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Original Article

Extending the shelf-life of refrigerated green mussel (*Perna viridis*) under modified atmosphere packaging

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Abstract

The effect of modified atmosphere packaging (MAP) on the keeping quality of green mussel stored at 4°C was investigated. Inhibition of the bacterial growth increased proportionally to the CO_2 concentration in the packaging, and maximum inhibition was achieved with 100% CO_2. Mussel stored under CO_2-enriched atmosphere had lower total volatile base, trimethylamine and TCA-soluble peptide contents than those stored in air ($P<0.05$). However, the increase in exudate loss was observed for samples packaged in high-CO_2 atmosphere suggesting the denaturation of muscle proteins by carbonic acid formed. The odor and flavor acceptability of CO_2-enriched packaged samples, particularly with 80 and 100% CO_2, was accepted throughout the storage of 12 days, compared with six days for those stored in air. Therefore, MAP with 80% CO_2, 10% O_2 and 10% N_2 was chosen as the optimum condition for extending the shelf-life of green mussel. Packaging with inclusion of O_2 should be considered to avoid the outbreak of strictly anaerobic toxin producing bacteria.

Keywords: extending, shelf-life, mussel, modified atmosphere packaging

1. Introduction

Immediately after death, several biochemical and microbiological changes are triggered in fishery products, especially with improper handling. The degradation of muscle structure was considered to be caused by enzymes (Gomez-Guillen and Batista, 1997; Visessanguan *et al.,* 2001; Godiksen *et al.,* 2009). Apart from endogenous proteinases, several microorganisms growing on muscle secrete a wide variety of hydrolytic enzyme, particularly proteinases. Those changes along with microbially induced activity are involved in the degradation of mussel muscle (Venugopal, 1990; Ozogul *et al.,* 2004). Moreover, the mussel flesh containing high glycogen and free amino acids content is used nutrient sources for microbial growth (Adams and Moss, 1995). The growth of microorganisms results in the short shelf-life and the poor sensory quality for consumption. Therefore, some techniques should be applied to extend the shelf-life of mussel products and safety concern should also be taken into consideration.

Modified atmosphere packaging (MAP) can be used as a supplement in ice or refrigeration to delay spoilage and extend the shelf-life of fresh fishery products, while maintaining a high-quality end product by extending transit and storage time (Sivertsvik *et al.,* 2002; Arkoudelos *et al.,* 2007; Kostaki *et al.,* 2009). MAP under various levels of CO_2 was used in the 1930s to extend the shelf-life of fresh fishery products kept in barrier package. CO_2 has bacteriostatic and fungistatic properties retarding the growth of microorganism. The shelf-life increased as a result of lag phase extension of several aerobic spoilage bacteria and retardation of enzymatic spoilage (Pantazi *et al*., 2008). It has been reported that

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shelf-life of fishery products under CO_2 atmosphere storage could be extended (Masniyom *et al.,* 2004; Kyykidou *et al.,* 2009). Bacteria spoilage in refrigerated fishery products under aerobic storage condition is caused by Gram negative psychrotrophic microorganisms such as *Pseudomonas, Shewanella,* and *Enterobacterioaceae* (Pantazi *et al.*, 2008). The spoilage microbial flora is effectively inhibited by atmosphere enriched with 20% or higher carbon dioxide concentrations (Lopez-Galvez *et al.,* 1998). The microbial flora of pink shrimp (*Parapenaeus longirostris*) was inhibited in 40% CO_2 atmosphere when compared with air atmosphere (Lopez-Caballero *et al.,* 2002). The shelf-life of mussel (*Mytilus galloprovincialis*) packaged in 80% CO_2/20% N_2 atmosphere could be extended for more than 14 days at 4°C (Goulas *et al.,* 2005). Therefore, CO_2-enriched atmospheres have been increasingly used for the distribution and storage of seafood. However, little information regarding the microbial safety, chemical, and sensorial changes of green mussel (*Perna viridis*) during refrigerated storage has been reported. Additionally, the information concerning the shelf-life extension using MAP of green mussel cultured in Thailand as well as in South-East Asia is scarce. Thus, the objective of this study was to determine the effect of MAP storage on the shelf-life of green mussel by monitoring microbiological, chemical and sensorial changes throughout the refrigerated storage at 4°C.

