Microbiological and Physical Assessment of Green Mussel (*Perna viridis*) Coated with Green Tea and Ascorbic Acid Stored under Modified Atmosphere Packaging

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ABSTRACT

This research aims to study the effect of modified atmosphere packaging (MAP) on the shelf life of cooked green mussel (*Perna viridis*) coated with green tea and ascorbic acid. The samples were packaged in air for the control sample (C000) and under different MAP conditions; 40%CO_2: 30%N_2: 30%O_2 (M433), 50%CO_2: 50%N_2 (M550) and 60%CO_2: 20%N_2: 20%O_2 (M622) stored at 4 ± 1 ˚C for 28 days. The results showed there were some significant declines in the microbiological and physical quality for all treatments with increasing storage time. M622 had the lowest total viable count (TVC) when compared with the other MAP treatments. Based on the total viable count (TVC) in all samples, the shelf life for M622 was more than 28 days, M550 and M433 were 24 days, while C000 was 22 days, respectively. Thus the composition of gas in the packages had an effect in retarding the autolytic and bacteria spoilage degradation of proteins. The high temperatures in the production process of cooked mussel could destroy the pathogens (Coliform bacteria, *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella* spp. and *Bacillus cereus*). The results indicated that pathogens were not found for all samples of cooked green mussel throughout storage.

Keywords: Microbiological assessment; Physical assessment; Modified atmosphere packaging; Green mussel; Green tea; Ascorbic acid

Introduction

Green mussel (*Perna viridis*) is a bivalve belonging to the family Mitilidae, found in Asia and has an important socioeconomic position in Thailand. The mussel is widely consumed, important economically and has a good nutritional value. In 100 g of mussel meat the nutritional value includes; protein 11.90 g, carbohydrate 3.69 g, fat 2.24 g, fatty acid

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0.507 g, minerals and vitamins (A6, B6, B12, E etc.) [1]. It is a filtration feeder and collects microbes. After mussel death microbiological activity and autolytic processes cause degradation of protein resulting in tissue softening impacting adversely on consumer acceptability [2]. A limitation for commercial products is its short shelf life of 2-3 days for raw mussels and 6-7 days for cooked products.

Therefore, improving the processing and storage in order to increase the shelf life of green mussel is very important. Researches have shown that storage of seafood under modified atmosphere alone or with added antioxidant and antimicrobial compounds can extend the shelf life of fresh Norway lobster (*Nephrops norvegicus*) [3], refrigerated wild mussel (*Mytilus galloprovincialis*) [4], fresh abalone (*Haliotis asinine*) [5] and smoked oyster (*Saccostrea cucullata*) [6]. Modified atmosphere packaging (MAP) involves storing products under different compositions of gases [7]. The gases in MAP can include carbon dioxide, nitrogen and oxygen. Therefore, adjustment of the ratio of the gases can retard microbial growth, protein degradation and prevent the collapse, deformation and fracture of the container [7]. MAP alone can retain the nutritional and sensorial quality of food but it also can prolong shelf life when combined with natural food additives. The natural food additives green tea and vitamin C are good alternative additives because of their familiar flavors and well known antioxidant and antimicrobial properties.

Green tea extracts contain polyphenolic compounds which have antioxidant, antimicrobial, anticarcinogenic and antimutagenic activities [8] to prolong shelf life of raw large yellow croaker (*Pseudosciaena crocea*) [9], refrigerated black sea bream (*Sparus macrocephalus*) [10] and fresh Pacific white shrimp (*Litopenaeus vannamei*) [11]. Ascorbic acid (vitamin C), can increase the shelf life of Cobia fillets (*Rachycentron canadum*) [12] and Newfoundland blue mussel (*Mytilus edulis*) [13]. Therefore, in this study effectiveness of various MAP with green tea and vitamin C coatings on cooked green mussel was tested for microbiological and physical quality. The aim of this study is to prolong the shelf life of cooked green mussel coated with green tea and vitamin C using MAP with different ratios of gases in the packages during refrigerated storage of up to 28 days.

