
ORIGINAL ARTICLE

Effect of lactic, acetic and citric acids on quality changes of refrigerated green mussel, *Perna viridis* (Linnaeus, 1758)

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Abstract

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Effect of lactic, acetic and citric acids on quality changes of refrigerated green mussel, *Perna viridis* (Linnaeus, 1758)

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Effect of lactic, acetic and citric acids on the quality changes and shelf-life extension of green mussel stored at 4°C was investigated. The inhibitory effect on bacterial growth was pronounced when the concentration of lactic, acetic and citric acids increased ($P<0.05$). Green mussel dipped with lactic acid had the lower total volatile base, trimethylamine, ammonia and TCA-soluble peptides contents than those dipped in acetic and citric acids. However, the increases in exudates loss and cooking loss were observed in samples dipped in organic acids, causing the denaturation of muscle protein by acids used. Thiobarbituric acid reactive substances (TBARS) increased as the organic acid concentration increased ($P<0.05$). Lactic acid dipped samples, particularly with 0.2 M, showed the greater acceptability than did those dipped in other acids throughout the storage of 27 days. The control sample had the acceptability only for 6 days of storage.

Key words : lactic acid, acetic acid, citric acid, quality, green mussel

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บทคัดย่อ

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ผลของกรดแลคติก อะซิติก และซิตริก ต่อการเปลี่ยนแปลงคุณภาพของหอยแมลงภู่แช่เย็น²
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การศึกษาผลของกรดแลคติก อะซิติก และซิตริก ต่อการเปลี่ยนแปลงคุณภาพของหอยแมลงภู่ระหว่างการเก็บที่อุณหภูมิ 4°C พบว่า การยับยั้งการเจริญของชุลินทรีย์มีความแปรผันโดยตรงกับความเข้มข้นของกรดแลคติก อะซิติก และซิตริกที่เพิ่มขึ้น ($P<0.05$) หอยแมลงภู่ที่แช่ด้วยกรดแลคติกมีปริมาณด่างที่ระเหยได้ทั้งหมด ไตรเมทิล เออมีน และโมโนนี และเปปไทด์ที่ละลายได้ในกรดไตรคลอโรอะซิติกน้อยกว่าตัวอย่างที่แช่ด้วยกรดอะซิติกและซิตริกอย่างไรก็ตาม ปริมาณน้ำอิสระและการสูญเสียน้ำหนักหลังจากการให้ความชื้นของหอยแมลงภู่ที่ผ่านการแช่ด้วยกรดอะซิติกเพิ่มขึ้น เนื่องจากกรดอะซิติกทำให้ปริมาณสูญเสียสภาพรวมชาติ ค่า TBARS เพิ่มขึ้น เมื่อความเข้มข้นของกรดอะซิติกเพิ่มขึ้น ($P<0.05$) หอยแมลงภู่ที่ผ่านการแช่ด้วยกรดแลคติกที่ระดับความเข้มข้น 0.2 โมลาร์ มีการยอมรับทางประสาทสัมผัสสูงกว่าตัวอย่างที่แช่กรดอย่างอื่น ตลอดระยะเวลา 27 วัน ของการเก็บรักษาตัวอย่าง ชุดควบคุมมีการยอมรับทางประสาทสัมผัสเพียง 6 วัน ของการเก็บรักษา

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Green mussel (*Perna viridis*) is a shellfish belonging to the *Mytilidae* family. It is very popular in tropical areas of South-East Asia, and cultured in the south of Thailand, especially in Pattani Bay. Iced storage has been widely used to prolong its shelf-life, particularly during transportation of mussel meat. Since ready-to-cook foods have become increasingly popular, mussel meat is available in supermarkets to serve the market demand. However, the limited shelf-life, primarily due to microbial growth, is a limiting factor for such perishable products (Jay, 1986).

Several intervention strategies have been developed to reduce the level of bacteria on fresh meat such as washing and sanitizing with chilled water, hot water, chlorinated water, food grade acids and salts, alone and in combination (Dubal, *et al.*, 2004). Acid treatments exert an impact on the microbiology of muscle food (Nakai and Siebert, 2003). A reduction in the number of micro-organisms is associated with an enhancement of quality and safety (Prasai *et al.*, 1991; Williams *et al.*, 1995). After acid treatment, shelf-life extension for various foods was reported (Kim *et al.*, 1995; Farid *et al.*, 1998). The addition of

sodium lactate at 1.8-4.8% delayed toxigenesis by *C. botulinum* in sous vide salmon at 12-16°C (Meng and Genigeorgis, 1994). Spray washing of catfish fillets with acetic, lactic and propionic acids inhibited the microbial growth of fillets (Fernandes *et al.*, 1998). These acids acted differently on microbial and chemical changes of seafoods, including fish and shellfish but the information is limited on the quality changes of green mussel treated with acids. Thus, the present work was undertaken to study the shelf-life of green mussel treated with different acids including lactic, acetic and citric acids during refrigerated storage.

Materials and Methods

Chemicals

Trichloroacetic acid was purchased from Riedal-deHaen (Seeize, Germany). 2-Thiobarbituric acid was obtained from Sigma Chemical Co. (St Louis, MO, USA). Malondialdehyde tetrabutylammonium was purchased from Fluka (Buchs, Switzerland). Folin-Ciocalteu's phenol and plate count agar were obtained from Merck (Darmstadt,

Germany).

