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EFFECT OF LACTIC ACID AND HOT WATER TREATMENTS ON QUALITY OF SHUCKED HARD CLAMS (*Meretrix casta*) DURING REFRIGERATED STORAGE

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Abstract: This research aims to investigate the quality and shelf life extension of shucked hard clams (*Meterix casta*) dipped in hot water and lactic acid. The samples were treated as follows: 1) dipping in control sterile tap water, 2) dipping in hot water at 65 ºC followed by 2.0 % LA, and 3) dipping in 2.0 % LA only. Dipping in hot water and LA was the most efficient method in delaying the proliferation of microorganisms, including total variable count. Chemical analysis indicated that the treated samples underwent a significant change in terms of pH value and total volatile base nitrogen content ($P<0.05$). However, assessment of sensory attributes indicated high scores for color, texture and odor of the samples that were dipped in hot water and LA. This method prevented the generation of undesirable chemicals, improved taste and presentation, and effectively extended the shelf life of shucked hard clams in refrigerated storage.

Keywords: Hard Clam (*Meretrix casta*), total volatile base nitrogen, shelf life, Lactic acid, hot water, dipping.

Introduction

Hard clams (*Meretrix casta*) are one of the most abundant shellfish harvested for food along the coast of Trang province, south Thailand, which faces the northern part of the Straits of Malacca. They are usually cooked fresh in their shells before the meat is removed and eaten as a local delicacy. In the seafood industry, large quantities of the clams are washed and packed for export. Some producers also shuck the clams and pack the meat. Although the clams have to be transported under refrigeration, the shelf life is still limited as the meat is susceptible to spoilage and decay.

Several intervention strategies have been developed to reduce the level of bacteria on the shell and meat, such as washing, sanitizing with hot or chilled water, and dipping in chlorine solution, besides adding food grade acids and salts, either alone or in combination. The Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) has approved the use of some organic acids and hot water as antimicrobial treatment for beef carcass. Lactic acid has low toxicity on humans and it is "generally recognized as safe" in the FSIS multipurpose food substances catalogue (USDA-FSIS, 1996). Topical spray washes with lactic or acetic acid solutions are widely employed in the meat industry as a post-harvest intervention to reduce bacterial pathogen loads in their products. Pathogen reduction after acid washing has been suggested from several factors, including the immediate decontamination of bacteria from meat surface (Castillo *et al.*, 1998), the bactericidal combination of acid concentration and application temperature (Anderson and Marshall, 2007), and from residual inhibitory effects that may initially be bactericidal due to lowered pH on the meat surface for a short time following an acid wash (Dorsa *et al.*, 1998a).

The effects of organic acids may depend on two factors; (1) pH and (2) the degree of dissociation of the acid. In addition, it is known that the antibacterial effect of lactic acid varies depending on the concentration of the acid, temperature of solution, method and time of

application (Smulders, 1995; Tamblyn & Conner 1997). The loss of quality and spoilage of seafood due to bacterial growth and endogenous enzymatic changes occur faster at ambient than chilled temperatures (Huss, 1995). Researchers have found the use of hot water or organic acid interventions in the processing of beef carcasses to be effective in reducing bacteria populations on the products (Reagan *et al.,* 1996; Dorsa *et al.*, 1998b). Therefore, the objective of this research is to determine the effects of lactic acid and hot water treatment on the chemical, microbiology (total viable and psychrotrophic count) and sensory attributes of shucked hard clams stored under refrigeration.

Materials and Methods

Sample Preparation and Sampling

The hard clams used in this study were obtained from local harvesters in Kantang district, Trang Province, Thailand. There were around 150 to 200 individuals per kg of clams. The samples were placed in nylon bags and transported to laboratory within one to two hours at ambient temperature. Upon arrival, the fresh hard clams were washed with water for 10 minutes. After that, they were boiled in water for three minutes and the meat was cut out from the shell. The shucked hard clam meat was divided and subjected to three treatments as follows:

1) Dipping in sterile tap water (as untreated control);
2) Dipping in 65°C of hot water, followed by a 2.0 % lactic acid (LA) solution for 30 s; and,
3) Dipping in a 2.0 % LA solution only for 30 s.