Materials and Methods

Mussel sample preparation

Green mussels of commercial size (average 25-28 mussel/kg) were obtained from a local farm in Ang-Sila, Chonburi, Thailand and transported in styrofoam boxes with ice (mussel to ice ratio 2:3) to the laboratory. The samples were washed with tap water and heated at 95 ± 2 ˚C for 5 mins. Each mussel was manually shucked using a sterile knife. The meat was dipped in 0.002% alginate–based coating incorporating 2.5% green tea and 1.25% vitamin C (v/v) [14] and packaged in plastic laminated bags (polyvinylidene chloride polyamide and cast polypropylene (PVDC/PA/CPP) center seal, size 180 x 30 x 250 mm, thickness 20/40 µm, water vapor permeability = 4g/m^2*24h, oxygen permeability = 10 cc/m^2*24 h at 20-25˚C) (S. Science Chemical Ltd., Thailand) (20 mussels/bags). Four compositions of gas mixtures were made up: C000 (air packaged), M433 (40%CO_2: 30%N_2: 30%O_2), M550 (50%CO_2: 50%N_2) and M622 (60%CO_2: 20%N_2: 20%O_2), using a gas mixer (MAP Mix 9001-3/200B, Denmark), sealed (UV 4350, Ultra Vac, Thailand) and stored at 4 ± 1 ˚C.

Microbiological analysis

A sample (25 g) was placed in a stomacher bag (LDPE granule extruded to 65 µm film thickness) (Seward Medical, UK) containing 225 mL of 0.85% sodium chloride solution [15] and mixed in a stomacher blender (Seward Medical, UK). The total viable counts (TVC) were determined by the pour plate method, using standard plate count agar (PCA) after incubation for 48 h at 35 ˚C [15] as follows: Coliform & *E. coli* using a 3M

petrifilm^{TM} count plate after incubation for 48 h and 24 h at 35 ˚C [16]; *C. perfringens* using tryptose sulfite egg-yolk cycloserine agar (TSC) by the streak plate method after incubation for 24 h at 35 ˚C [17]; *S. aureus* using Baird parker medium (BPA) by the spread plate method and incubation for 48 h at 35 ˚C [18]; *V. parahaemolyticus* by adding 225 mL of alkaline peptone water and incubation for 6-8 h at 35 ˚C, using Thiosulfate -Citrate-Sucrose Agar (TCBS Agar) by the streak plate method (2 plates) and incubation for 18-24 h at 35 ˚C followed by streak plate on TCBS Agar and incubation for 24 h at 35 ˚C [17]; *Samonella* spp. using lactose broth after incubation for 24 h at 35 ˚C [18]; and *B. cereus* using mannitol egg-yolk phenol red poly myxin agar (MYP) by the streak plate method after incubation for 24 h at 35 ˚C [18]. Analysis of TVC was performed every 2 days and pathogenic bacteria (*E. coli* and Coliform, *C. perfringens*, *S. aureus*, *V. parahaemolyticus*, *Salmonella* spp. and *B. cereus*) on day 0.

Physical analysis

A 5.0 g sample of the sample was thoroughly homogenized with 50 mL of distilled water and the homogenate was used to measure the pH value using a digital pH meter (713 pH Meter, Metrohm, Switzerland) at ambient temperature. The color of cooked male and female green mussel was measured using a colorimeter (CM 3500d, Konica Minolta, Japan) and reported in CIE system color profile of L* (lightness), a*(redness/ greenness) and b* (yellowness/blueness). Before analysis, the colorimeter was standardized at the measurement port with black glass tile and then with white glass tile [19]. For each sample, the color was measured at three positions on the center part of a cooked green mussel. Following color analysis, plugs were positioned in the center part of the cooked green mussel on the calibrated texture analyzer platform (TA-XT2i, Stable Micro Systems Ltd., Surrey, England). Warner–Bratzler shear force (WBSF) values were cut on the center part of cooked green mussel. The peak shear-force of each sample (n=3) was measured using a texture analyzer (Zwick, model BZ2.5/TH1S, Germany) fitted with a Warner-Bratzler V-Bladeshear cell, using a pre-test speed of 2.00 mm/s, a post-test speed of 2.50 mm/s test speed of 2.00 mm/s. [20], and mean values were expressed in terms of peak force (Newtons per gram of muscle at the point of maximum load before sample breaking).