2. Materials and Methods

2.1 Chemicals

Trichloroacetic acid was purchased from Honeywell Riedel-de Haen (Seelze, Germany). 2-thiobarbituric acid was obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). Malondialdehyde tetrabutylammonium was purchased from Fluka (Buchs, Switzerland). Folin-Ciocalteu's phenol and plate count agar were obtained from Merck (Darmstadt, Germany).

2.2 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 hrs after harvesting. Upon arrival, mussels were washed with tap water and the flesh meat was removed from the shells. Meat were placed on polystyrene trays and inserted in vacuum bag (15 cm × 25 cm) with gas permeability (O_2 transmission rate of 46.6 $cm^3m^{-2}day^{-1}$ at 38°C, 1 atm pressure) and packaged in modified atmosphere using a Henkovac type H 1502 (Netherlands). Different gas mixtures were used as follows: 40% CO_2, 10% O_2, 50% N_2 (M1); 60% CO_2, 10% O_2, 30% N_2 (M2); 80% CO_2, 10% O_2, 10% N_2 (M3); 100% CO_2, (M4). The meat kept in air (C) was used as the control. For all treatments, a meat/gas ratio of 1:2 (v/v) was used. All samples were stored at 4 °C for 15 days and were taken for microbiological, chemical, physical, and sensory analyses every three days.

2.3 Microbiological analyses

Green mussel samples (25 g) 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. Total viable counts were determined by plate count agar (PCA, Merck) with the incubation at 35°C for two days (Arashisar *et al.,* 2004). Lactic acid bacteria were counted in double-layer in man rogaso sharpe (MRS) agar and incubated at 35°C for three days according to the method of Ordonez *et al.* (2000). Microbial counts were expressed as log colony-forming unit (CFU)/g.

2.4 Chemical analyses

2.4.1 pH measurement

The pH measurements were 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.

2.4.2 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.

2.4.3 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.

2.4.4 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×*g* for 20 min (Sorvall, Newtown, CT, U.S.A.) 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.

2.5 Physical analyses

2.5.1 Determination of exudate loss

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

2.5.2 Determination of cooking loss

Cooking loss was determined as the water loss which was the percentage of weight loss in sample after steaming for 1 min, compared with the initial weight (Masniyom *et al*., 2007).

2.6 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-point hedonic scale (Mailgaad *et al.,* 1999): 1, extremely dislike; 2, dislike very much; 3, moderately dislike; 4, slightly dislike; 5, neither like nor dislike; 6, slightly like; 7, moderately like; 8, like very much; 9, like extremely. The evaluation of odor was carried out at the moment of opening the pack. For cooked samples, the samples were wrapped with aluminum 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-point hedonic scale.

2.7 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).

3. Results and Discussion

3.1 Effect of MAP 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 (4 log CFU/g). Total Viable Count (TVC) of all samples increased with increasing time of storage at 4°C ($P<0.05$) (Figure 1(A)). TVC of mussel stored in air (control) increased rapidly from an initial value of 4 to 7 log CFU/g within 15 days and was generally higher than those of mussels kept under CO_2-enriched atmospheres ($p<0.05$). In contrast with the control, viable counts of CO_2-enriched samples were typically below 7 log CFU/g and remained