The samples were packed into sterile plastic bags and stored at 4±2°C for further study. They included microbiological and chemical analyses, besides sensory attributes, every three days for 15 to 18 days.

Chemical analysis

The pH measurement was carried out using a pH meter. A total of 10g of hard clams were homogenized thoroughly in 90 ml of distilled water at 12,000 rpm for 1 min before pH determination (Woyewoda *et al.*, 1986). Total volatile base nitrogen (TVBN) content was determined using the Conway microdiffusion method (Conway, 1962). The samples (5 g) were homogenized in 25 ml of trichloro acetic acid (TCA) at a concentration of 4 g per 100 ml. The homogenate was filtered through Whatman No. 1 paper (Whatman Plc, Maidstone, UK) and 1 ml of the filtrate was placed in the outer ring of the Conway diffusion disc. A 1 % boric acid solution with bromocresol green indicator was pipetted to the inner ring and the reaction was initiated by adding 1 ml of saturated K_2CO_3 to the filtrate in the outer ring. The disc was then sealed and placed in a dessicator at 37°C for 60 minutes. The inner ring solution was then titrated with 0.02 mol/l HCl using a micro-burette until the color turned from green to pink.

Microbiological Examination

A total of 25 g of hard clam tissue were transferred to a stomacher bag filled with 225 ml sterile Butterfield's phosphate-buffered water under aseptic conditions in a laminar flow to obtain a stock dilution of 10^{-1}. The stock was further diluted for culturing of microrganisms. Total viable count (TVC) and psychrotrophic count were determined by using the pour plate method. Plate count agar were used and incubated at 35°C for 48 hours and at 7°C for 10 days, respectively (BAM, 2001). To detect *Escherichia coli,* the lauryl sulfate tryptose (LST) broth and EC broth were used for preliminary screening, followed by selective streaking on eosin methylene blue (EMB) agar. Typical *E.coli*-like colonies were confirmed by IMViC test (BAM, 2002). For detection of *Vibrio parahaemolyticus,* glucose salt teepol broth (GSTB) was used for the preliminary screening. Growth of *V. parahaemolyticus* in GSTB tubes was confirmed by streaking onto thiosulfate citrate bile salts sucrose (TCBS) agar, according to the methods described by BAM (2004).

Evaluation of Sensory Attributes

The sensory qualities of samples were performed using the scoring test by 10 trained panellists using modified guidelines by Jeong *et al.* (1990), Aaraas *et al.* (2004) and Songsaeng *et al.* (2010). Three parameters on a scale of 0 (extremely undesirable) to 9 (extremely desirable) were evaluated (1, reject; 2, extremely poor; 3, very poor; 4, poor; 5, acceptable (border line); 6, good; 7, very good; 8, extremely good; and, 9, excellent). The panellists were asked to give a score for each of color, odor and texture while the samples were raw. Individual samples from each treatment were placed on dishes, covered with aluminium foil and stored at 4°C for at least 15 minutes prior to presentation.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 16.0 (SPSS Inc, Chicago, IL, USA). The data was analyzed using one-way ANOVA with Duncan's multiple range test ($P<0.05$).

Results and Discussion

Chemical Quality

Changes in pH could be used as a post-mortem indicator of glycogen breakdown to lactic acid and the degradation of muscle components, such as proteins and nucleotides, during long-term storage (Jay, 2000). The initial pH values recorded for treated samples were below 6.5, while it was 7.12 for the control group (Figure 1).

Shucked hard clams dipped in hot water and LA showed a slight decrease in pH than those dipped in 2 % LA alone. This decrease might be due to the relatively high level of glycogen in the molluscs, and the fact that the spoilage of shellfish was partly a fermentative process (Balasundari *et al.*, 1997).

The decrease in pH values during cold storage could be attributed to breakdown of the clams' glycogen content through glycolysis. However, in the absence of oxygen, the pyruvate end-product would be converted into lactic acid instead of being turned to acetyl Co-A for the citric acid cycle. The presence of lactic acid would cause the pH in the clam homogenates to drop (Muchenje *et al.*, 2009). Treating the clams with hot water and LA solution resulted in lower and slower change in pH, which could affect the solubility of myofibrillar proteins that cause the production of volatile bases, which might delay the decreasing rate of pH (Huss, 1995).