Statistical analysis

The software package IBM SPSS statistics was used for statistical analysis. All experiments were replicated 3 times on different samples and analysis of variance (ANOVA) was performed and mean comparisons by Duncan's multiple range test. *P* values less than 0.05 were considered statistically significant. The results are reported as mean values ± standard deviation (S.D.).

Results and Discussion

Changes in microbiological quality

Total viable counts (TVC)

Fig.1 shows the TVC of cooked mussel stored under MAP in a refrigerator. In the first stage (day 0-10), the TVC of all samples were almost constant (3.02-3.45 log CFU/g). The TVC of samples progressively increased when the CO_2 concentration decreased as follows: C000 after 10 days, M433 after 12 days, M550 after 20 days and M622 after 22 days. The effect of CO_2 on aerobic bacteria, anti-microbial property of ascorbic acid and polyphenolic compounds in green tea were against microbial growth [21]. TVC of cooked mussel stored under MAP containing CO_2 (M433, M550 and M622) was less when compared to those stored in the air (C000) ($p < 0.05$).

According to the TVCs on day 14 to day 20, the TVCs of M622 and M550 were significantly different from M433 and C000 ($p < 0.05$) and the TVCs of M622 and M550 were different ($p > 0.05$). M622 and M550 could maintain TVC for 20 days. In the storage time range day 22-28 (Fig.1). The rate of TVC increase showed a significant increase as the

CO_2 concentration decreased. Thus M622 had the lowest TVC when compared with the other MAP treatments ($p < 0.05$). These results demonstrated that CO_2 higher concentrations in packages increased the antibacterial compounds. It is likely that O_2 and N_2 in these packages do not affect microbial growth since, although O_2 is important for aerobic bacteria, these MAP treatments would involve mainly effect by CO_2. N_2 is used to replace oxygen in packaging to slow spoilage of food and protect shrinkage of the package. Similar results for increased TVC were reported on fresh oyster (*Saccostrea cucullata*) [22]. These was also agreement that 60%CO_2 retards the shelf life of raw sea bass (*Dicentrarchus labrax*) [23]. TVC reached a value of 6 log CFU/g, considered the upper microbiological limit for good quality cooked seafood [24], on day 22 for C000, day 24 for both M433 and M550 and day 28 for M622.

Pathogens

Pathogens (Coliform bacteria, *Escherichia coli*, *Clostridium perfringens*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella* spp. and *Bacillus cereus*) were not found throughout storage. This could be due to high temperatures in the production process that destroy the microbe metabolic processes [2].

Similar results were reported in dipping shrimp (*Penaeus indicus*) in boiling water for 3 mins which caused microbial activity to decrease to safe level [25] and similar results were not found for the microbial activity in beef, after boiling for 20 min [26].

Changes in physical quality

pH value

pH could be an indicator for spoilage of mussels due to autolytic and microbiological processes causing degradation of proteins and deamination of non-protein nitrogen compounds [22]. Fig.2 shows the pH of all samples with increasing storage time.

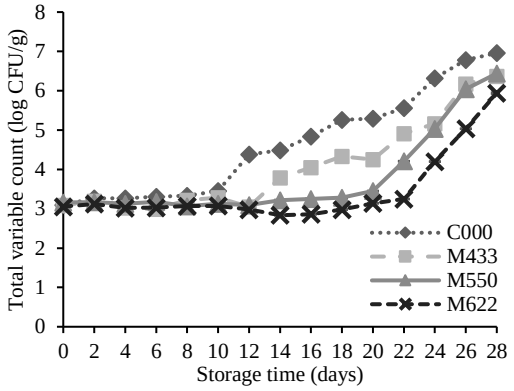


Fig. 1. The effect of modified atmosphere packaging on total viable counts (TVC) of cooked mussel coated with green tea and vitamin C during refrigerated storage at 4 ± 1 °C: C000 (air packaged; ◆), M433 (40%CO_2: 30%N_2: 30%O_2; ■), M550 (50%CO_2: 50%N_2; ▲) and M622 (60%CO_2: 20%N_2: 20%O_2; ×). Bars represent the standard deviation (n = 3) but are smaller than the symbols.

