

Bactericidal effects of organic acids as sanitizing agent on iced storage shrimp

Abstract

The microbial population and sensory of *Penaeus vannamei* white shrimp were observed during iced storage period in order to keep fresh and prolong its shelf life through treatments by levulinic acid plus sodium dodecyl sulfate (SDS) and lactic acid. Results indicated that the microbial growths in shrimp meat and shrimp intestine were relatively slow. However, the microbial growths in shrimp shell and shrimp head were rapid during iced storage, especially after the 8th day. At the same time, shrimp's qualities begun to deteriorate on the surface, from shrimp shell and head to the interior of shrimp meat. Treatments by levulinic acid plus SDS and lactic acid effectively reduced the microbial growth in the shrimp meat, head, and shell ($p < 0.01$). The log reduction is related with sanitization concentration, i. e., there were 0.87, 1.06, 0.73, 0.91 log (CFU/g⁻¹) reductions of shrimp shell and head in treatments of dipping 0.5% levulinic acid plus 0.05% SDS, 1.0% levulinic acid plus 0.1% SDS, 0.5% and 1.0% lactic acid when compared with the control group, respectively. Results revealed that levulinic acid plus SDS and lactic acid can be used as sanitizing agents and their antibacterial effects in the shrimp head and shell were better than that obtained from the shrimp meat ($p < 0.05$). The shrimp treated with levulinic acid and SDS had better color, flavor and texture conditions than the control.

Keywords: White shrimp, levulinic acid plus sodium dodecyl sulfate, lactic acid, iced storage

Highlights:

1. Organic acids-based chemicals used as sanitizing agents on shrimp.
2. Treatments by lactic acid and levulinic acid plus sodium dodecyl sulfate (SDS) substantially reduced the microbial growth.
3. The treatment by levulinic acid plus SDS extended shelf life of treated shrimp.

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Introduction

White shrimp (*Penaeus vannamei*) is one of the three highest aquaculture productions in the world. Ecuadorian white shrimp is the most outstanding species among many species in shrimp farming.¹ This type of shrimp, because of its high meat yield, and nutrient-rich flavors is favored by consumers all over the world. Due to the characteristics of high protein, nutrient-rich flavors and high water content, white shrimp is highly prone to be deteriorated. Quality deterioration of shrimp normally occurs during handling of the catch (postmortem), which mainly caused by microbial contamination and autolytic enzymes. The predominant potential spoilers are identified as: *Enterobacter* and *Acinetobacter* at room temperature, *Pseudomonas* and *Aeromonas* at refrigerated storage and, *Aeromonas* and *Enterococcus* at ice storage.^{2,3} Heinsz et al.⁴ reported that *Acinetobacter* spp. were the main organisms responsible for the spoilage of shrimps (*Penaeus aztecus*) harvested from Georgia coastal waters. The dominant microorganisms in shrimps (*Penaeus merguensis*) harvested from Pakistan at sensory rejection times were found to be *Vibrio* spp. at high storage temperatures (15-35°).⁵ All these reports on shrimp spoilage showed that even for the same seafood product, spoilage may develop differently, depending on geographical origin and other unknown factors interacting with microbial growth.⁶ Some undesirable changes such as melanosis formation, protein denaturation, and drip loss in muscle texture can

occur during the iced storage and even freeze/thaw process, thus, negatively affect the product quality and consumer acceptability. Therefore, how to preserve the various shrimp products is a concern. Qian⁷ found that the PPO activity of shrimp inoculated *Shewanella putrefaciens* was about one time higher than other samples and greater melanosis. Traditional preservation methods such as cold storage, freezing and chilling are used to maintain the quality and extend the shelf life of shrimp. However, these methods for shrimp preservation cannot suppress effectively spoilage.⁸ Recent studies indicated that the combination by various preservation methods for obtaining improved effects on microbial inactivation of shrimp is an area with increased interest.⁹ Globally, the demand for high quality minimally processed food products is increasing.