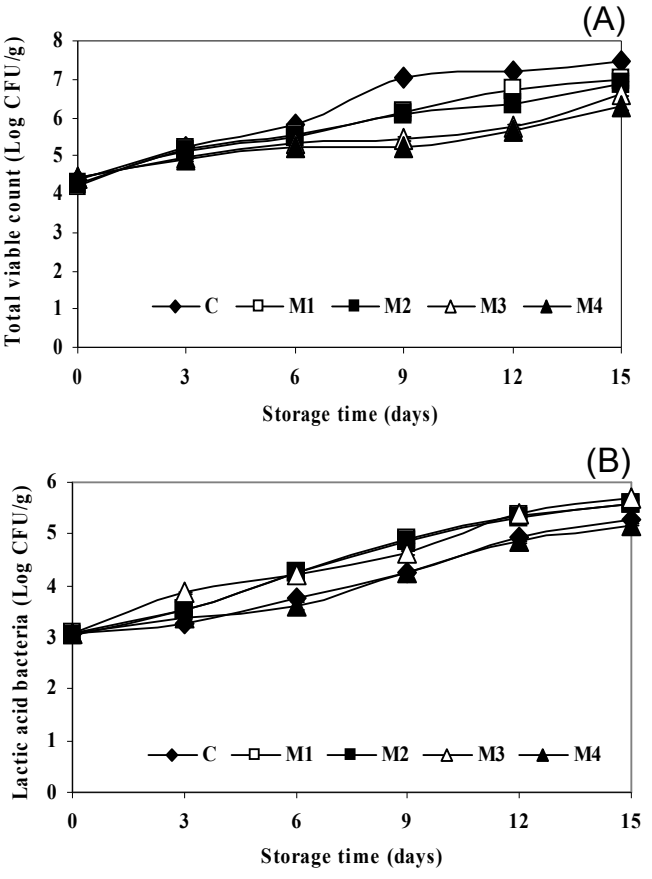

Figure 1. Changes in total viable count (A) and lactic acid bacteria count (B) of green mussel kept under different conditions during storage at 4°C: C = control (♦), M1 = 40% CO_2, 10%O_2, 50% N_2 (□); M2 = 60% CO_2, 10% O_2, 30% N_2 (■); M3 = 80% CO_2, 10% O_2, 10% N_2 (Δ); M4 = 100% CO_2, (▲). Bars represent the standard deviation from triplicate determinations.

constant throughout the storage time. ICMSF (1986) recommended the upper acceptability limit of TVC for fresh water fish and seafood at the value of 7 log CFU/g. The storage time of samples kept in air was estimated to be six days. However, the shelf-life of mussel was extended to 9 and 12 days when packed under 40-60% and 80-100% CO_2 atmosphere, respectively. Lower TVC of sample kept under MAP indicated that CO_2 at a concentration higher than 40% effectively inhibited the microbial growth. CO_2 commonly becomes more effective as antibacterial agent when its concentration is increased (Farber, 1991). It retards the microbial growth of spoilage bacteria, such as *Pseudomonas* spp. and *Shewanella* spp. Although, *Phosphobacterium phosphoreum* is more resistant to CO_2 but microbial growth is inhibited by higher CO_2 concentration (Sivertsvik *et al.,* 2002; Emborg *et al.,* 2005). Thus, CO_2-enriched atmosphere have been used in the preservation for fresh fishery products. This was probably because CO_2 enters into mass action equilibrium for enzymatic decarboxylation, leading to inhibition of the metabolic activity of microbial flora as a result of an extension in lag phase and a reduction in logarithmic phase of spoilage bacteria (Farber, 1991; Lalitha *et al.,* 2005; Pantazi *et al*., 2008). Our result was in agreement with Goulas *et al.* (2005) who reported that TVC was retarded when mussels (*Mytilus galloprovincialis*) were kept in 80% CO_2/20% N_2-enriched atmospheres. It has been reported that 60% CO_2/ 40% N_2 inhibited the microbial growth in sardines during refrigerated storage (Ozogul *et al.,* 2004), 80% CO_2/10% N_2/ 10% O_2 in sea bass slices (Masniyom *et al.,* 2004) and 100% CO_2 in spotted shrimp (*Pandalus platyceros*) (Layrisse and Matches, 1984). Our results showed that 80-100% CO_2-enriched atmospheres were effective in shelf-life extension for mussel. Moreover, Masniyom *et al.* (2008) reported that the pathogenic bacteria including *Vibrio parahaemolyticus*, *S. aureus* and *Salmonella* spp. were not found in the mussels from a farm in Pattani.