The cooked mussel in MAP (M433, M550 and M622) had a significantly lower pH than sample C000 ($p < 0.05$). This could be due to CO_2 which dissolves to become carbonic acid. Microbes are not resistant to acid environments. The dissolving of CO_2 could retard the production of microbial degradation products. Similar pH increases were reported in fresh chub mackerel (*Scomber colias japonicus*) stored under MAP [27].

During storage, M433 had the lowest pH of all other MAPs ($p < 0.05$).

Although high amounts of CO_2 inhibited microbial growth, CO_2 negatively affected sensory aspects of consumed mussels. Cooked mussel in these packages would have an acidic smell and sour flavor. Therefore, M550 was the best of the treatments since 50 - 60% CO_2 inhibits microbial activity and was more consumer acceptable.

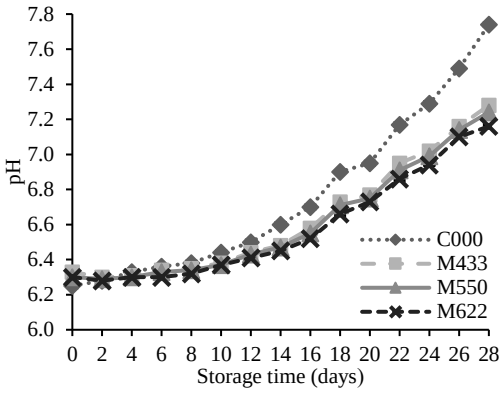


Fig. 2. The effect of modified atmosphere packaging on pH value of cooked mussel coated with green tea and vitamin C during refrigerated storage at 4 ± 1 °C: C000 (air packaged; ◆), M433 (40%CO_2: 30%N_2: 30%O_2; ■), M550 (50%CO_2: 50%N_2; ▲) and M622 (60%CO_2: 20%N_2: 20%O_2; ×). Bars represent the standard deviation (n = 3).

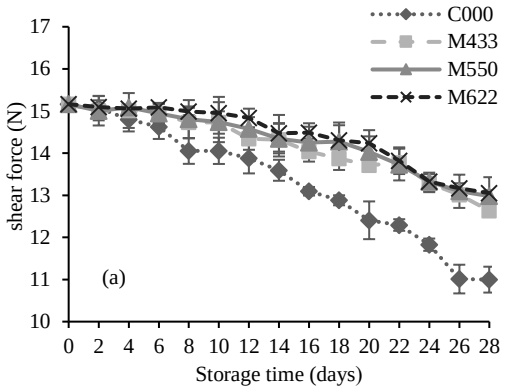
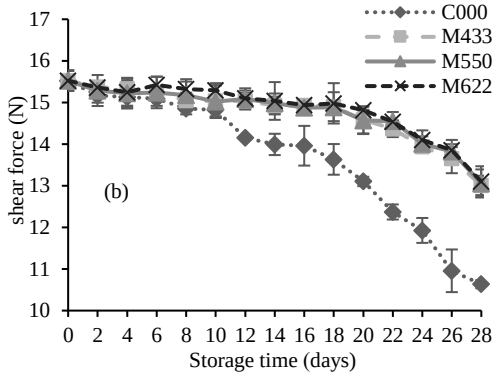


Fig. 3. The effect on the shear force of modified atmosphere packaging on (a) male and (b) female of cooked mussel coated with green tea and vitamin C during refrigerated storage at 4 ± 1 °C: C000 (air packaged; ◆), M433 (40%CO_2: 30%N_2: 30%O_2; ■), M550.



(50%CO_2: 50%N_2; ▲) and M622 (60%CO_2: 20%N_2: 20%O_2; ×). Bars represent the standard deviation (n = 3).

