

Effect of garlic juice on quality changes of oyster (*Crassostrea belcheri*) meat during chilled storage

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Abstract. Surat-thani oyster, a big and thin-shell bivalve mollusks, has been registered as Geographical Indicators, GI, as its good taste and delicacy as well as nutritious. Eaten style is raw then there is high risk to face with some disease as oyster is filter feeder. Physical, chemical, microbiological and sensory qualities after the oyster meat treated with the garlic juice at 0, 2 and 3 ml, respectively were monitored. Though initial pH of the control, untreated with garlic juice, was higher compared with the sample treated with 3 ml garlic juice, pH of it (control) was significantly lower ($p < 0.05$) than the sample treated with the juice during storage. The total volatile base (TVB), chemical quality, values of all samples increased when increased storage time but not over 35 mg/100 g sample. During chilled storage, lactic acid content of all samples increased as storage time increased but the sample treated with the juice was higher compared with the control. A thiobarbituric acid reactive substance (TBARS), rancidity indicator, of the control was higher than the sample treated with the juice. K-value (%) of all oyster meats increased however; the lowest value was found in the control at end of storage, 12 days. Ammonia content of all samples slightly increased during storage. Lactic acid content of the treated sample was higher than control after stored for 12 days. Both mesophilic and lactic acid bacteria of all samples tended to increase during storage. Mesophilic of control, sample-injected with garlic juice 2 ml and 3 ml increased from 3.89 ± 0.04 , 3.62 ± 0.18 to 5.89 ± 0.06 and 3.57 ± 0.02 to 6.04 ± 0.10 log CFU/g, respectively at end of storage. Lactic acid bacteria of control and sample-injected with garlic juice 2 ml and 3 ml changed from 1.71 ± 0.02 to 2.94 ± 0.04 , 1.79 ± 0.09 to 4.63 ± 0.08 and 1.84 ± 0.04 to 4.82 ± 0.10 log CFU/g, respectively. However, psychrophilic, coliforms, fecal coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.* and *Vibrio spp.* were low throughout the storage. Consumer acceptability scores were higher than borderline (>5) at the end of the storage.

Key words: oyster, garlic juice, quality, safety

Introduction

Oyster is one of the delicious, nutritious seafood and may be the most expensive bivalve mollusks (Bussarawit & Simonsen, 2006). Oyster is often eaten raw or lightly cooked then there is high risk to have direct implications for disease transmission since bivalve mollusks are filter-feeders that obtain food from the environment by filtering seawater through their gills (Lee *et al.* 2008). The pathogenic bacteria, biological toxins and harmful chemicals accumulate in the oyster are the results of limits of consumers safety. In addition, oyster is perishable product therefore quality and economic losses are normally found and necessary to have a proper manage.

Garlic has been proven to be effective against a plethora of gram-positive, gram-negative and acid-fast bacteria. These include *Pseudomonas*, *Proteus*, *Staphylococcus aureus* (Cavallito & Bailey 1944), *Escherichia coli*, *Salmonella* (Johnson & Vaughn 1969), *Klebsiella* (Jezowa *et al.* 1966), *Micrococcus*, *Bacillus subtilis* (Sharma *et al.* 1977), *Clostridium* (De Witt *et al.* 1979), *Mycobacterium* (Delaha & Garagusi 1985) and *Helicobacter* (O'Gara *et al.* 2000). Garlic clove is served as a side dish to consumer when raw oyster is ordered. It is believed that garlic will help to enhance the oyster taste and flavor as well as to protect diarrhea from eating raw meat.

The objective of this study was to determine the effect of garlic juice on physical, chemical and microbiological qualities and sensorial score of oyster meat during chilled storage.

Materials and Methods

Reagents

White-scar oysters (*C. belcheri*), two years old, 250–300 g in weight and 13–15 cm in length, were obtained in March, 2012 from Bandon Bay, Surathani Province, Thailand,. After harvesting, the live oysters were packed in nylon sacks and transported to the laboratory (4–5 h). It was then washed and cleaned using a brush to remove dirt deposited on the outside of the shell with care to prevent a joint between the valves. Oysters were aseptically shucked from their shells. Fresh garlic (*Allium sativum* L.) was purchased from local markets in Hat

Yai, Thailand. All chemicals, reagents, and media were of analytical grade from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany).