Green mussel preparation

Green mussels (*Perna viridis*) with an average size of 30-40 individuals/kg were purchased from a farm in Pattani, Thailand. The samples were transported in ice with an ice/sample ratio of 1/2 (w/w) to the Department of Technology and Industries, Prince of Songkla University, within 2 h after harvesting. Upon arrival, mussels were washed with tap water and the flesh meat was removed from the shells.

Acid pretreatments of green mussel

Lactic, acetic and citric acids were individually dissolved in distilled water to obtain the final concentrations of 0.1 and 0.2 M. Fresh meat was soaked in two volumes of solution (4°C) for 10 min and drained for 10 min at 4°C. After being drained, the fresh meat weighing approximately 500 g was placed and sealed in polyethylene bag. The control sample was soaked in distilled water under the same condition. All samples were stored at 4°C and taken for microbiological, chemical, physical and sensory analyses every 3 days up to 27 days.

Microbiological analyses

Green mussel samples (25g) were collected aseptically in a stomacher bag and 10 volumes of sterile saline solution (0.85%) were added. After homogenizing in a Stomacher M400 (Seward, UK), a series of ten-fold dilutions was made using saline solution. Mesophilic and psychrophilic bacterial counts were determined by plate count agar (PCA) with the incubation at 35°C for 2 days (Speck, 1976) and 7°C for 7 days (Cousin *et al.*, 1992), respectively. Microbial counts were expressed as log CFU/g.

Chemical analyses

The pH measurement

The pH measurement was carried out using a Cyberscan model 500 pH meter (Euteon Instruments, Singapore). The samples (2 g) were homogenized thoroughly with 10 ml of distilled

water and the homogenate was subjected to pH determination.

Determination of total volatile base (TVB) and trimethylamine (TMA)

TVB and TMA were determined by the Conway's method as described by Conway (1950). The samples (2 g) were homogenized with 10 ml of 4% trichloroacetic acid. The homogenate was filtered through a Whatman No.1 filter paper and the filtrate was used for analyses. Sample extract (1 ml) was placed in the outer ring. The inner ring solution of 1% boric acid containing the Conway indicator was then pipetted into the inner ring. To initiate the reaction, K_2CO_3 (1 ml) was mixed with the sample extract. The Conway unit was closed and incubated at 37°C for 60 min. The inner ring solution was then titrated with 0.02 M HCl until the green color turned to be pink. TMA was determined with the same procedure as TVB, but 10% formaldehyde was added to the extract to tie up ammonia.

Determination of ammonia

Ammonia was assayed according to the method of Parris and Foglia (1983). Ground samples (10 g) were placed in a 600 Kjeldahl flask containing 200 ml of distilled water, 10 g of carbonate-free MgO and several drops of antifoam. The mixture was distilled and the distillate (100 ml) was collected in 20 ml of 0.1 M HCl. The titration was performed using 0.05 N NaOH and methyl red was used as an indicator. Ammonia content was calculated and expressed as mg/g sample.

Determination of TCA soluble peptides

TCA soluble peptides were determined according to the method described by Morrissey *et al.* (1993). The samples (3 g) were homogenized with 27 ml of 5% TCA using a homogenizer (IKA, Labortechnik, Malaysia). The homogenate was kept in ice for 1 h and centrifuged at 5,000 ×g for 5 min. Soluble peptides in supernatant were measured using the Lowry method (Lowry *et al.*, 1951) and expressed as μmol tyrosine/g muscle.

Determination of thiobarbituric acid-reactive substances

Thiobarbituric acid-reactive substances (TBARS) were determined according to the method of Buege and Aust (1978). Chopped samples (0.5 g) were homogenized in 2.5 ml of the mixture containing 0.375% TBA, 15% TCA and 0.25 M HCl. The mixture was heated in the boiling water for 10 min, followed by cooling with the running tap water. The mixture was centrifuged at 3,600 $\times g$ for 20 min (Sorvall, Newtown, CT, USA) and the absorbance was measured at 532 nm using UV 1601 spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). TBARS were calculated from the standard curve of malondialdehyde and expressed as mg malondialdehyde/kg muscle.

Physical analyses

Determination of exudate loss

Exudate loss was measured as the percentage loss of sample weight, compared to the initial weight (Pastoriza *et al.*, 1996).

Determination of cooking loss

Cooking loss was determined as the percentage loss of sample weight after steaming for 1 min, compared with the initial weight (Pastoriza *et al.*, 1996).

Sensory evaluation

The sensory evaluation was performed by 15 trained panelists. The assessment was conducted for the odor and flavor of raw mussel samples using a 9-points hedonic scale (Mailgaad *et al.*, 1999): 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. The evaluation was carried out at the moment of opening the pack. For cooked samples, the samples were wrapped with aluminium foil, cooked in steaming pot until the core temperature of each sample reached 70°C. Stick water was drained and allowed to cool to room temperature (25-28°C). The flavor acceptability of cooked samples was evaluated using a 9-points hedonic scale.

Statistical Analysis

All experiments were run in triplicate. Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences between means (Steel and Torrie, 1980). Statistical analysis was performed using the Statistical Package for Social Science (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL).