Organic acids are widely used in the food industries for reduction of spoilage bacteria and foodborne pathogens. The antibacterial effects of chlorine, lactic acid, citric acid, acetic acid, levulinic acid and other acids have been confirmed. Among them, chlorine is the most used water disinfectant due to the low cost, the reliable availability, the good effectiveness against suspended vegetative bacteria and some enteric viruses. However, the use of high chlorine concentrations may lead to the production of excessive amounts of harmful disinfection by-products (DBPs) in the water.^{10,11} Partially due to the possible generation of DBPs, the use of chlorine in fresh-cut produce washing is prohibited altogether in some European Union countries.¹² Lactic acid is also one of the most commonly used disinfectant as it is

odorless and cheap compared with other acids. There are lot of reports to approve it as antibacterial interventions for beef, pork and others.¹³ Lactic acid (>0.5%) could effectively reduce pathogens and improve the color and safety of minimally processed lotus sprouts.¹⁴ 4% Lactic acid and 4% sodium metasilicate are effective against pathogens on beef and pork steaks.¹⁵ In addition, levulinic acid (4-oxo pentanoic acid) is an organic acid that has GRAS status for direct addition to food as a flavor additive or adjunct (21 CFR, 172.515). Levulinic acid has a pKa of 4.61 as well as a much higher boiling point than lactic and acetic acids. The latter feature would allow use of levulinic acid at higher temperatures with minimal evaporation, which in theory could provide maximum bacterial kill while minimizing problems with corrosion of equipment and facilities. Levulinic acid plus SDS to inactivate *Listeria monocytogenes*, *Salmonella*, and *Escherichia coli* O157:H7 during mechanical slicing on slicers,¹⁶ washing of contaminated gloves¹⁷ and surface of stainless steel¹⁸ were evaluated effectively.

Therefore, we believe that physical and antibacterial properties of levulinic acid plus SDS may be used as an effective alternative to chlorine, lactic acids or acetic acids for keeping freshness of iced storage shrimp. In this study, our objectives are: 1) to investigate the trends of microbial growth in shrimp head, shrimp shell and shrimp meat respectively during iced storage; 2) to assess the antimicrobial effects of levulinic acid plus SDS and lactic acids at various concentrations as sanitizing agent for iced storage shrimp; 3) to observe the quality and shelf life of treated shrimp.

Materials and methods

White shrimp

The frozen shrimp (*Penaeus vannamei*) with head-on were purchased from a local retail store. These shrimps were offshore farm raised. The weight was about 30-40 shrimps/kg and the size was about 13-18 cm length and 2-4 cm width.

Chemicals and chemical treatment

Lactic acid (Sigma Chemicals, St Louis, MO), levulinic acid (Sigma Chemicals), and sodium dodecyl sulfate (SDS, Sigma Chemicals) with distilled water were to yield two kinds of concentration.

1) Low concentrations: 0.5% (V/V) levulinic acid plus 0.05% (W/V) SDS, which pH value was 2.88; 0.5% (V/V) lactic acid, which pH value was 2.37.

2) High concentrations: 1.0% (V/V) levulinic acid plus 0.1% (W/V) SDS, which pH value was 2.68; 1% (V/V) lactic acid, which pH value was 2.12.

Treatment of organic acids on iced storage shrimp

Frozen white shrimps were transported to laboratory and were randomly divided into seven groups (Table 1). The treatment methods of organic acid are slightly modified as we reported previously.¹⁹

The shrimps placed on the crushed ice (0±0.5) in seven different coolers for iced storage experiments. The shrimp samples were removed for each treatment daily up to 14 days for microbial counts and sensory evaluation. Before microbial determination, individual shrimp was separated three parts, including shrimp head, shrimp shell and shrimp meat, respectively.

Table 1 List of treatment methods of different shrimp groups

Groups	Treatments	methods	pH of acid
A	The negative control	Water	6.47
B	0.5%Levulinic acid +0.05%SDS	dip for 60 seconds	2.88
C	1%Levulinic acid+ 0.1%SDS	dip for 60 seconds	2.68
D	0.5%Lactic acid	dip for 60 seconds	2.37
E	1.0%Lactic acid	dip for 60 seconds	2.12
F	0.5%Levulinic acid +0.05%SDS	spray 20 times/kg	2.88
G	1.0%Levulinic acid+ 0.1%SDS	spray 20 times/kg	2.68