Garlic juice preparation and Oyster preparation

The fresh garlic washed with tap water was blended with blender in a Philips HR-2068 blender (Thailand) before brought to blended with sterilized distilled water at a ratio of 1:3 (garlic:water). The oyster meat was washed with tap water before divided into three groups as control sample and oyster meat treated with garlic juice 2 ml and 3 ml, respectively. The sample was subjected to analyze for physical, chemical and microbiological qualities and sensorial acceptability during chilled storage.

Physical and chemical analysis and Total volatile base (TVB)

pH

Samples was blended with sterilized distilled water at a ratio of 1:5 (sample:water) and allowed to dissolve for 2 min before measuring their pH measured with a pH meter (Mettler 350, Singapore). Two-gram sample of oyster meat was blended with 8 ml of 4% trichloro acetic acid, filtered with Whatman paper No 41 before subjected to TVB analyses using the Conway method (Conway & Byrne, 1936).

Thiobarbituric acid reactive substances (TBARS) value

Ten-gram sample of oyster meat was blended with 50 ml distilled water for 2 minutes then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 ml of distilled water. This was added to a distillation tube containing 2.5 ml 4 N HCl and a few drops of antifoam agent. Five-ml of 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid (called a TBA reagent) was added to a vial containing 5 ml of distillate and mixed well. The vials were capped and heated in boiling water for 30 minutes and cooled to room temperature. The absorbance was measured at 352 nm using a spectrophotometer (Fernandez-Lopez *et al.* 2005).

Determination of K-value using ion exchange chromatography

K value was determined using anion-exchange column chromatography (Uchiyama & Kakuda, 1984). A ground sample (1 g) was subjected to a serial extraction using 10% PCA and 5% PCA. The final extract (2 ml) was adjusted to pH 9.4 using 0.5 N NH₃. The prepared extract (pH 9.4) was loaded onto an anion-exchange column (1×10 cm²) containing Resin-AG (R) 1-X⁴, 400 mesh Cl-form. The column was rinsed using deionised water (20 ml). The elution was performed using 45 ml of solution A (0.001 N HCl). The eluate was collected, and the volume was made up to 50 ml using solution A. Thereafter, the column was eluted with 45 ml of solution B (0.01 N HCl containing 0.6 M NaCl). The resulting eluate was made up to 50 ml using solution B. Both eluates were read at 250 nm using the corresponding eluent (solution A or B) as the blank. K value was calculated as follows:

$$\text{K-value (\%)} = \frac{A}{A+B} \times 100$$

Where A is A₂₅₀ of eluate A representing the amount of inosine (HxR) and hypoxanthine (Hx), and B is A₂₅₀ of eluate B representing the amount of ATP, ADP, AMP, and IMP.

Determination of ammonia content

Ammonia content was determined as described by Parris & Foglia (1983). Groud sample-10 g was placed in a 600 ml round bottom flask containing 200 ml of distilled water; 10 g carbonate-free MgO and a few drops of antifoam. The mixture was distilled and the distillate 100 ml was collected in an erlenmeyer flask containing 20 ml of 0.1 N HCl. Collected distillate was titrated using 0.05 N NaOH and methyl red was used as an indicator. Ammonia content was calculated and expressed as mg/g sample.

Acidity and Bacteriological analysis

Acidity in oyster meat was estimated by titration Method AOAC (1980). Groud sample 5 g was placed in a 250 ml round bottom flask containing 50 ml of distilled water. Titrated using 0.1 N NaOH and phenolphthalein was used as an indicator. Lactic acid content was calculated and expressed as Lactic acid (%). The oyster meat was analyzed for total viable count, coliforms, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp, *Vibrio cholera*,

Vibrio parahaemolyticus, *Vibrio vulnificus* (BAM, 2001) and Lactic acid bacteria (De Man *et al.* 1960).

Statistical analyses

Data were subjected to Analysis of Variance (ANOVA) and mean comparison was performed using the Duncan's New Multiple Range Test. Statistical analyses were carried out using the SPSS statistical software version 6.