Results and Discussion

Effect of acid pretreatment on microbiological changes of green mussel during refrigerated storage

The initial prime quality of green mussel used in this study was observed, as indicated by a low initial number of bacteria ($<10^4$ CFU/g). The initial mesophilic bacteria counts in green mussel varied with treatments. Samples pretreated with 0.2 M lactic acid had the lowest count (2.36 log CFU/g), whereas the control contained 3.63 log CFU/g (Figure 1 (A)). This indicated that dipping green mussel in the different pretreatment solutions resulted in the reduction of the initial mesophilic bacteria counts differently. Mesophilic bacteria counts of all samples increased with increasing time at 4°C. Mesophilic bacteria counts of the control sample increased rapidly from an initial value of 10^3 to 10^8 CFU/g sample within 12 days and were generally higher than other treatments ($P<0.05$). Among all treatments, samples pretreated with 0.2 M lactic acid had the lowest mesophilic bacteria count, especially when kept for a longer time. Williams *et al.* (1995) found a significant decrease in bacterial counts, along with shelf-life extension of fresh catfish (*Ictalurus nebulosus*) filleted treated with sodium lactate during 8 days storage at 1°C. Lin and Chuang (2001) reported that lactic acid was more inhibitory toward microorganisms than acetic acid in pork loin chop. However, the maximal counts of samples dipped in acetic and citric acids were typically below 10^6 CFU/g sample and remained at this level up to 27 days. Lower mesophilic bacterial counts of sample indicated that acids effectively inhibited the growth of

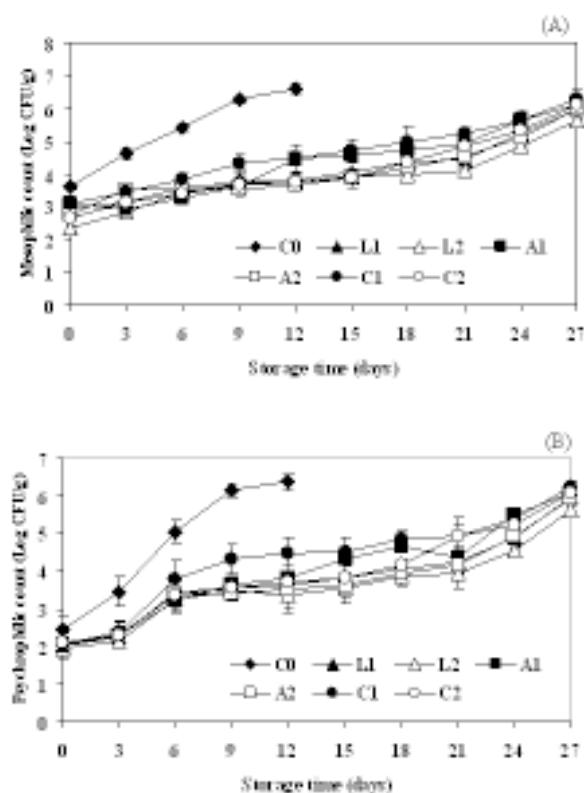


Figure 1. Changes in mesophilic (A) and psychrophilic (B) counts in green mussel dipped in different acids during storage at 4°C: control (◆), 0.1 M lactic (▲), 0.2 M lactic (△), 0.1 M acetic (■), 0.2 M acetic (□), 0.1 M citric (●) and 0.2 M citric (○) acids. Bars represent the standard deviation from triplicate determinations.

mesophilic bacteria. From the result, lactic acid was the most effective acid in inhibiting the microbial growth, followed by acetic acid, while citric acid was markedly less effective. The anti-microbial properties of acid are attributed to the undissociated molecule and to a reduction of pH below the level at which the growth of many bacteria is inhibited (Nykanen *et al.*, 1998). Undissociated weak acids possess the ability to cross membranes of microorganisms, become dissociated inside the cell and acidify the cell interior. The intracellular dissociation of acid results in denaturation of enzymes and disruption of

membrane (Freese *et al.*, 1973). From the result, mesophilic counts in samples pretreated with lactic acids were lower than in other samples. The results revealed that lactic acid inhibited the growth of bacteria effectively. The lower capacity of acetic and citric acids to enter bacteria cell is compensated by their greater capacity to dissociate inside the cell and thus acidify the cell cytoplasm (Young and Foegeding, 1993). In addition, acetic acid has been reported to dissipate, leading to the lower capacity (Samelis *et al.*, 2002). The higher bacterial count of mussel treated with citric acid might result from higher molecular size and the lower capacity of citric acids to enter bacteria cell (Ouattara *et al.*, 1997).

Higher counts of psychrophilic bacteria were also observed in samples without acid pretreatments, compared with those dipped in acids (Figure 1 (B)). However, psychrophilic bacterial counts in samples pretreated with 0.2 M lactic acid were lower than those of other samples. Our result was in agreement with Samelis *et al.* (2002) who reported that lactic acid is higher in effectiveness than acetic acid against inhibiting the growth of microorganisms. Fernandes *et al.* (1998) found that lactic, acetic and propionic acids reduced microfloral suspensions on the channel catfish. Organic acid interventions may reduce the number or alter the composition of background flora on fresh meat or other foods, thereby leading to potentially compromised safety of decontaminated food products.