Generally, LAB became the dominant spoilage flora in MAP and vacuum packaged mussels (Banks *et al.*, 1980). LAB of all samples increased with increasing time of storage at 4°C ($P<0.05$) (Figure 1(B)). However, LAB counts in samples with 100% CO_2 atmosphere were lower than in other samples. The lower LAB in 100% CO_2 sample indicated that the high concentration of CO_2 not only inhibited the microbial growth of spoilage bacteria but also inhibited LAB. This finding was similar to the studies in chub mackerel under MAP of Stamatis and Arkoudelos (2007) and in hake steaks (Ordonez *et al.,* 2000). This presumed to be due to dissolution and proportional with the concentration of CO_2 into the product to inhibit bacterial growth (Gill and Penney, 1988). The growth of LAB was concomitant with the increase in lactic acid concentration. Lactic acid is known as a natural antimicrobial agent (Church and Parson, 1995). Generally, spoilage flora is replaced, probably to a large extent, by CO_2-resistance organisms, lactic acid bacteria and *Brochotrix thermophacta* (Stamatis and Arkoudelos, 2007).

3.2 Effect of MAP on chemical changes of green mussel during refrigerated storage

Changes in pH of mussel as affected by concentration of CO_2 during refrigerated storage are presented in Figure 2. The initial pH of mussel sample was 6.2. For the samples kept under MAP, a slight decrease in pH was observed. A slight decline in pH was observed in mussel kept under 80 and 100% CO_2 packing after six days of the storage. This may be due to some atmospheric dissolution in the liquid phase of the muscle tissue, which is associated with increased carbonic acid. Carbonic acid can produce an appreciable drop in pH. Changes in pH could depend on the amount of CO_2 dissolved and the buffering capacity of tissue. A decrease in surface pH has been studied with rockfish fillets kept in 80% CO_2 (Parkin *et al.,* 1981) and sea bream kept in 40% CO_2 (Goulas and Kontominas, 2007), while it was not observed in rainbow trout fillets stored in an atmosphere containing 40% CO_2 (Arashisar *et al*., 2004). The pH of mussel in air decreased to 5.9 during 15 days of storage, presumably owing to accumulation of lactic acid generated in anoxic condition from glycogen (Pacheco-Aguilar *et al.,* 2008). De Vido *et al.* (2001) found that postmortem pH in the adductor muscle of scallop was decreased after chilled storage. However, Riaz and Qadri (1985) reported that an increase in pH of lobster stored on ice was probably due to the production of alkaline metabolites from bacterial accumulation.

Total volatile base (TVB) contents of sample packed under MAP and air are shown in Figure 3 (A). Generally, the higher TVB content was found in control when compared with CO_2-enriched atmosphere samples throughout storage ($P< 0.05$). Samples stored under a higher content of CO_2 showed a lower TVB content in which the lowest TVB content was found in sample kept under 100% CO_2 atmosphere. For the samples kept in air, TVB increased rapidly and reached 0.25 mg/g after nine days of storage. However, samples kept under-enriched atmosphere, particularly with

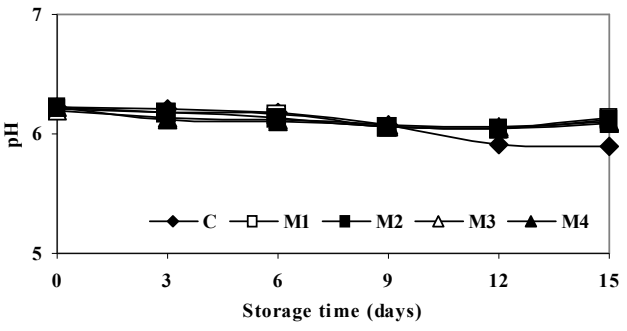

Figure 2. Changes in pH of green mussel kept under different conditions during storage at 4 °C: C=control (♦), M1=40% CO_2, 10% O_2, 50% N_2 (□); M2=60% CO_2, 10% O_2, 30% N_2 (■); M3=80% CO_2, 10% O_2, 10% N_2 (Δ); M4=100% CO_2, (▲). Bars represent the standard deviation from triplicate determinations.