Results and Discussion

pH of meat is a indicator for the postmortem changes of glycogen to lactic acid and the degradation of muscle components e.g. proteins and nucleotides during storage (Jay, 2000). The initial pH value of the control sample was 6.68 and gradually decreased ($P < 0.05$) during storage. Similar to results of pH of oyster meat treated with garlic 2 ml and 3 ml which decreased from 5.61 to 6.72 and from 5.59 to 6.85, respectively Figure 1. This result was in agreement with the finding of Balasundari *et al.* (1997) who reported that The initial pH value of the control samples was 6.30, which was in agreement with the reports by other authors (Balasundari *et al.* 1997). During storage, the pH values decreased slightly. This decrease might be due to the relative high level of glycogen in oysters and the fact that the spoilage of mollusk shellfish is partly fermentative. At the end of the shelf-life determined by APC, pH values were 6.05 at 10°C (day 6), 6.06 at 5°C (day 10), and 6.06 at 0°C (day 18), respectively. This result was similar to that of Banks *et al.* (1977) who suggested the pH value of about 6.0 as the lower limit of acceptability for oysters. A decrease of pH values of the oyster meat might be due to the relative high level of glycogen and the fact that the spoilage of mollusk shellfish is partly fermentative as reported by (Cao *et al.* 2009).

Total volatile base (TVB), a decomposed both protein and non-protein nitrogenous compounds (trimethylamine (TMA), dimethylamine (DMA) and ammonia), of all samples increased with increased storage time, probably caused by bacterial and endogenous proteolytic enzymatic actions (Hernandez-Herrero *et al.* 1999). The initial TVB value of the control sample was 1.56 mg /100 g whereas the oyster meat injected with garlic 2 ml and 3 ml were 2.34 and 2.91 mg /100 g, respectively (Figure 2). At 12 day of storage the TVB values of control and sample-injected with the juice 2 ml and 3 ml were 6.7, 13.4 and 15.6 mg N/100 g respectively. The higher TVB value of the sample injected with the juice may because of volatile sulfur compound containing in the garlic juice as reported by Pakawatchai *et al.* 2011. It pointed out that positive fault interpretation will be occurred if the sample was treated with the garlic juice. However, TVB values of all samples increased when increased storage time even there was lower 30 mg N/100 g which was considered to be unfit for human consumption (El-Marrakchi *et al.* 1990; Harpaz *et al.* 2003).

Changes in the TBARS of the oyster meat stored at 4 ° C were showed in Figure 3. It was found that TBARS of all treatments increased as storage time increased. Not surprising, TBARS of oyster treated with garlic juice was lower than control sample ($P < 0.05$). This may be due to antioxidant property of sulfur compounds derived from garlic (Yang *et al.* 1993; Pakawatchai *et al.* 2009). Initial K-value was around 12.5-16.66 % for all oyster meats. As storage time increased K-value increased (Figure 4). However, control sample had the lowest K-value, 50% compared with other samples at the end of storage, 12 days. Pacheco-Aguilar *et al.* (2000) reported that final K-value of shrimp meat was 50.7% after storage in ice for 15 days. An increase of ammonia content of all samples during chilled storage was found as showed in Figure 5. This may be due to the role of autolysis and bacterial growth (Paarup *et al.* 2002). However, there was no significant different in ammonia content in any sample even oyster meat treated with garlic juice seemed to be higher.

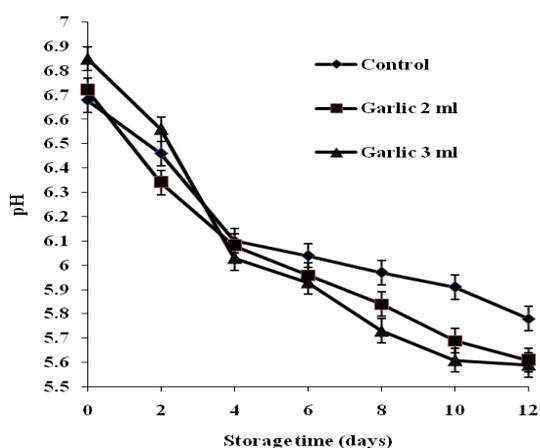


Figure 1. Effect of garlic juice on pH values of oyster meat during storage at 4 °C

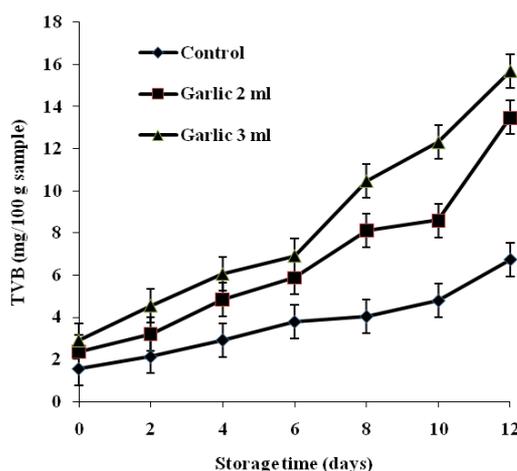


Figure 2. Effect of garlic juice on total volatile base (TVB) values in oyster meat