Effect of acid pretreatment on chemical changes of green mussel during refrigerated storage

Changes in pH of mussel as affected by organic acid pretreatment during storage are presented in Figure 2. The initial pH of mussel sample was 6.2. For the samples pretreated with organic acids, a decrease in pH was observed. Among all organic acids used, sample pretreated with citric acid had a lower pH when compared with those pretreated with lactic and acetic acids. The pH of acid pretreated samples correlated with the pKa of the acids used (Farid *et al.*, 1998). During storage, pH of control sample increased

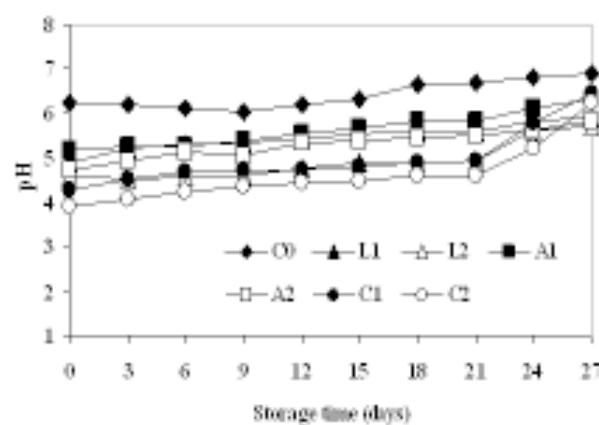


Figure 2. Changes in pH in green mussel dipped in different acids during storage at 4°C: control (◆), 0.1 M lactic (▲), 0.2 M lactic (△), 0.1 M acetic (■), 0.2 M acetic (□), 0.1 M citric (●) and 0.2 M citric (○) acids. Bars represent the standard deviation from triplicate determinations.

throughout the storage time ($P<0.05$), presumably due to the production of basic amines and metabolites by bacteria (Pastoriza *et al.*, 1996; Masniyom *et al.*, 2004). A sharp increase in pH was observed in the control sample up to 12 days and associated with the increase in mesophilic and psychrophilic bacterial counts (Figure 1). For samples pretreated with acids, the increased pH was much slower than that observed in the control. Therefore, the pH of mussel depended on the pK_a and the concentration of acids used.

Total volatile bases (TVB) and trimethylamine (TMA) contents of sample with and without acid pretreated are depicted in Figure 3 (A) and (B), respectively. Generally, the higher TVB content was found in control when compared with acid pretreated samples throughout storage ($P<0.05$). Samples pretreated with lactic acid showed the lowest TVB content, followed by those pretreated with acetic and citric acids. At the same concentrations used, citric acid was less effective than lactic and acetic acids. For the control, TVB content increased rapidly and reached 0.20 mg TVB/g after 9 days of storage. However, all samples dipped

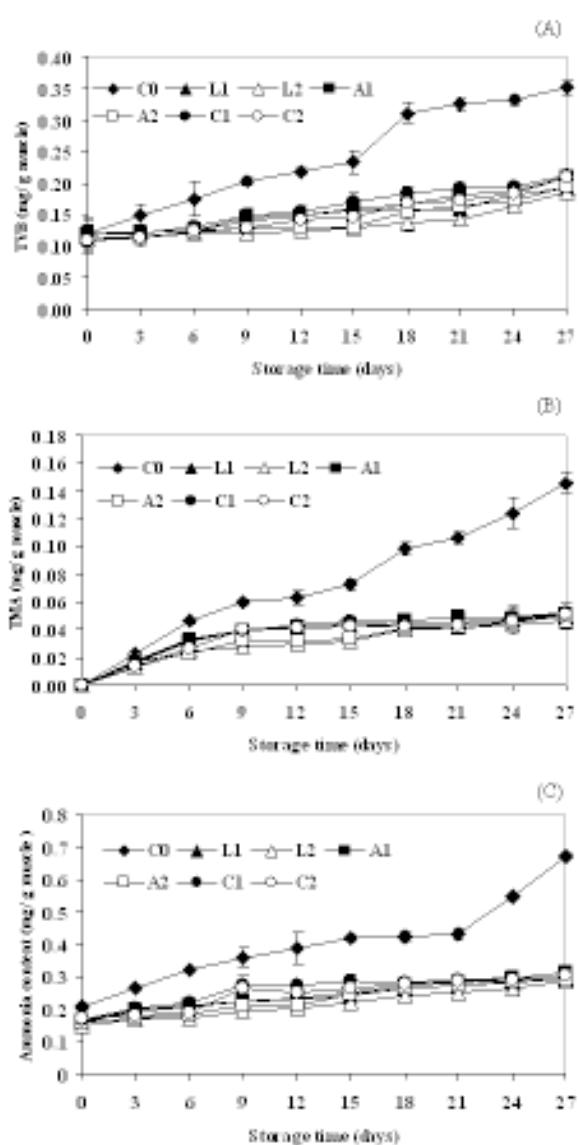


Figure 3. Changes in TVB (A), TMA (B) and ammonia (C) contents in green mussel dipped in different acids during storage at 4°C: control (◆), 0.1 M lactic (▲), 0.2 M lactic (△), 0.1 M acetic (■), 0.2 M acetic (□), 0.1 M citric (●) and 0.2 M citric (○) acids. Bars represent the standard deviation from triplicate determinations.

with acids had TVB contents less than 0.20 mg/g within 27 days of storage. TVB usually includes

trimethylamine and ammonia. TVB and TMA are products of bacterial spoilage and the content is often used as an index to assess the keeping quality and shelf-life of seafood products (Connell, 1990; Goulas *et al.*, 2005). TVB values of fresh and good quality fish are less than 0.12 mg/g. TVB values in the range 0.20-0.25 mg/g and above 0.25 mg/g indicate fish that are slightly decomposed/ edible and decomposed/ inedible, respectively (Lannelongue *et al.*, 1982). The result was in accordance with that of Goulas *et al.* (2005) who reported a TVB value of 0.25 mg/g for air packed mussel sample after 11 days of storage at 4°C.