Microbial counts

Whole shrimps aseptically cut into three parts: shrimp meat, shrimp head and shrimp shell, after which a 25 g every part of shrimp sample was transferred aseptically to a stomacher bag and diluted 10 times with 0.1% peptone solution. The mixture homogenized for 30s using a stomacher (Seward Laboratory Stomacher 400, England) to get the first dilution from which successive decimal dilutions were prepared. Total Viable Counts (TVC) measured as aerobic plate counts were enumerated on plate count agar (PCA, Fisher Scientific) in duplicate and incubated at 37° for 24 h.⁴ For the PCA plates, 1 mL of the appropriate dilution was inoculated into a Petri dish, then approximately 15-20 mL of the molten (50°) medium was poured into the Petri dish.²⁰ After setting, the Petri dish was overlaid with approximately 10-mL of the same molten medium. Samples treated with 0.1% peptone water only were used as the negative control.

Sensory evaluation

Shrimp samples from each treatment group taken for sensory evaluation, which including color, flavor, and texture of the iced storage shrimp were analyzed by 10 panelists.²¹ Melanosis and presence of yellow-greenish coloration were evaluated as color scores. Melanosis was evaluated according to a scale of 1 to 10, where 10-9=complete absence of black spots; 8-6=a few small spots on the carapace; 5-3=considerable spotting on the carapace; 2-1= substantial spotting over the entire shrimp. The presence of yellow-greenish coloration beneath the head cuticle was scored according to a scale from 10 to 1, where 10-9=typical pink color; 8-6=yellow pale/yellow coloration; 5-3= green pale/green coloration and 2-1=dark. Odor was evaluated as flavor scores of shrimps according to a scale from 1 to 10, where 10=sea and none acid odor; 9-8=typical and none acid odor; 7-6=neutral or little of odor; 5-4= slightly ammonia or acid odor; 3-2=ammonia or obviously acid odor; 1= off-odor or serious acid odor. The tight junction between abdomen and cephalothorax was scored as the texture scores, which scale from 10 (tight) to 1 (loose). The total sensory scores were the means of shrimp's color, flavor, and texture scores.

Statistical analysis

Statistical analysis performed with SPSS 11.5 for Windows (SPSS Inc. Chicago, IL, USA). The data presented as mean ± standard error. Data were analyzed for analysis of variance (ANOVA) to determine significant differences (P<0.05) and for correlation coefficients (CORREL) to determine the correlation between two group data.

Results and discussion

Change of microbial growth and sensory evaluation in white shrimp during iced storage

The ratio in whole shrimp of shrimp meat, head and shell were about $53.58 \pm 1.53\%$, $34.04 \pm 1.28\%$, $12.37 \pm 1.91\%$, respectively. Shrimp meat occupied more than half of the whole shrimp. The proportions of shrimp shell and head occupied the other half of the shrimp. Structurally, the shell is tightly attached to the epidermis by attachment fibers (intracuticular fibers), and the epidermis is securely attached to the muscle by extensive interdigitating.²² Shrimp is highly perishable, and inappropriate preservations are prone to diminish the meat quality e.g., freshness, texture, color, flavor. The spoilage of shrimp mostly caused by bacteria. Thus, it is very important to reduce the microbial growth on the surface of shrimp to keep fresh of shrimp.

This research investigated the changes of microbial growth on shrimp shell, shrimp head, and shrimp meat during iced storage. As Figure 1 showed, the microbial counts of shrimp shell and shrimp head showed similar changes during iced storage. They were both slowed down during iced storage of first 5 days and then gradually increased in the next 5 days. The correlation coefficient between shrimp shell and shrimp head was 0.97. It means there was very strongly positive correlation of the change of microbial growth between shrimp shell and shrimp head. Normally, the bacteria on shrimp shell grew as that on shrimp head. Therefore, we will study the microbial growth of the shrimp head and shell together in the subsequent experiment of organic acid as sanitizing agent for iced storage shrimp. On the other hand, the change of the microbial growth in shrimp meat was not obvious during the iced storage. Results indicated that there were no correlations between shrimp meat and shrimp head, shrimp shell. The correlation coefficients were only 0.74 and 0.70, respectively. Moreover, the microbial count in shrimp meat was lower than that of shrimp shell and shrimp head during iced storage ($p < 0.01$). The microbial counts in shrimp head, shell and meat increased from 2.46, 3.41, 3.59 log (CFU g⁻¹) on the 0th day to 2.87, 4.36, 4.38 log (CFU g⁻¹) on the 10th day, respectively. They increased 0.41, 0.95, and 0.79 log (CFU g⁻¹) during 10 days of storage, respectively. The microbial counts in shrimp head and shrimp shell had more increments of 1.49 Log CFU/mg and 1.51 Log CFU/mg than shrimp meat at the end of storage, respectively. The results demonstrated that the shrimp has growth that is more microbial on its surface. Then, it might begin to deteriorate from surface to interior of shrimp during iced storage. Shrimp and shrimp products, including ready-to-eat shrimp, can support the survival and/or growth of bacterial foodborne pathogens and there are reports of foodborne disease outbreaks where shrimp has been implicated.²³ Furthermore, the presence of pathogens has also caused significant numbers of shrimp product detentions and recalls.²⁴ Therefore, it is necessary to properly sanitize the surface of shrimp.

