

Effect of Modified Atmosphere Packaging on Qualities and Shelf Life of Precooked Baby Clam (*Paphia undulata*)

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ABSTRACT

The effect of modified atmosphere (20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂, 60% CO₂ + 20% O₂ + 20% N₂) on the quality and shelf life of precooked baby clams was investigated. Baby clams were blanched in boiling water until the temperature in the center of meat reached 73 ± 2 °C for 1 min. The precooked samples were packed in nylon laminated with polyethylene (Nylon/PE) bags and kept at 4 ± 2 °C. The results indicated that as the storage time increased, the total plate count, % drip loss, total volatile base nitrogen (TVB-N), trimethylamine (TMA) and pH of the precooked products, regardless of the atmospheric conditions, increased while their firmness and sensory scores decreased. All products, regardless of the modified atmosphere packaging conditions, showed better qualities and had longer shelf life compared with those packed under atmospheric conditions. Precooked clam meat in 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and 60% CO₂ + 20% O₂ + 20% N₂ packages could be kept at 4 ± 2 °C for 15, 21 and 24 d, respectively whereas the products packed under atmospheric conditions had a shelf life of 10 d. The results revealed that 60% CO₂ + 20% O₂ + 20% N₂ was the most effective storage condition for refrigerated precooked baby clam. The product was orange-yellow in color, elastic and slightly stiff with no exotic flavor, and was liked moderately by the panelists.

Keywords: baby clam, blanching, shelf life, modified atmosphere packaging, precooked product

INTRODUCTION

Baby clam (*Paphia undulata*), as with other seafood, is high in calcium, iodine and zinc but low in calories. Clam consumption, however, is considered healthy for the circulatory system because of the lack of significant levels of saturated fat. Clam is a versatile ingredient and is often used as a part of the cuisine of many cultures. Common

methods of preparation include boiling and frying. Baby clam may be purchased loose or in the shell. However, the shelf life of refrigerated clam is relatively short; it is susceptible to microbiological and chemical deterioration and hence, the consumption of this product is expected to be shortly after purchase. The extension of product shelf life is very important in order to increase the market and satisfy consumer demands. Modified

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atmosphere packaging (MAP) combined with refrigeration is a widely used food preservation method. The gases normally used for preservation include combinations of O₂, CO₂ and N₂. Since vegetative cells of *Clostridium botulinum* emerge and produce toxin in an anaerobic environment, the presence of O₂ is very important during storage as it maintains aerobic conditions. Different concentrations of CO₂ had some effects on bacteria (Church, 1998). High concentrations of CO₂ for fish should be avoided since the gas dissolves into the fish juice and deforms the package (Stenström, 1985). However, the use of MAP with an enhanced CO₂ level was successful in extending the shelf life of many fishery products by retarding microbial growth (Layrisse and Matches, 1984; Stenström, 1985; Emborg *et al.*, 2002). N₂, usually used as filling gas in the package, can delay oxidative rancidity and inhibit the growth of aerobic microorganisms by displacing the oxygen in packs (Church, 1998). Gas mixtures have been recommended to preserve a variety of fishery products such as seabream (Goulas and Kontominas, 2007), rainbow trout (Arashisar *et al.*, 2004) and shrimp (Lu, 2009). Thus, the present study was undertaken to determine the effects of MAP with various gas mixtures on microbiological, physicochemical and sensory changes of baby clam meat during storage at 4 ± 2 °C. The knowledge from this research may help in improving the quality and extending the shelf life of precooked baby clam.

MATERIALS AND METHODS

Sample preparation

Fresh baby clams (120–140 clam/kg) purchased from a local fish market in Samut Sakhon province were packed on ice and transported to the laboratory within 2 h. The clam shells were scrubbed until they were perfectly clean. The baby calms were blanched in boiling water and held until the temperature in the center

of meat reached 73 ± 2 °C for 1 min. After cooling, the precooked baby clam meat (about 60 ± 5 g) was placed on a polystyrene (PS) tray and packed in a nylon/LLDPE pouch (85 µm, thickness; oxygen transmission rate (OTR), 101.4 cm³/m² day at relative humidity (RH) = 0% and temperature = 23 °C; carbon dioxide transmission rate (CO₂TR), 74.5 cm³/m² day at RH = 0% and temperature = 23 °C; and water vapor transmission rate (WVTR), 1.64 g/m² day at RH = 100% and temperature = 23 °C). Gas mixtures were prepared using a gas mixer (WITT MM-2G, Germany). The following MAP conditions were applied: 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and 60% CO₂ + 20% O₂ + 20% N₂. The gas concentrations in the packages were monitored by a gas analyzer (Servomex, Model 1450, UK). Pouches were heat-sealed using a vacuum sealer (Multivac C200, Germany) and kept at 4 ± 2 °C. For the Control, identical clam meat samples were packed in air and kept under the same conditions. For analysis of quality and storage life, samples from each treatment were randomly taken at intervals of 2 and 3 d for Control samples and modified atmosphere samples, respectively.