TMA is produced by the reduction of trimethylamine oxide (TMA-O) by TMA-O reductase producing organisms and possibly through the action of intrinsic enzymes (Connell, 1990). It is also used as a quality indicator for fish. TMA concentration is normally used as a limit for acceptability (Adam and Moss, 1995). Masniyom *et al.* (2004) reported that seabass (*latus calcalifer*) was deteriorated when the TMA concentration exceeded 0.05 mg/g tissue. The highest concentration of TMA was observed for the control sample ($P<0.05$), followed by those treated with citric, acetic and lactic acids, respectively. When using the TMA concentration of 0.05 mg/g as a limit, the control sample was rejected after 9 days of storage. From the result, no marked differences in TVB and TMA contents of samples pretreated with different acids were observed. Since the reduction of TMAO to TMA is a property of Gram-negative microorganisms (Adams and Moss, 1995), the slow rate of TMA production in acid dipped samples was most likely due to an inhibition of aerobic, Gram-negative bacteria growth, including TMA-producing microorganisms, by acids. However, even after 27 days of storage, samples pretreated with acids had TMA less than 0.05 mg/g.

The ammonia content of mussel during storage is shown in Figure 3(C). The ammonia content in the control increased sharply after 6 days of storage ($P<0.05$), while it increased slightly in the samples with acids. In general, acids at higher concentrations led to the higher retardation of ammonia formation. After 9 days of storage,

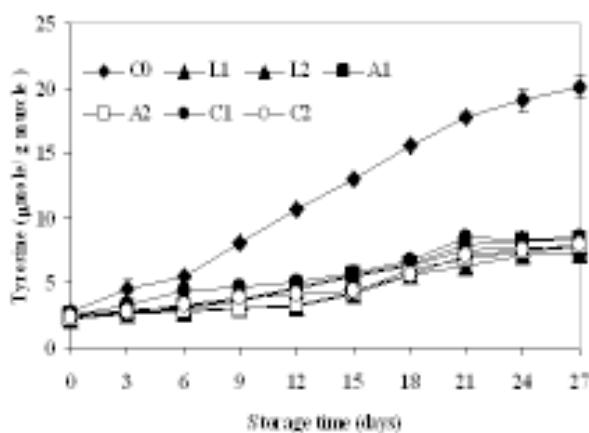


Figure 4. Changes in trichloroacetic acid soluble peptide contents in green mussel dipped in different acids during storage at 4°C: control (◆), 0.1 M lactic (▲), 0.2 M lactic (△), 0.1 M acetic (■), 0.2 M acetic (□), 0.1 M citric (●) and 0.2 M citric (○) acids. Bars represent the standard deviation from triplicate determinations.

ammonia content of the control reached 0.36 mg/g. This presumably resulted in the poor sensory quality of the control. Wang and Brown (1983) found that crayfish stored at 4°C began to have a bad odor when ammonia levels reached 0.2-0.3 mg/g, whereas fresh samples contained the ammonia at levels of 0.07-0.1 mg/g. The majority of ammonia formed in most stored fish originated from enzymatic deamination of free amino acids and from decomposition of nucleic bases (Huang *et al.*, 1992). Thus, an increase in ammonia reflects the decomposition of muscle.

A sharp increase in TCA-soluble peptides in the control sample was observed after 6 days of storage ($P<0.05$) (Figure 4). However, a slight increase in TCA-soluble peptides in the samples treated with acids were observed throughout the storage. From the results, TCA-soluble peptides of samples pretreated with lactic acids were slightly lower than those treated with acetic or citric acids at the same concentration throughout the storage. Degradation of muscle protein might be caused by either endogenous or microbial proteinases during

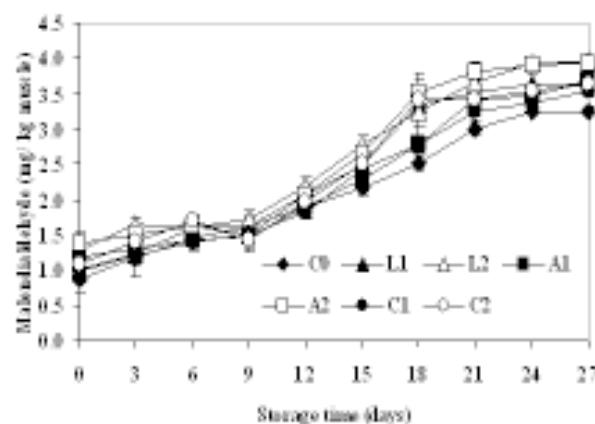


Figure 5. Changes in TBARS value (measured as malondialdehyde) in green mussel dipped in different acids during storage at 4°C: control (◆), 0.1 M lactic (▲), 0.2 M lactic (△), 0.1 M acetic (■), 0.2 M acetic (□), 0.1 M citric (●) and 0.2 M citric (○) acids. Bars represent the standard deviation from triplicate determinations.

refrigerated storage. TCA-soluble peptide has been used as the index for the protein degradation of fish muscle (Benjakul *et al.*, 1997). Venugopal *et al.* (1983) reported that protease from *Pseudomonas marinoglutinosa* hydrolyzed actomyosin at 0-2°C. Microorganisms responsible for fish spoilage were dominated by Gram-negative, such as *Pseudomonas*, *Shewanella* spp. (Hobbs, 1991). Organic acids used might retard the growth of microorganisms producing some proteolytic enzymes. As a consequence, a lower degradation was obtained as evidenced by the lower rate of TCA-soluble peptide formation. Therefore, acid pretreatment was shown to be the promising means to prevent the degradation of muscle proteins during prolonged storage.