Shear force

Shear force is an important physical property for consumer acceptability. Fig.3 shows the shear force value of cooked mussel under different packaging with increasing storage time. On day 0 the initial shear force of all samples was 15.16 ± 0.17 N for males and 15.52 ± 0.24 N for females. After day 20 the shear force showed a significant decrease in samples of MAP (M433, M550, M622) compared to the air package (C000) storage ($p < 0.05$) because the antimicrobial action can slow down the protein degradation of autolytic processes. Protein denaturation causes autolytic and bacteria spoilage by degradation of myofibrillar protein resulting in the softening of muscle. MAP may retard protein denaturation in muscle tissue in mussels. Similar results were found on shucked fresh oyster (*Saccostrea cucullata*) in MAP where storage decreased shear force [22]. Also, the blanched cockles (*Anadara granosa*) under MAP showed lower shear force than the control package [28].

Color

Fig. 4, 5, 6 shows the color values (L*, a* and b*) of cooked male and female green mussel under MAP. It was shown the color values in all samples decreased with increasing storage time. The values of freshly cooked mussel were L* (brightness) 31.3 ± 0.9 (male) and 31.9 ± 0.6 (female), a* (redness) 3.8 ± 0.5 (male) and 18.1 ± 0.5 (female), b* (yellowness) 21.5 ± 0.4 (male) and 26.0 ± 0.3 (female).

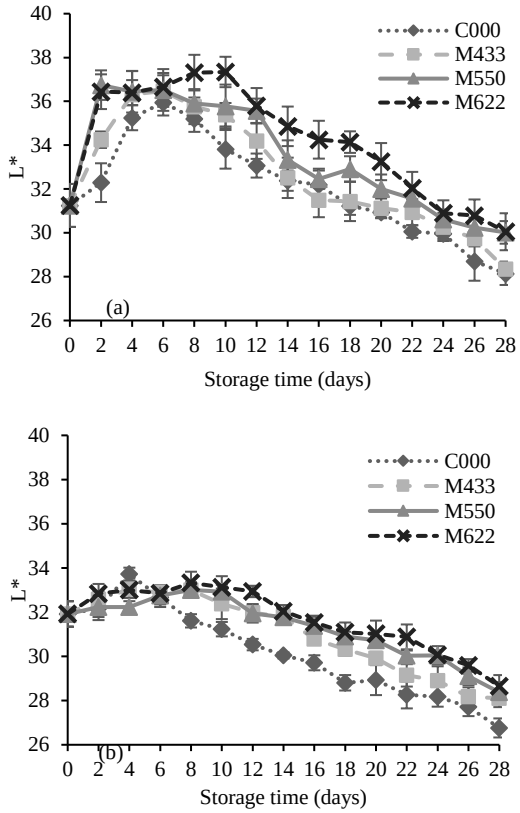


Fig. 4. The effect of modified atmosphere packaging on (a) L* of male, (b) L* of female, cooked mussel coated with green tea and vitamin C during refrigerated storage at 4 ± 1 °C: C000 (air packaged; ◆), M433 (40%CO_2: 30%N_2: 30%O_2; ■), M550 (50%CO_2: 50%N_2; ▲) and M622 (60%CO_2: 20%N_2: 20%O_2; ×). Bars represent the standard deviation (n = 3) but are smaller than the symbols.