On the first day, the bacterial count was 4.57 log (CFU g⁻¹), and on 10th day, the bacterial count was still only 4.78 log (CFU g⁻¹). There was only 0.21 log (CFU g⁻¹) rising of microbial counts during the completely iced storage period. Our results revealed that the bacterial count in shrimp intestine was high, but the bacterial growth rate was not obvious ($P > 0.05$). Therefore, the result demonstrated that the microbial growth rate of shrimp intestines was relatively stable without any external contamination during iced storage.

The sensory index of shrimp, including color, flavor, and texture state all decreased significantly during iced storage of 10d (Figure

2). Especially after the 3rd day, some shrimps showed melanosis phenomena on their surfaces. After the 5th day, all shrimps showed obvious melanosis and softening phenomena on the surface. Due to continuous reproduction of residual bacteria in shrimp during iced storage, the phenomena of melanosis and spoilage were appeared on the shrimp surface.²⁵ Quality loss in shrimp is due to the onset of melanosis and microbial spoilage.²⁶ Melanosis is caused by the formation of melanin.²⁷ Although the presence of melanins is not harmful, it considerably reduces the market value of the crustacean. Therefore, the treatment with proper preservation is of great significance for prolonging the shelf life of iced storage shrimp.

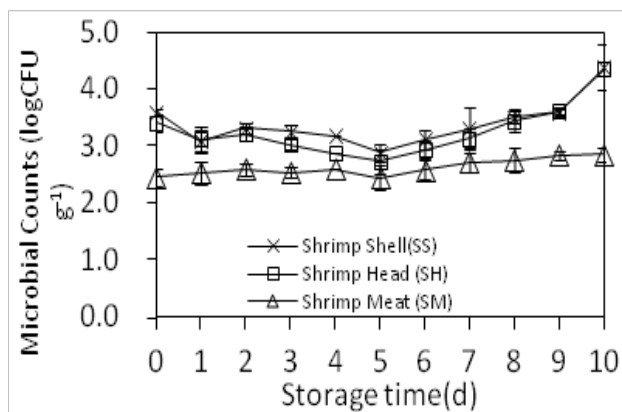


Figure 1 Microbial counts of shrimp during storage time.

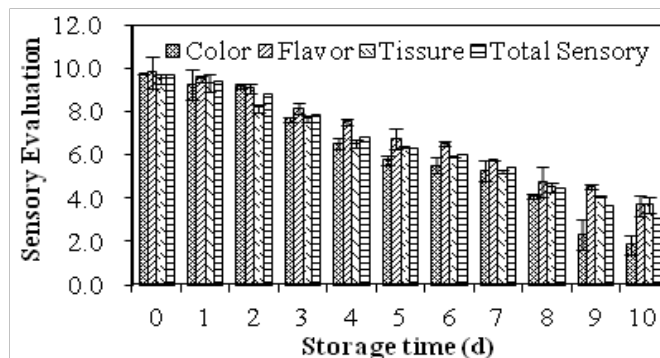


Figure 2 Sensory evaluation of shrimp during storage time.