TBARS values in mussel pretreated with acids are shown in Figure (5). The control sample showed the lower TBARS, compared with other samples, throughout the storage. Increase in TBARS was observed in all samples when the storage time increased ($P<0.05$), indicating the lipid oxidation. Lipid in muscle typically has a high

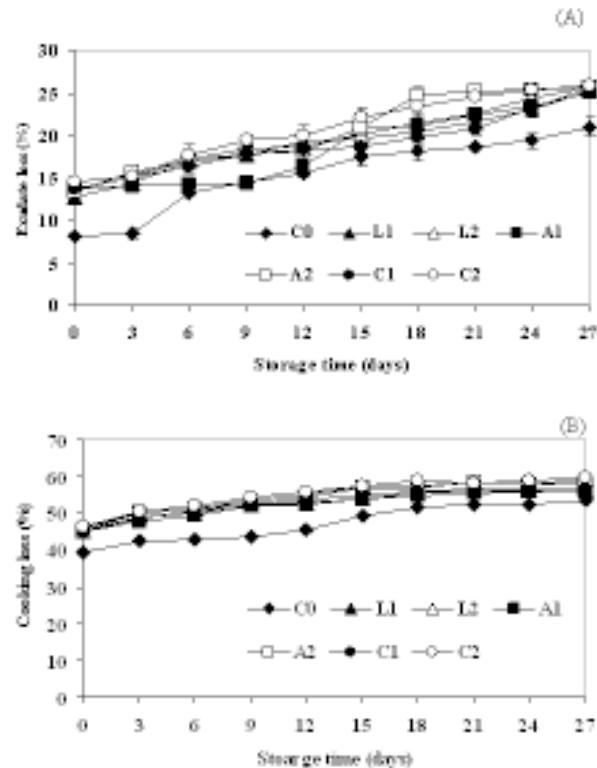


Figure 6. Changes in exudates loss (A) and cooking loss (B) in green mussel dipped in different acids during storage at 4°C: control (◆), 0.1 M lactic (▲), 0.2 M lactic (△), 0.1 M acetic (■), 0.2 M acetic (□), 0.1 M citric (●) and 0.2 M citric (○) acids. Bars represent the standard deviation from triplicate determinations.

percentage of polyunsaturated fatty acids and is consequently prone to oxidative reaction (Budge and Parrish, 2003). Moreover, the increase in TBARS values in all acid treated samples probably caused by the induced denaturation of muscle protein, leading to the releases of not only from heme iron, a potential pro-oxidant in muscle system. Samples pretreated with citric acid showed lower TBARS than those treated with lactic and acetic acids. Citric acid has been shown as antioxidant (chelating agent) (Khayat and Schwall, 1983). Moreover, the lower TBARS values of the control samples might result from the direct microbial

utilization of malonaldehyde and other TBARS or result from reactions between TBARS and the amine compounds produced by bacterial metabolism (Rhee *et al.*, 1997). In addition, the interaction between amines and malonaldehyde in muscle probably caused to form complexes with TBA, resulting in decreased TBARS (Kikugawa *et al.*, 1984.).

Effect of acid pretreatment on physical changes of green mussel during refrigerated storage

Exudate loss and cooking loss of samples pretreated with different acids are shown in Figure 6 (A) and 6 (B), respectively. An increase in exudate and cooking loss were observed in all samples when the storage time increased ($P < 0.05$). The exudate loss of mussel pretreated with citric acid was higher than that of samples pretreated with lactic and acetic acids. Generally, the higher the concentration of acids used, the greater exudates and cooking loss were found. This might be due to a greater loss of water holding capacity of the muscle protein at lower pH values (Stammen *et al.*, 1990). The exudates loss of muscle contributed to the lower acceptability due to the fewer taste constituents remained as well as the shrinkage of samples. Lin and Chuang (2001) reported that lactic and acetic acids were the effective agents in decreasing water holding capacity in pork loin chops. Thus, acid pretreatment which lowered the pH of mussel might cause the increased exudate levels and cooking loss. From the result, acid soaking resulted in the microbial inhibition, but it caused the inferior quality as evidenced by the increased free drip and cooking loss.

Effect of acid pretreatment on sensory property of green mussel during refrigerated storage

Fresh mussel was generally considered to possess very high acceptability. Samples pretreated with lactic acids exhibited the higher score for odor (Figure 7 (A)) and flavor (Figure 7 (B)), compared to the control and the samples pretreated with acetic and citric acids. The odor and flavor scores of the control sample decreased more rapidly than those of samples pretreated with all