TVBN is a group of biogenic amines formed in non-fermented food products during storage, and it is widely used as an indicator for fish spoilage. A TVBN level of 30 mg N/100 g could be considered the upper limit for spoilage, above which fishery products would become unfit for human consumption (Harpaz *et al.*, 2003). The changes in TVBN of shucked hard

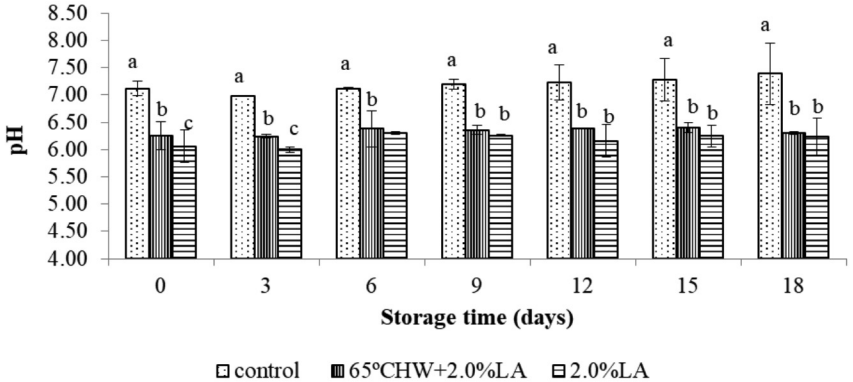


Figure 1: Effect of hot water and lactic acid treatments on pH of hard clams during cold storage (4±1°C). Samples washed in clean water served as controls in the experiment

clams stored at 4±1°C are shown in Figure 2. At day zero, the TVBN value of control samples was 7.82 mg N/100g. A significant difference between control and treated samples was already detected ($P<0.05$) on that day.

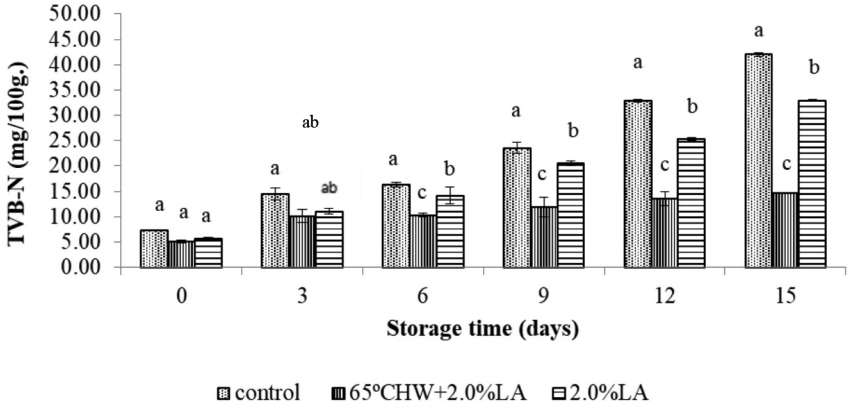
During the storage, TVBN values in control samples increased rapidly compared with treated ones. For the samples dipped in hot water and LA, TVBN values increased the slowest ($P<0.05$) up to day 12 and remained almost the same on day 15. On day 12 of storage, the TVBN level for control samples had exceeded the threshold at 32.89 mg/100 g, whereas the sample treated with hot water and LA had around half the value. The samples treated with LA alone also had increased TVBN levels, but it was slightly lower than the control and higher than the sample treated with hot water and LA solution.

The chemical analysis demonstrated the significant reduction of TVBN in treated hard clams when compared with control samples. Molluscs differed in their chemical composition from fish and crustaceans in that they contained higher levels of carbohydrate (glycogen) and a lower quantity of nitrogen. The reason that TVBN values of treated shucked hard clams were relatively low at the end of the shelf-life might be because relatively high levels of glycogen had been converted to lactic acid, instead of proteins being broken down into amine bases.