The initial lactic acid content was 0.28% and gradually increased ($P < 0.05$) in all treatments during storage (Figure 6). An increase of lactic acid content agreed with a decrease of pH as affected of glycolysis pathway and fermentation of lactic acid bacteria (Aarnikunnas, 2006). It meant that beside the glycolysis pathway, garlic juice may provide more carbon source particularly in term of prebiotic led to suitable condition for the growth of lactic acid bacteria that would be explained later. Mesophilic bacteria of oysters were shown in Table 1. The initial mesophilic bacteria of control, sample-injected with garlic juice 2 ml and 3 ml were 3.89, 3.62 and 3.57 log CFU/g, respectively and increased during storage particularly in treated sample at 4°C for 12 days.

It seemed like garlic juice which claimed as antibacterial agent was not well function in this situation or may enhance some bacterial growth instead. As well known that *Lactic acid bacteria* are generally classified in mesophilic group (Lars, 2004). Therefore, a higher increase of lactic acid bacteria in sample with injected garlic juice may account for mesophilic bacteria. It pointed out that using only total viable count either mesophilic bacteria or psychrophilic bacteria count may cause positive false interpretation led to wrong conclusion in some condition. Gibson & Rastall (2006) reported that garlic and *Allium* family is a good source of prebiotic for probiotic bacterial which mainly is lactic acid bacteria group. Fecal coliforms, *E. coli* of oysters were less than 1.8 MPN/g and *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* of oysters storage temperatures were less than 3 MPN/g, while *S. aureus* and *Salmonella spp.* were not detected in 25g throughout the storage time. In addition, psychrophilic bacterial counts were less than 30 log CFU/g. There was some scientific data reported that the psychrophile composition of shellfish related to the oyster habitat can vary depending on different factors such as salinity, environmental condition, bacterial load in the water, water temperature, diet, method of catch and chilling conditions (Vasconcelos & Lee, 1972). Therefore, it meant that oyster from various locations or even from the same location at different times may show a different bacterial concentration or composition. To confirm that using garlic juice would have more safety benefit than oyster inoculated with test pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* etc. would be further investigated. As a whole it was found that oyster treated with garlic juice did not have negative effect for consumer preference. Then using garlic juice may be a potent alternative way to control oyster quality.

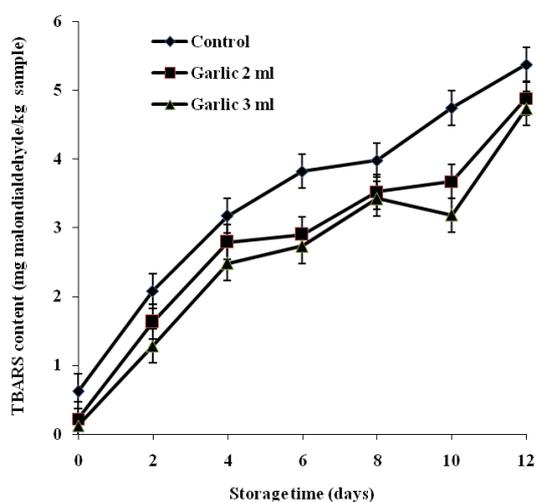


Figure 3. Effect of garlic juice on 2-thiobarbitulic acid reactive substances (TBARS) values in oyster meat during storage at 4 °C

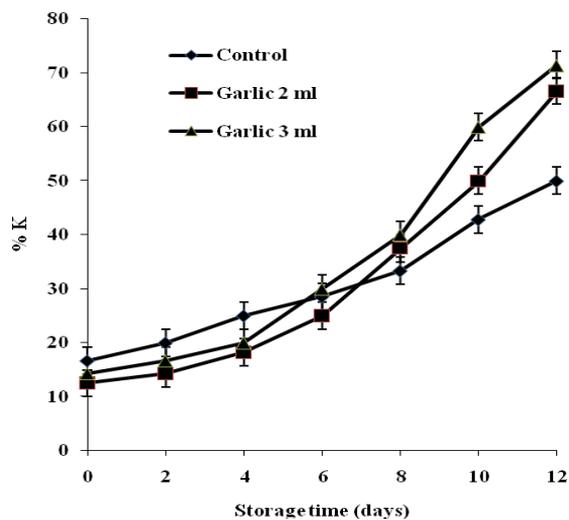


Figure 4. Effect of garlic juice on K value in oyster meat during storage at 4 °C.

