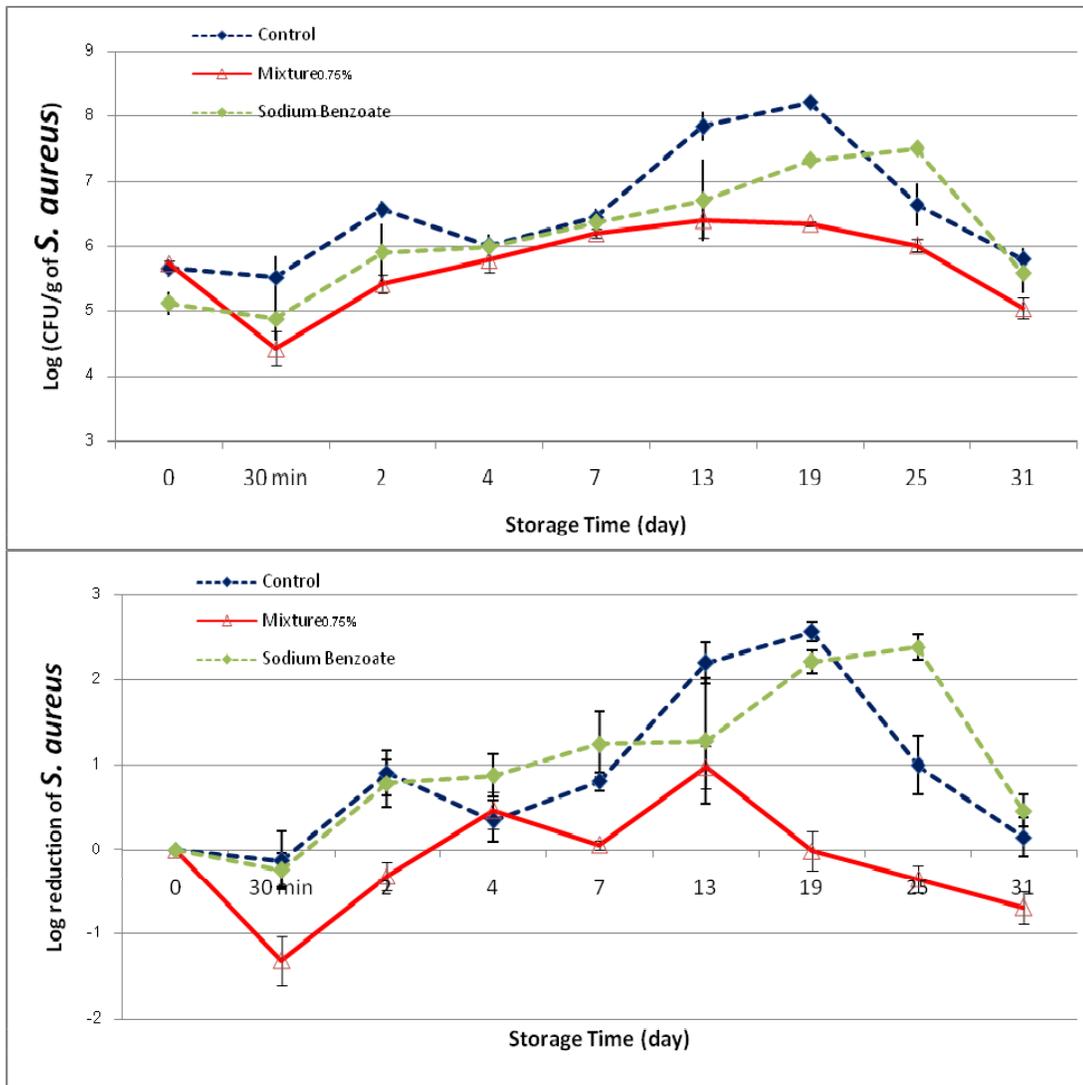


Figure 8. Effects of the mixture of organic acids and their salts on growth of *Staphylococcus aureus* in treated samples and controls as indicated by total viable count (a) and log reduction (b) during storage ($p \leq 0.05$).

Similarly, the addition of an organic acid and salt mixture was significantly effective at maintaining the microbial load of *E. coli* O157:H7 at lower levels than both negative and positive controls for 31 days (Fig. 9a). Samples treated with organic acids and salts mixture of 0.75% showed about 1 log reduction of *E. coli* O157:H7, where none was observed in both controls (Fig. 9b). This was previously reported [8, 9].