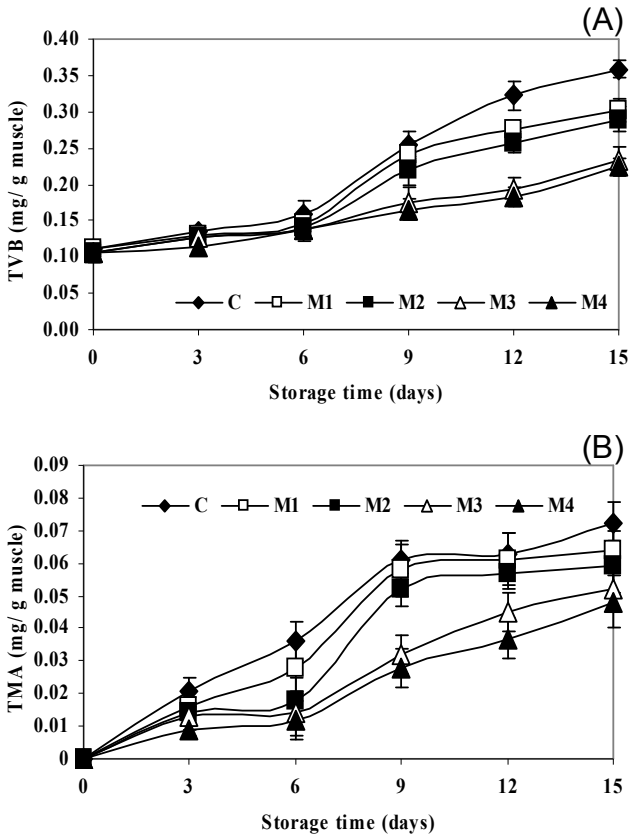

Figure 3. Changes in TVB (A) and TMA (B) contents of green mussel kept under different conditions during storage at 4°C: C = control (♦), M1 = 40% CO_2, 10% O_2, 50% N_2 (□); M2 = 60% CO_2, 10% O_2, 30% N_2 (■); M3 = 80% CO_2, 10% O_2, 10% N_2 (Δ); M4 = 100% CO_2, (▲). Bars represent the standard deviation from triplicate determinations.

80 and 100% CO_2 had TVB less than 0.20 mg/g within 12 days. For samples kept under 40 and 60% CO_2, TVB reached 0.20 mg/g after nine days of storage. TVB usually includes trimethylamine (TMA) and ammonia. TVB is produced by bacterial spoilage and the content is often used as an index to assess the keeping quality and shelf-life of seafood products (Riaz and Qadri, 1985; Connell, 1990). TVB values of fresh and good quality fish are less than 0.12 mg/g. Higher TVB values in the range of 0.20-25 mg/g and above 0.25 mg/g indicate that fish are slightly decomposed and inedible (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.

The TMA content of mussel during storage is shown in Figure 3(B). The TMA content of the control increased sharply after six days of storage ($P<0.05$), while it increased slightly in the samples kept under 80-100% CO_2 atmosphere. In general, MAP at higher CO_2 concentrations led to the higher retardation of TMA formation. According to Wang and Brown (1983), who observed the effect of MAP on TMA content of crayfish, TMA content in 80% CO_2/20% air was lower than that of air kept fish at the end of storage. In addition, a low TMA content was noticeable in rainbow trout under 100% CO_2 atmosphere (Arashisar *et al.,* 2004). After six days of storage, TMA content of the control reached 0.061 mg/g. Lannelongue *et al.* (1982) shown that a TMA content of 0.05 mg/g was the limit for acceptability of sword-fish. This presumably resulted in the poor sensory quality of the control. Since the reduction of TMAO to TMA is a property of Gram-negative microorganisms, the slow rate of TVB and TMA production in MAP stored samples was most likely due to an inhibition of aerobic, Gram-negative bacteria growth, including TVB and TMA producing microorganisms, by CO_2-enriched atmosphere (Ruiz-Capillas and Moral, 2001).