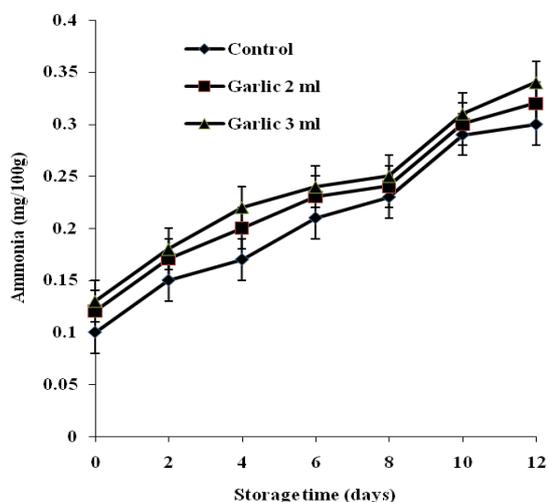


Figure 5. Effect of garlic juice on amino values in oyster meat during storage at 4 °C

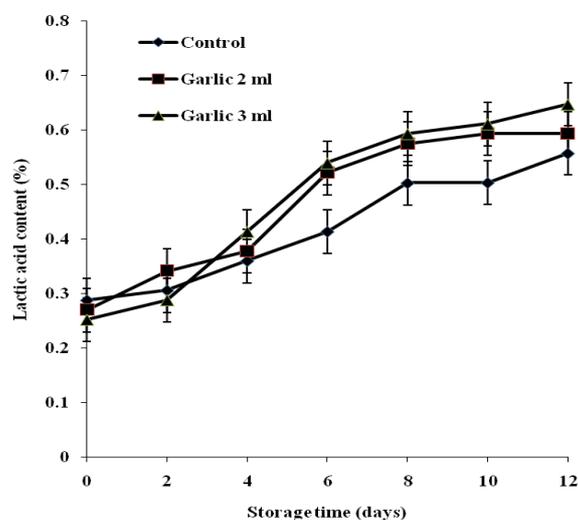


Figure 6. Effect of garlic juice on lactic acid content in oyster meat storage at 4 °C

Table 1. Effect of garlic juice on microorganism values in oyster meat during storage at 4 °C for 12 days.

Bacterial type	Storage time (days)	Treatment		
		T1	T2	T3
Mesophile (log10 cfu/g)	Day 0	3.89±0.04	3.62±0.18	3.57±0.02
	Day 12	6.2±0.04	5.89±0.06	6.04±0.10
Psychrophile (log10 cfu/g)	Day 0	ND	ND	ND
	Day 12	<30	<30	<30
Coliforms (MPN/g)	Day 0	21	14	24
	Day 12	540	540	540
Fecal coliforms (MPN/g)	Day 0	<1.8	<1.8	<1.8
	Day 12	<1.8	<1.8	<1.8
<i>E. coli</i> (MPN/g)	Day 0	<1.8	<1.8	<1.8
	Day 12	<1.8	<1.8	<1.8
<i>S. aureus</i> (MPN/g)	Day 0	ND	ND	ND
	Day 12	ND	ND	ND
<i>V. parahaemolyticus</i> (MPN/g)	Day 0	<3.0	<3.0	<3.0
	Day 12	<3.0	<3.0	<3.0
<i>V. cholerae</i> (MPN/g)	Day 0	<3.0	<3.0	<3.0
	Day 12	<3.0	<3.0	<3.0
<i>V. vulnificus</i> (MPN/g)	Day 0	<3.0	<3.0	<3.0
	Day 12	<3.0	<3.0	<3.0
<i>Salmonella spp.</i> (MPN/g)	Day 0	ND	ND	ND
	Day 12	ND	ND	ND
<i>Lactic acid bacteria</i> (log10 cfu/g)	Day 0	1.71±0.02	1.79±0.09	1.84±0.04
	Day 12	2.94±0.04	4.63±0.08	4.82±0.10

ND=not detected, T1 = Control, T2 = Injected with garlic juice 2 ml, T3 = Injected with garlic juice 3 ml

Conclusions

According to the results of this study, it was found that garlic juice may have a negative and positive effect on the oyster meat when using chemical and microbiological aspect as a tool for quality determination. However, using the garlic juice did not reduce consumer acceptability. Therefore, to confirm the antibacterial property of garlic juice for in term of microbial safety then the pathogenic such as *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* etc. would be innoculated into the oyster meat for investigated further.

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