Microbial growth in shrimp meat using different organic acid as sanitizing agent

The average microbial counts of shrimp meat in group A, B, C, D, E, F, and G during the whole iced storage period were 2.40, 2.21, 1.64, 2.23, 1.94, 2.06, and 1.95 log (CFU g⁻¹), respectively (Figure 3). The average microbial counts in group B, C, D, E, F, and G were lower than group A, which were 0.19, 0.76, 0.17, 0.46, 0.34, and 0.45 log (CFU g⁻¹) reductions, respectively. In addition, the microbial growths in-group C, E, and G were even lower than group B, D, and F, which were 0.57, 0.29, and 0.11 log (CFU g⁻¹) reductions, respectively. Results showed that the treatments with both levulinic acid plus SDS and lactic acid could decrease the microbial growth whatever using dipping or spraying method ($p < 0.01$). But the higher concentration of organic acids with dipping method demonstrated better bactericidal effect in shrimp meat, i. e., dipping with 1% levulinic acid plus 0.1% SDS had better antibacterial effect than 0.5% levulinic acid plus 0.05%

SDS ($p < 0.01$), and dipping with 1% lactic acid had better antibacterial effect than 0.5% lactic acid ($p < 0.05$). Both dipping method and spraying method can reduce the microbial growth in shrimp meat. However, there was not obvious difference between 1.0% levulinic acid plus 0.1% SDS and 0.5% levulinic acid plus 0.05% SDS with spraying method ($p > 0.05$). A bactericide containing levulinic acid and sodium dodecyl sulfate (SDS) has been previously judged as an effective sanitizer in the presence of organic matter, i. e., pathogen inactivation increased as levulinic acid concentration increased.²⁸

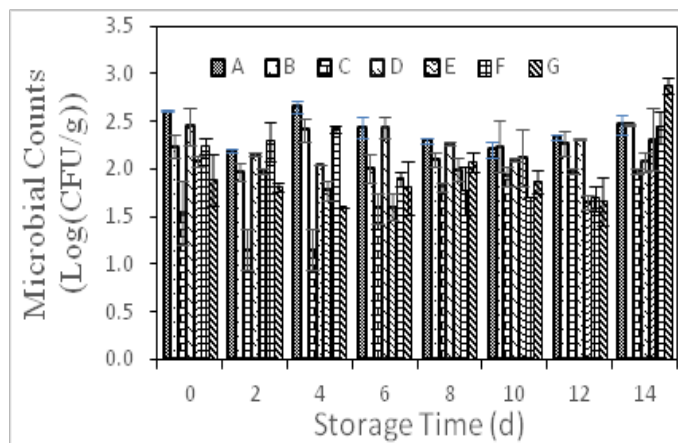


Figure 3 Change of microbial growth in shrimp meat using organic acid as sanitizing agent.

Microbial growth in shrimp head and shell using different organic acid as sanitizing agent

The average microbial counts of shrimp head and shell in group A, B, C, D, E, F, and G during the whole iced storage period were 3.13, 2.26, 2.07, 2.40, 2.22, 2.27, and 1.97 log (CFU g⁻¹), respectively (Figure 4). The average microbial counts in group B, C, D, E, F, and G were lower than group A, which were 0.87, 1.06, 0.73, 0.91, 0.86, and 1.16 log (CFU g⁻¹) reductions, respectively. The average microbial growths in group C, E, and G were even lower than group B, D, and F, which were 0.19, 0.17, and 0.30 log (CFU g⁻¹) reductions, respectively. The results revealed that both levulinic acid plus SDS and lactic acid can greatly reduce the microbial growth in shrimp head & shell no matter using either dipping or spraying ($p < 0.01$). However, the higher concentration of organic acid did not demonstrate better effects of reducing the microorganisms in shrimp head and shell ($p > 0.05$). It can be inferred that both 0.5% levulinic acid plus 0.05% SDS and 0.5% lactic acid can get a better bactericidal effect on the shrimp shell and head. As Figure 5, both dipping method and spraying method can reduce microbial growth in shrimp head and shell, but had not obvious difference ($p > 0.05$). Our results demonstrated that levulinic acid plus SDS and lactic acid can be used as sanitizing agents and their antibacterial effects in the shrimp head and shell were better than that obtained from the shrimp meat ($p < 0.05$). The use of levulinic acid plus SDS as a wash solution may have practical application for killing foodborne enteric pathogens on skin of fresh produce and poultry.¹⁹