A sharp increase in TCA-soluble peptide content in the control sample was observed after six days of storage ($P<0.05$) (Figure 4). However, a slight increase in TCA-soluble peptide content in the samples stored under 80-100% CO_2 were observed throughout the storage. From the results, TCA-soluble peptide contents of samples kept under 100% CO_2 were slightly lower than those stored under other conditions when the storage time increased. Degradation of muscle protein might be caused by either endogenous or microbial proteinases during refrigerated storage. TCA-soluble peptide content 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). CO_2-enriched packaging used might

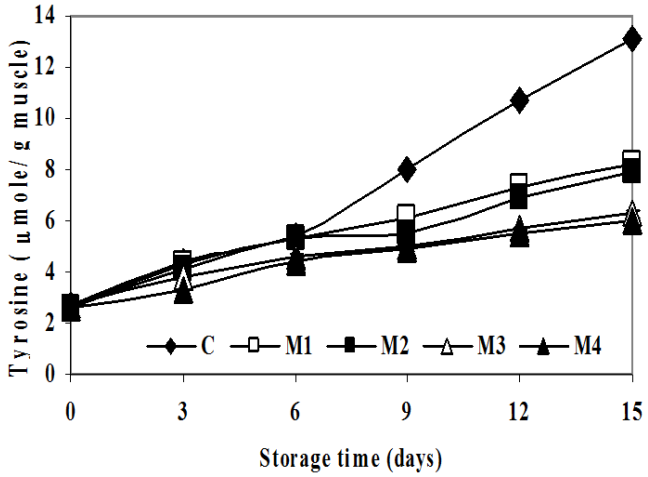

Figure 4. Changes in trichloroacetic acid soluble peptide contents of green mussel kept under different conditions during storage at 4°C: C = control (♦), M1 = 40% CO_2, 10% O_2, 50% N_2 (□); M2 = 60% CO_2, 10% O_2, 30% N_2 (■); M3 = 80% CO_2, 10% O_2, 10% N_2 (Δ); M4 = 100% CO_2, (▲). Bars represent the standard deviation from triplicate determinations.

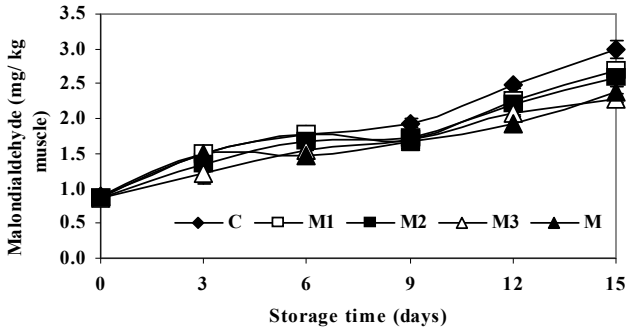

Figure 5. Changes in TBARS value (measured as malondialdehyde) of green mussel kept under different conditions during storage at 4°C: C = control (♦), M1 = 40% CO_2, 10% O_2, 50% N_2 (□); M2 = 60% CO_2, 10% O_2, 30%N_2 (■); M3 = 80% CO_2, 10% O_2, 10% N_2 (Δ); M4 = 100% CO_2, (▲). Bars represent the standard deviation from triplicate determinations.

delay 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, a high CO_2 concentration was shown to be the promising means to prevent the degradation of muscle proteins during prolonged storage.

The TBARS value is a measure of malondialdehyde, one of the degradation products of lipid hydroperoxide, from polyunsaturated fatty acid (Goulas and Kontominas, 2007). TBARS values of mussel stored under MAP and air are shown in Figure 5. The CO_2-enriched sample showed the higher TBARS, compared with control samples, throughout the storage ($P<0.05$). Samples kept under 80-100% CO_2 showed higher TBARS value than the others but this was not caused the off-odor and off-flavor development. The results indicated that a high content CO_2 caused the lipid oxidation. Sinnhuber and Yu (1958) reported that TBARS values of 4-7 mg malondialdehyde/kg indicated poor quality fish. Samples stored under 80 and 100% CO_2 atmosphere had TBARS values of less than 4 mg malondialdehyde/kg. This result is in agreement with the observation by Pastoriza *et al.* (1996) in hake slices under MAP. It was postulated that the carbonic acid formed in the sample kept under MAP may induce the denaturation of muscle protein at the surface, leading to the release of free haem iron, a potential prooxidant in the muscle system. Moreover, an increase in TBARS was observed in all samples when the storage time increased ($P<0.05$), indicating the marked lipid oxidation. Lipid in seafood typically has a high percentage of polyunsaturated fatty acids and is consequently prone to oxidative reaction (Budge and Parrish, 2003). From the result, lower TBARS values of the control samples might result from the direct microbial utilization of malonaldehyde and other TBARS or might result from reactions between TBARS and the amine compounds produced by bacterial metabolism via maillard reaction (Rhee *et al.,* 1997). In addition, the interaction between amines and malondehyde in muscle probably caused to form complexes with TBA, resulting in decreased TBARS (Kikugawa *et al.,* 1984). Thus, CO_2-enriched packaging effectively inhibited the spoilage caused by microbial flora, but it could not prevent the lipid oxidation.