Microbiology Quality

The hard clam sample treated with hot water and LA had TVC significantly lower than control and treatment with LA alone (Figure 3). The dipping in hot water combined with LA extended the shelf life of hard clam samples for 24 days, whereas shelf-life of the control samples was only six days. Rapid increases were observed in TVC values of the control while slight increases were observed in treated samples. A TVC value of 7 log CFU/g could be considered the upper acceptability limit for fresh food (ICMSF, 1986). The TVC of control increased from approximately 2.96 log CFU/g (day 0) to unacceptable level after nine days only. Samples treated with hot water and LA had acceptable TVC counts until day 18, whereas it was until day 15 for samples treated with LA alone.

E. coli and *V. parahaemolyticus* were found to be less than 3 MPN/g throughout the storage period (data not shown). Pychlorphile of sample treated in hot water and LA, and LA alone slightly increased during the storage period. However the psychlorphile increase was not as great as TVC.



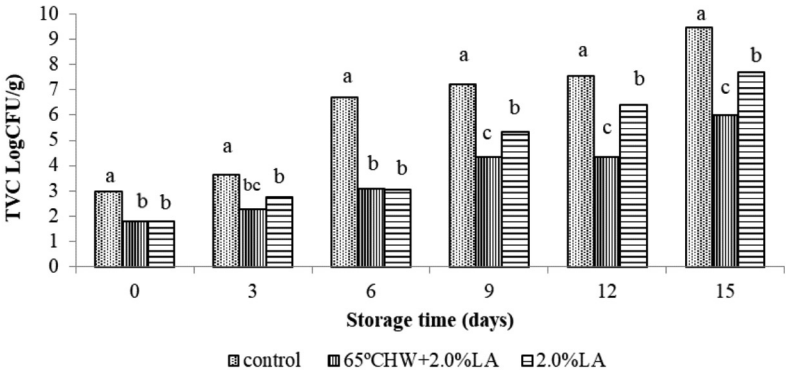


Figure 3: Effect of hot water and lactic acid treatments on total viable count of microorganisms during cold storage (4±1°C). Samples washed in clean water served as controls in the experiment

Sensory Quality

The color, odor and texture of shucked hard clams treated in hot water and LA slightly decreased compared with the control and sample treated with LA only. (Figures 4 to 6). The acceptable shelf-life was considered from the sensory score of more than 2.0. The results showed that the color score of shucked hard clams treated in hot water and LA was acceptable for 18 days, while it was 15 days for LA alone and six days for control.

Meanwhile, the odor score for the hot water and LA treated sample was acceptable for 12 days, which was significantly longer compared with LA treatment alone and control, which were nine and six days, respectively ($p<0.05$).

The texture score was also significantly different ($p<0.05$). The samples dipped in hot water and LA had the highest score. Therefore the combined use of hot water and LA solution had the potential to retard microbial growth and metabolism as shown by the sensory index indicators on spoilage, and control of odor during storage at 4±1°C.

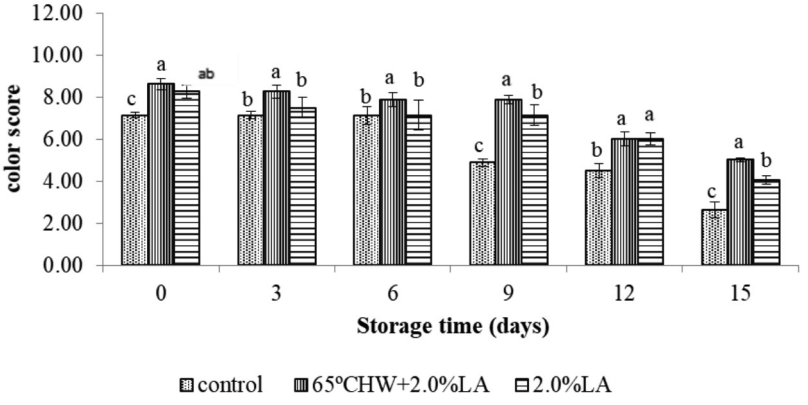


Figure 4: Effect of hot water and lactic acid treatments on color score of hard clams stored under refrigeration (4±1°C). Samples washed in clean water served as controls in the experiment