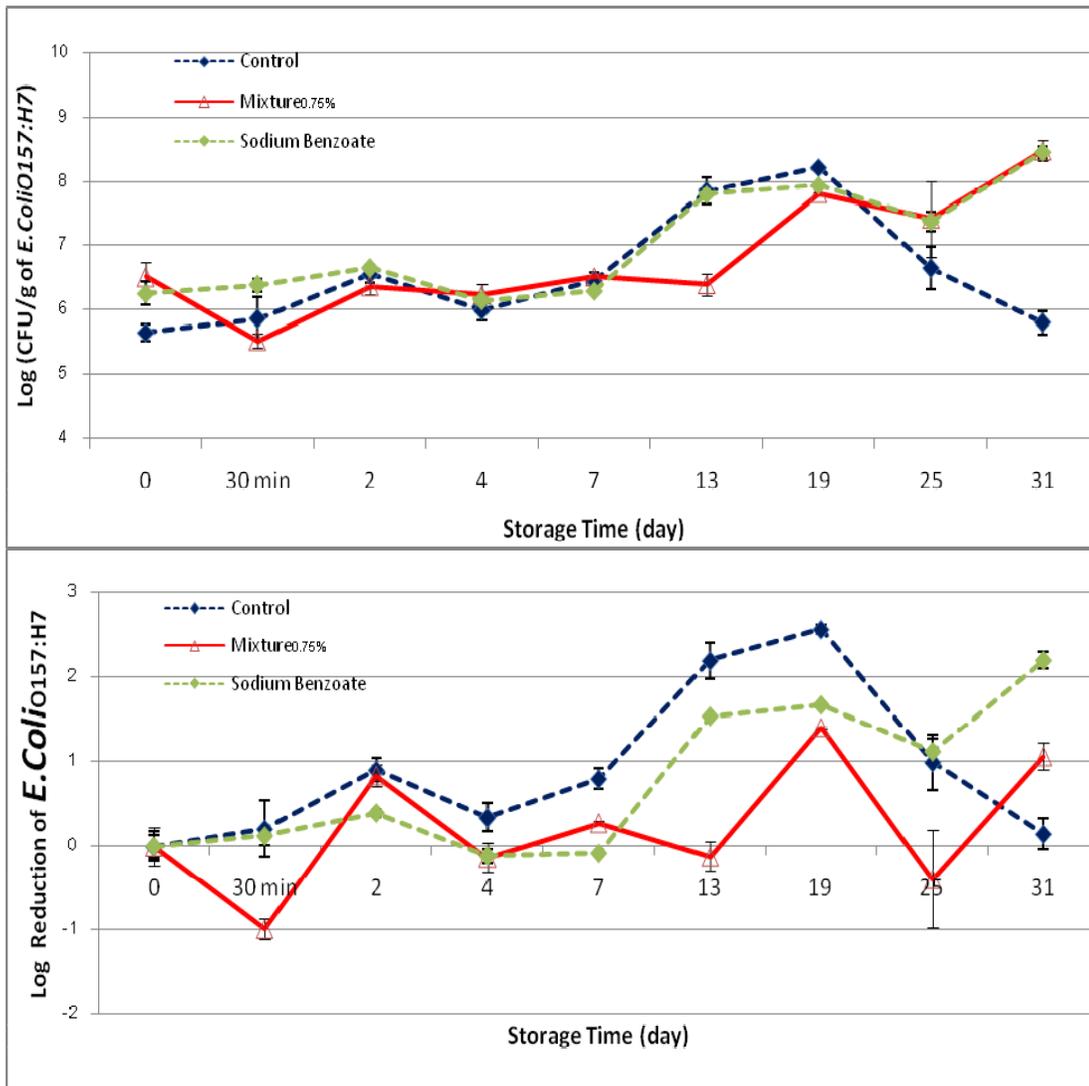


Figure 9. Effects of the mixture of organic acids and their salts on growths of *Escherichia coli* O157:H7 in treated samples and controls as indicated by total viable count (a) and log reduction (b) during storage ($p \leq 0.05$).

In this study, *E. coli* O157:H7 seemed to be more resistant to the lethal effects of organic acids and salts than *S. aureus*. As reviewed by Smulders and Greer [8], this may be related to the inherent properties of *E. coli* or to its attachment mechanism to meat tissues (binding to collagen). Despite the result, organic acids and salts mixture demonstrated a promising trend in suppressing growth of *S. aureus* and *E. coli* O157:H7 at a better level than untreated and preservative-treated controls.

Conclusion

The organic acids and salts mixture solution significantly extended the shelf-life of treated *moo yor* for at least 31 days while shelf-life of control was 26 days. Physical and chemical properties of samples were slightly affected by the organic acids and salts mixture, but this had little effect on panelist's acceptance. The mixture solution of 0.75% offered promising inhibitory effects and growth suppression towards *S. aureus* and *E. coli* O157:H7 throughout storage. Thus, a mixture of organic acid and salts solution with an appropriate concentration has great potential in shelf-life extension and growth inhibition of *S. aureus* and *E. coli* O157:H7 in refrigerated *moo yor* without altering product acceptance.

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