3.3 Effect of MAP on physical changes of green mussel during refrigerated storage

Exudate loss and cooking loss of samples kept with different conditions are shown in Figure 6 (A) and 6 (B), respectively. Increase in exudate and cooking losses were observed in all samples when the storage time increased ($P<0.05$). The exudate and cooking loss of mussel kept under 100% CO_2 was found to be the highest. Generally, the higher the concentration of CO_2 used, the greater the exudate and cooking loss was 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). Moreover, the pI of muscle protein in fish is between 5.7 and 6.0 and depends on the species, season, and type of muscle (Toom *et al.,* 1982). The

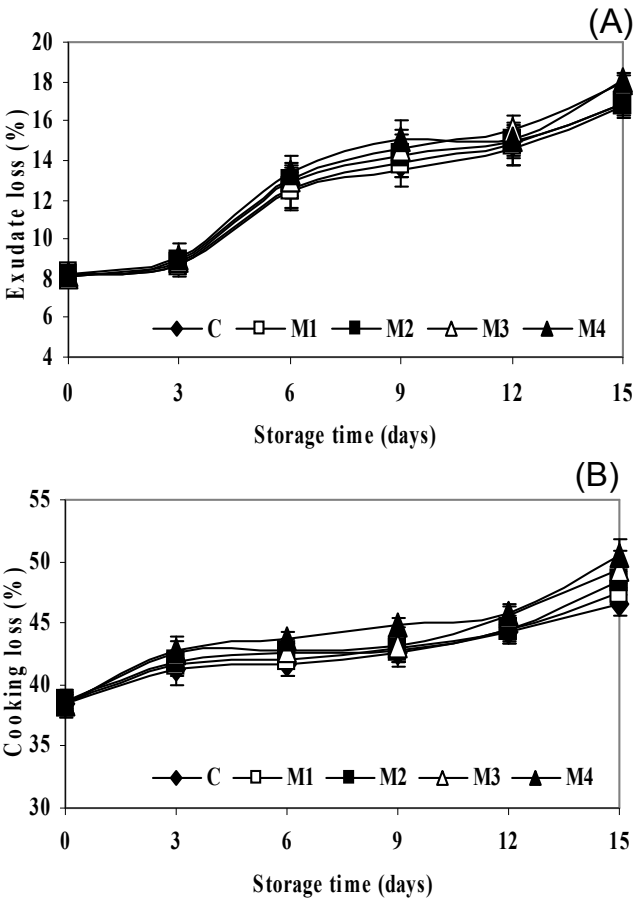

Figure 6. Changes in exudate loss (A) and cooking loss (B) of green mussel kept under different conditions during storage at 4°C: C = control (♦), M1 = 40% CO_2, 10% O_2, 50% N_2 (□); M2 = 60% CO_2, 10% O_2, 30% N_2 (■); M3 = 80% CO_2, 10% O_2, 10% N_2 (Δ); M4 = 100% CO_2, (▲). Bars represent the standard deviation from triplicate determinations.

pH changes of mussel under MAP caused the induced denaturation of muscle protein, leading to the increase of exudates and cooking loss. The exudates loss of muscle contributed to the lower acceptability due to the fewer taste constituents remained as well as the shrinkage of samples. Our result was in agreement with Layrisse and Matches (1984) who reported that shrimp stored under 100% CO_2 had an increased weight loss, when kept for a longer time. Thus, a high CO_2 concentration caused higher exudate and cooking loss. The results show, that keeping mussels under a high concentration of CO_2 resulted in the microbial inhibition but it caused the inferior quality as evidenced by the increased free drip and cooking loss.