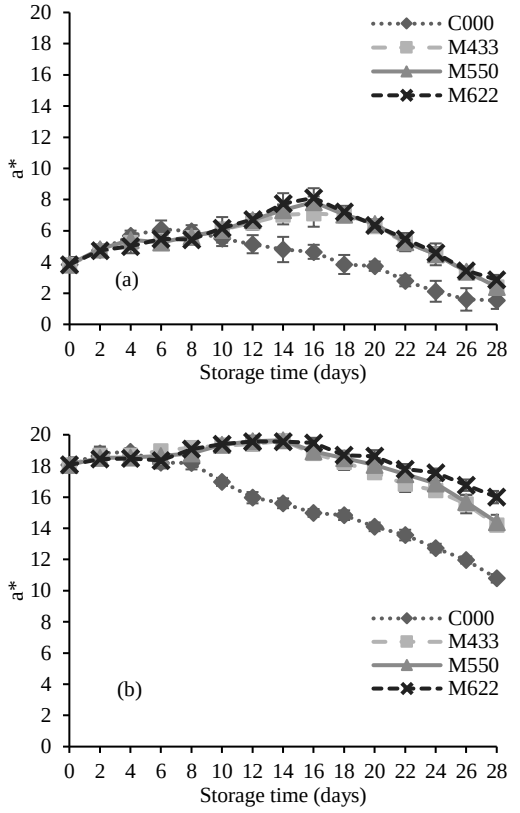


Fig. 5. The effect of modified atmosphere packaging on (a) a* of male, (b) a* of female, cooked mussel coated with green tea and vitamin C during refrigerated storage at 4 ± 1 °C: C000 (air packaged; ◆), M433 (40%CO_2: 30%N_2: 30%O_2; ■), M550 (50%CO_2: 50%N_2; ▲) and M622 (60%CO_2: 20%N_2: 20%O_2; ×). Bars represent the standard deviation (n = 3) but are smaller than the symbols.

Initially all samples had low L* and a* values due to the green tea while b* was high. When the color of green tea began to fade L* and a* increased with increasing storage time. After mussel death, microbial spoilage and autolytic processes cause degradation of proteins. The value of L*, a* and b* in all samples were declined significantly.

The samples in MAP had higher color values than C000 but significantly decreased during storage ($p < 0.05$). The concentrations in the packages that increased the antibacterial compounds and retarded protein denaturation were in agreement with the finding on lingcod (*Ophiodon elongates*) fillets in MAP which found that the rate of change of color was slower than in the control sample [29]. Also, decreased color in shucked fresh oyster (*Saccostrea cucullata*) was observed over time [22].

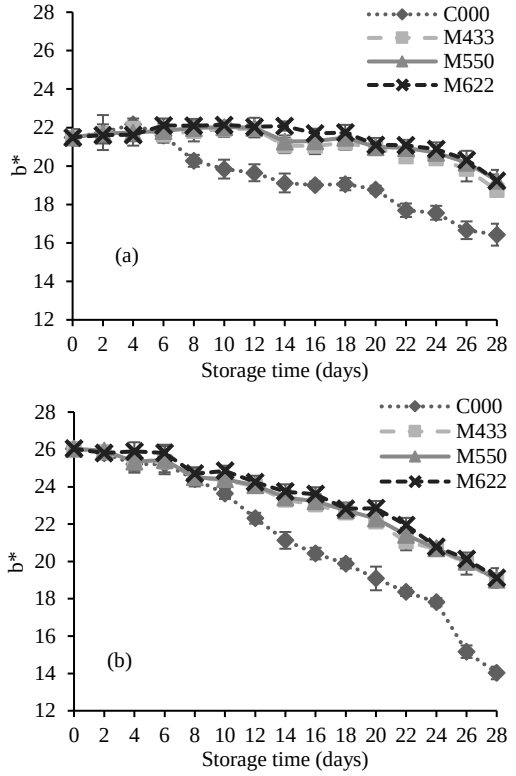


Fig. 6. The effect of modified atmosphere packaging on (a) b* of male, (b) b* of female, cooked mussel coated with green tea and vitamin C during refrigerated storage at 4 ± 1 °C: C000 (air packaged; ◆), M433 (40%CO_2: 30%N_2: 30%O_2; ■), M550 (50%CO_2: 50%N_2; ▲) and M622 (60%CO_2: 20%N_2: 20%O_2; ×). Bars represent the standard deviation (n = 3) but are smaller than the symbols.

Conclusion

In this study it was demonstrated that modified atmosphere packaging (MAP) can prolong the shelf life of cooked green mussel. Sample M622 (60%CO_2: 20%N_2: 20%O_2) had the best conditions that inhibited microbial growth and retarded the physical properties. Based on the microbiological results, the shelf life of M622 was more than 28 days, M550 (50%CO_2: 50%N_2) and M433 (40%CO_2: 30%N_2: 30%O_2) were 24 days, while C000 (air packaged) was 22 days, respectively.

Acknowledgements

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