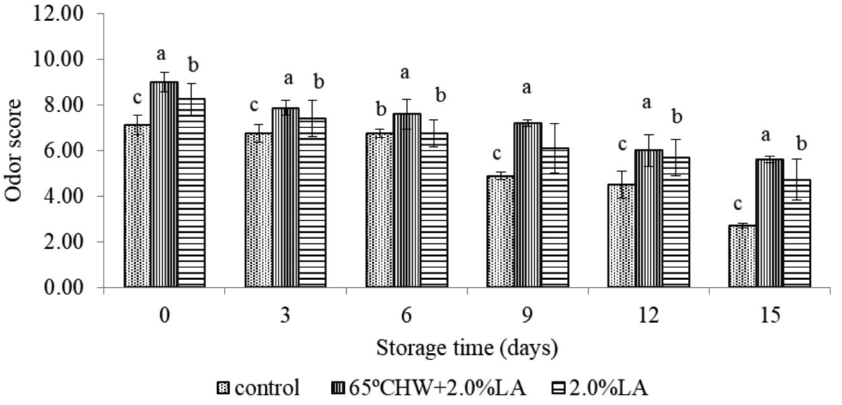


Figure 5: Effect of hot water and lactic acid treatments on odor score of hard clams stored under refrigeration (4±1°C). Samples washed in clean water served as controls in the experiment

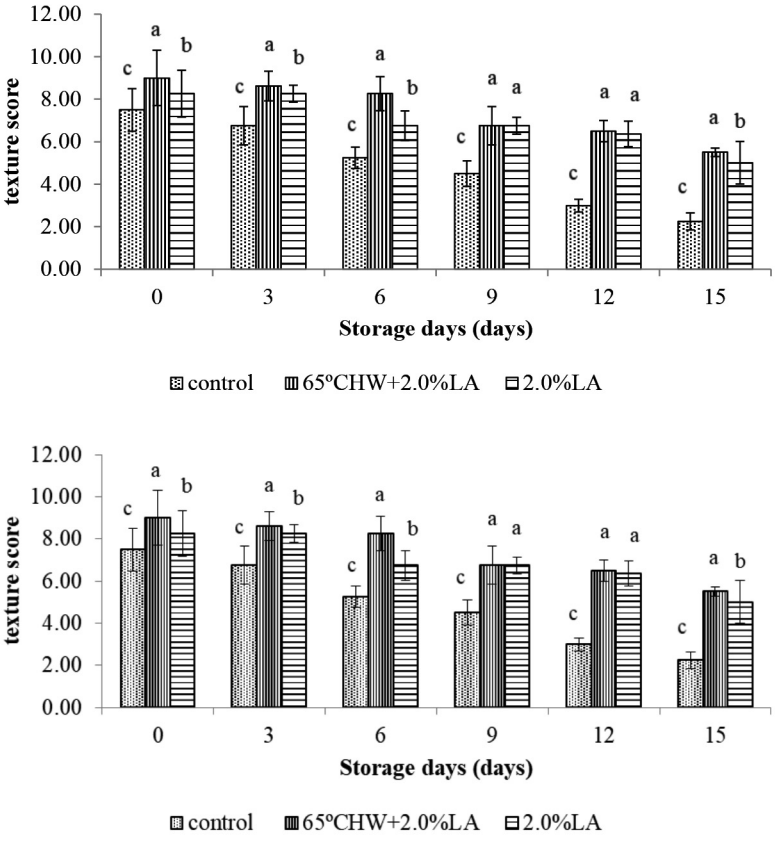


Figure 6: Effect of hot water and lactic acid treatments on texture score of hard clams stored under refrigeration (4±1°C). Samples washed in clean water served as controls in the experiment

Conclusion

This study demonstrated that dipping of shucked hard clams in hot water, followed by LA solution is an optimal treatment to prolong the shelf life and quality of samples in cold storage (4±1°C). The clams showed slower deterioration in quality compared with dipping in LA alone and clean water as control. In terms of microbiological and sensory quality, the treatment could more than double the storage period of the clams compared to controls.

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