3.4 Effect of MAP on sensory property of green mussel during refrigerated storage

Fresh mussels were generally considered to possess very high acceptability. The odor and flavor scores of the control sample decreased more rapidly than those of samples kept under CO_2-enriched atmospheres during storage (Figure 7 (A) and (B)). Although the control sample had lower levels of exudate loss and TBARS than those of samples kept under MAP, 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 six days of storage but those kept under 80 and 100% CO_2 could be accepted within 12 days of storage. While under 40-60% CO_2 packing, samples were rejected after nine days of storage. The overall acceptability in odor and flavor of all samples decreased with increasing storage 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 keeping the mussel under 80% CO_2-enriched atmospheres effectively extended the shelf-life of mussel with a high acceptability. However, it caused some changes in weight loss and lipid oxidation of mussel. Therefore, it suggested that the use of MAP might prevent bacteria growth and maintain overall quality, leading to the safety and a prolonged shelf-life of mussel.

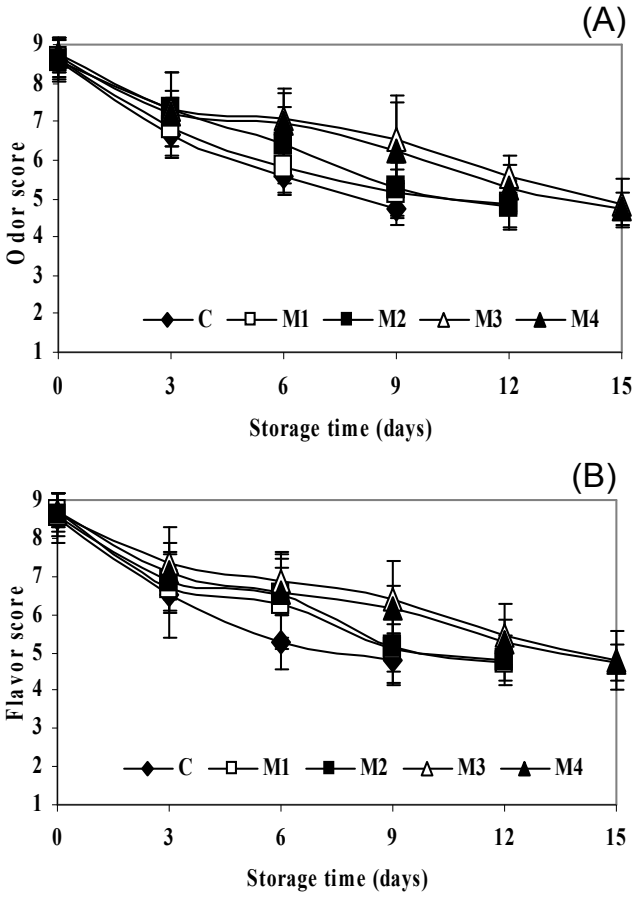

Figure 7. Changes in odor (A) and flavor (B) score of green mussel kept under different conditions during storage at 4 °C: C=control (♦), M1=40% CO_2, 10% O_2, 50% N_2 (□); M2=60% CO_2, 10% O_2, 30% N_2 (■); M3=80% CO_2, 10% O_2, 10% N_2 (D); M4=100% CO_2, (▲). Bars represent the standard deviation from fifteen determinations.

4. Conclusion

CO_2-enriched atmosphere packing has a potential shelf-life extension of refrigerated mussel. Appropriate MAP with the optimal concentration may provide the cornerstone of processing aids that could improve the microbiological and biochemical quality during storage. Microbial growth in mussel stored under 80% CO_2 was retarded, leading to the delayed spoilage, however, exudate loss still occurred. Thus, MAP with 80% CO_2, 10% O_2 and 10% N_2 could be the most desirable concentration to use for modified atmosphere storage of green mussel by maintaining their odor and flavor attributes.

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