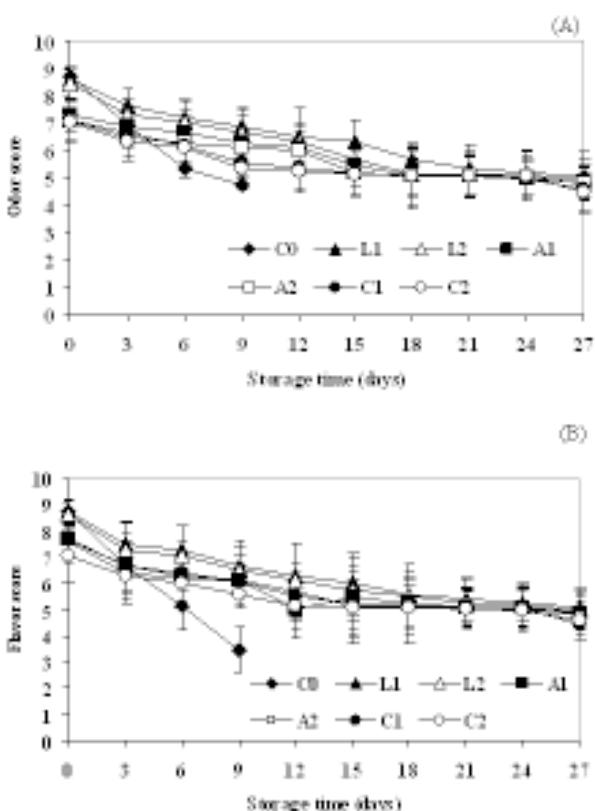


Figure 7. Changes in (A) odor and (B) flavor score in green mussel dipped in different acids during storage at 4°C: control (◆), 0.1 M lactic (▲), 0.2 M lactic (△), 0.1 M acetic (■), 0.2 M acetic (□), 0.1 M citric (●) and 0.2 M citric (○) acids. Bars represent the standard deviation from triplicate determinations.

acids during storage. Although the control sample had lower levels of exudate loss and TBARS than those of samples pretreated with acids, it gave rise to a faster loss of fresh mussel characteristics, along with deterioration of visual aspect of the mussel. From the result, samples were rejected after 6 days of storage but those pretreated with lactic acids could be accepted within 27 days of storage. The overall acceptability in odor and flavor of all samples decreased with increasing time. Generally, sensory evaluation is frequently applied in estimating the quality of seafood and correlated with microbiological and chemical analyses

(Karungi *et al.*, 2004). Our results indicated that soaking the mussel in acid solutions effectively extended the shelf-life of mussel with high acceptability. Therefore, it suggested that the selected organic acids might prevent bacteria growth, leading to the safety and prolonged shelf-life of mussel.

Conclusion

Appropriate organic acid with the optimal concentration may provide the cornerstone of sanitizing formulation or processing aids that could improve the microbiological quality and safety of mussel during refrigerated storage. Microbial growth in mussel treated with lactic acid 0.2 M was retarded, leading to the delayed spoilage, however, exudates loss still occurred.

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References

Adams, M.R. and Moss, M.O. 1995. Food Microbiology. pp. 156-251. The Royal Society of Chemistry, Cambridge.

Benjakul, S., Seymour, T.A., Morrissey, M.T. and An, H. 1997. Physicochemical changes in Pacific whiting muscle proteins during iced storage. *J. Food Sci.* 62: 729-733.

Budge, S.M. and Parrish, C.C. 2003. Fatty acid determination in cold water marine samples. *Lipids.* 38: 781-791.

Buege, J.A. and Aust, S.D. 1978. Microsomal lipid peroxidation. In S. Fleischer and L. Packer. *Methods in Enzymology* (pp. 302-310). New York: Academic Press.

Connell, J.J. 1990. Methods of assessing and selecting for quality. In *Control of Fish Quality* 3rd edn. Connell, J.J. pp. 122-150. Oxford, UK: Fishing New Books.

Conway, E.T. 1950. Microdiffusion and volumetric error. London: Crosby Lockwood and Son Ltd.

Cousin, M.A., Jay, J.M. and Vasavada, P.C. 1992. Psychrotrophic microorganism. In C. Vanderzand and D.F. Splitstoesser, *Compendium of Methods for the Microbiological Examination of Foods* (pp. 153-168). Washington, DC: American Public Health Association.

Dubal, Z.B., Paturkar, A.M., Waskar, V.S., Zende, R.J., Latha, C., Rawool, D.B. and Kadam, M.M. 2004. Effect of food grade organic acids on inoculated *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* in sheep/ goat meat storage at refrigeration temperature. *Meat Sci.* 66: 817-821.

Farid, M., Bal, A'A. and Marshall, D.L. 1998. Organic acid dipping of catfish fillets: effect on color, microbial load and *Listeria monocytogenes*. *J. Food Prot.* 61: 1470-1474.

Fernandes, C.F., Flick, G.J., Cohen, J. and Thomas, T. B. 1998. Role of organic acids during processing to improve quality of channel catfish fillets. *J. Food Prot.* 61: 495-498.

Freese, E., Sheu, C. W. and Galliers, E. 1973. Function of lipophilic acids as antimicrobial food additives. *Nature.* 241: 321-325.

Goulas, A.E., Chouliara, I., Nessi, E., Kontominas, M.G. and Savvaidis, I.N. 2005. Microbiological, biochemical and sensory assessment of mussels (*Mytilus galloprovincialis*) stored under modified atmosphere packaging. *J. Appl. Microbiol.* 98: 752-760.

Hobbs, G. 1991. Fish: microbiological spoilage and safety. *Food Sci. Technol. Today.* 5: 166-173.

Huang, Y., Kouadio, K. and Gates, K.W. 1992. Effect of packaging on chemical changes and quality of refrigerated spot (*Leiostomus xanthurus*). *J. Aqua. Food Prod. Technol.* 2: 111-127.

Jay, J.M. 1986. *Modern Food Microbiology*. 4th edn. New York: Van Nostrand Reinhold. p. 278.