Sensory index of shrimp using different organic acid as sanitizing agent

The sensory score of control shrimp decreased sharply and appeared melanosis phenomenon during iced storage and only gained 5.75 on the 4th day of iced storage, which had reached unacceptable

level due to the melanosis showed on control shrimp's surface (Figure 5). Similar results were documented in the sodium metabisulfite treated shrimp during 4°C storage.²⁹ However, the shrimp treated with levulinic acid plus SDS had better color, flavor and texture conditions than the control. The shrimp treated with levulinic acid plus SDS began to appear melanosis until on the 6th day. Both dipping method and spraying method showed the similar treatment effects for iced storage shrimp. Especially the shrimp sprayed with 1% levulinic acid+ 0.1% SDS showed the best color, flavor and texture conditions ($p < 0.01$). At the same time, the shrimp dipped with lactic acid begun to appear melanosis phenomenon on the 6th day, which were more obvious on the 8th day. However, the shrimp treated with 1% lactic acid showed paler on its surface and had a more pungent odor than others had. The odor may limit use of lactic acid with high concentration on fresh produce.³⁰ All of the shrimps appeared melanosis, softened textures, and lost their commercial values when stored for more than 14 days. The results suggested that the treatments with levulinic acid plus SDS could improve the sensory condition of iced storage shrimp. Levulinic acid has shown considerable promise as an antimicrobial intervention for fresh produce in recent years, particularly when used in combination with sodium dodecyl sulfate (SDS).³¹

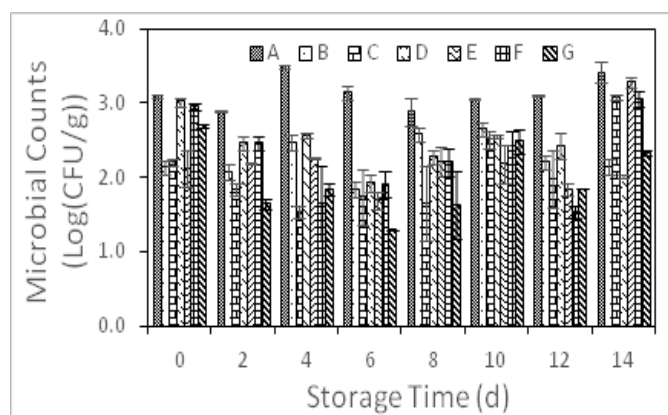


Figure 4 Change of microbial growth in shrimp head & shell using organic acid as sanitizing agent.

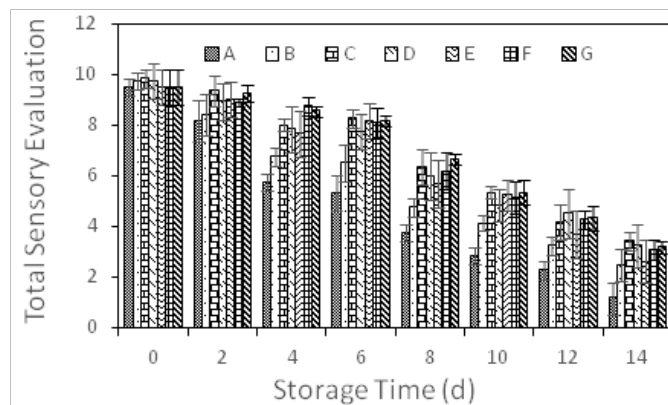


Figure 5 Changes of total sensory index of shrimp using organic acid as sanitizing agent.

Conclusion

The microbial growths in shrimp meat and shrimp intestine were relatively slow and the microbial growth in shrimp shell and head was significantly fast, especially after the 8th day of iced storage.

Therefore, it is necessary to sanitize the surface of shrimp in order to extend the shelf life. Both levulinic acid plus SDS and lactic acid can effectively reduce the microbial growth in the shrimp meat, shrimp head and shrimp shell ($p < 0.01$). The shrimp treated with levulinic acid plus SDS obtained better color, flavor and texture conditions than the control, which can enhance bright-brown color of shrimp and inhibit melanosis formation for more than 2-4 days when compared with the control.

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Conflicts of interest

The authors declare that no conflicts of interest

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