Karungi, C., Byaruhanga, Y.B., and Muyonga, J.H. 2004. Effect of pre-icing duration on quality deterioration of iced Nile perch (*Lates niloticus*). *Food Chem.* 85: 13-17.

Khayat, A., and Schwall, D. 1983. Lipid oxidation in seafood. *Food Technol.* 37 (7): 130-140.

Kikugawa, K., Ido, Y. and Mikami, A. 1984. Studies on peroxidized lipids VI fluorescent products

derived from the reaction of primary amines, malondehyde and monofunctional aldehydes. J. American Oil Chem. Soc. 61: 1574-1581.

Kim, C.R.H., Hearnsberger, J.O., Vickery, A.R., White, C.H., and Marshall, D.L. 1995. Extending shelf life of refrigerated catfish fillets using sodium acetate and monopotassium phosphate. J. Food Prot. 58: 644-647.

Lannelongue, M., Finne, G., Hanna, M.D., Nickelson, F. and Vanderzant, C. 1982. Microbiological and chemical changes during storage of swordfish (*Xiphias gladius*) steaks in retail packages containing CO₂ enriched atmospheres. J. Food Prot. 45: 1197-1203.

Lin, K.W. and Chuang, C.H. 2001. Effectiveness of dipping with phosphate, lactate and acetic acid solutions on the quality and shelf life of pork loin chop. J. Food Sci. 66: 494-499.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 256-275.

Mailgaad, M., Civille, G.V. and Carr, B.T. 1999. Sensory evaluation techniques. Boca Raton, Florida: CRS Press.

Masniyom, P., Benjakul, S. and Visessanguan, W. 2004. Combination effect of pyrophosphate and modified atmosphere on quality and shelf-life extension of refrigerated seabass slices. Lebensm.-Wiss.u. Technol. 38: 745-756.

Meng, J. and Genigeorgis, C. A. 1994. Delaying toxinogenesis of *Clostridium botulinum* by sodium lactate in "sous vide" products. Lett. Appl. Technol. 19: 20-23.

Morrissey, M.T., Wu, J.W., Lin, D.D. and An, H. 1993. Protease inhibitor effects on torsion measurements and autolysis of Pacific whiting surimi. J. Food Sci. 58: 1050-1054.

Nakai, S.A. and Siebert, K.J. 2003. Validation of bacterial growth inhibition models based on molecular properties of organic acid. Int. J. Food Microbiol. 86: 249-255.

Nykanen, A., Lapretelainen, A., Hietanen, R-M and Kallio, H. 1998. The effect of lactic acid, nisin, whey permeate, sodium chloride and related combinations on aerobic plate count and the sensory characteristics of rainbow trout. Lebensm.-Wiss.u-Technol. 31: 286-290.

Ouattara, B., Simard, R.E., Holley, R.A., Piete, G.J.P. and Begin, A. 1997. Inhibitory effect of organic acids upon meat spoilage bacteria. J. Food Prot. 60: 246-253.

Parris, N and Foglia, T.A. 1983. Simplified alcoholic extraction procedure for ammonia in meat tissue. J. Agric. Food Chem. 31: 887-889.

Pastoriza, L., Sampedro, G., Herrera, J.J. and Cabo, M.L. 1996. Effect of modified atmosphere packaging on shelf life of iced fresh hake slices. J. Sci. Food Agric. 71: 541-547.

Prasai, R.K., Acuff, G.R., Lucia, L.M., Hale, D.S., Savell, J.W. and Morgan, J.B. 1991. Microbiological effects of acid decontamination of beef carcasses at various locations in processing. J. Food Prot. 54: 868-872.

Rhee, K.S., Krah, L.M., Lucia, L.M. and Acuff, G.R. 1997. Antioxidation/antimicrobial effect and TBARS in aerobically refrigerated beef as related to microbial growth. J. Food Sci. 62: 1205-1210.

Samelis, J., Sofos, J.N., Kendall, P.A. and Smith, G. 2002. Effect of acid adaptation on survival of *Escherichia coli* O157: H7 in meat decontamination washing fluids and potential effects of organic acid interventions on the microbial ecology of the meat plant environment. J. Food Prot. 65: 33-40.

Speck, M.L. 1976. Compendium of methods for the microbiological examination of foods. American Publish Health Association, Washington, D. C.

Stammen, K., Gerdes, D. and Caporaso, F. 1990. Modified atmosphere packaging of seafood. Food Sci. Nut. 29: 301-331.

Steel, R.G.D., and Torrie, J.H. 1980. Principle and Procedures of Statistic, 2nd edn, New York: McGraw-Hill.

Venugopal, V., Alur, M.P. and Lewis, N.F. 1983. Extracellular protease from *Pseudomonas marinoglutinosa*: some properties and its action on fish actomyosin. J. Food Sci. 48: 671-675.

Wang, M.Y. and Brown, W.D. 1983. Effects of elevated CO₂ atmosphere on storage of fresh water crayfish (*Pacifastacus leniusculus*). J. Food Sci. 48: 158-162.

Williams, S.K., Rodrick, G.E. and West, R.L. 1995. Sodium lactate affects shelf life and consumer acceptance of fresh catfish (*Ictalurus nebulosus, marmoratus*) fillets under simulated retail condition. *J. Food Sci.* 60: 636-639.

Young, K.M. and Foegding, P.M. 1993. Acetic, lactic and citric acids and pH inhibition of *Listeria monocytogenes* Scott A and the effect on intracellular pH. *J. Appl. Bacteriol.* 75: 515-520.