Microbiological analysis

Each sample (25 g) was removed aseptically and transferred to 225 mL of sterile 0.1% peptone water solution for total plate count, *E. coli* and coliform determination, and to phosphate buffer saline (PBS) for *Vibrio* sp. determination. Each sample was homogenized for 30 sec. A ten-fold dilution was prepared using sterile peptone water as needed for plating. For microbial enumeration, 0.1 mL samples of serial dilution of clam meat homogenates were spread on the surface of dry media. Total plate count measurements were performed on plate count agar (Merck, Germany). The samples were incubated at 30 ± 2 °C for 3 d. *E. coli* and coliform were determined on Petrifilm™ *E. coli* count and Petrifilm™ rapid coliform count (3M, Germany),

respectively, after incubation at $35 \pm 2^\circ\text{C}$ for 1 d. *Vibrio* sp. was determined by enriching in alkaline peptone water (APW) and incubating at $35 \pm 2^\circ\text{C}$ for 8 h. Isolations were performed on thiosulfate citrate bile salt sucrose agar (TCBS, Merck, Germany) at $37 \pm 1^\circ\text{C}$ for 24 h. Suspected colonies were tested biochemically by the methods as described in the Food and Drug Administration Bacteriological Analytical Manual ([cited: 21 September 2009]. [Available from: <http://www.cfsan.fda.gov/~ebam/bam-5.html>]).

Physicochemical analysis

Drip loss was determined by weighing one set of samples at the beginning of the experiment and during each evaluation on the same calibrated scale. A cutting test was performed using a texture analyzer (Stable Micro Systems, TA-XT2i, England) at a displacement speed of 2.0 mm/s, using a Warner Bratzler blade (HDP/BSW) and 25 kg of load cell. The maximum shear force (cutting strength) was calculated from the force distance curves and expressed as N/g sample. Total volatile base nitrogen (TVB-N) and trimethylamine (TMA) were determined according to the method proposed by Conway (1962) and expressed as mg N/100 g sample. The pH value was recorded using a pH meter (Metrohm, Switzerland). Samples were thoroughly homogenized with 10 mL of distilled water and the homogenate was used for pH determination.

Sensory evaluation

The sensory quality of precooked baby clam meat was evaluated at each sampling time by ten panelists who had been trained for a period of 1 m to become familiar with the sensory attributes of precooked baby clam (that is, the color, flavor and texture). Clam meat was randomly drawn from each experimental code and reheated in a microwave oven at high power (700 W) for 4 min. Along with the test samples, throughout the experiment, the panelists were

presented with a freshly precooked clam meat sample (previously stored at -20°C) which served as the reference sample. Panelists were asked to evaluate each sample in terms of color (5 = orange-yellow to 1 = extensive black discoloration), flavor (5 = sweet/natural flavor to 1 = spoiled), texture (5 = extremely firm to 1 = mushy) and overall liking (5 = like very much and 1 = dislike very much). An overall liking score of 3.0 was taken as the lower limit of acceptability. The product was defined as unacceptable (a score of less than 3.0) after one or more of the following criteria: development of any first off-odor; or under the application of finger pressure, the muscle returned less than half way (muscle texture); or slime was produced; or after the first discoloration (meat color).

Statistical analysis

Duplicate experiments were carried out. All analyses were performed in triplicate. The experimental data were analyzed using analysis of variance (ANOVA). Means and standard deviations were calculated for all data, and when the F-values were significant at the $P \leq 0.05$ level, mean differences between pairs were separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Microbiological analysis

The change in total plate count of refrigerated clam meat packed under different atmospheric conditions significantly ($P \leq 0.05$) increased with storage time (Figure 1). The results also showed that the antimicrobial effect increased with increased CO_2 concentration. As shown in Figure 1, the lag phase of bacterial growth increased as the CO_2 concentration increased. The antimicrobial action of CO_2 resulted from the gas being absorbed onto the surface of the food, forming carbonic acid (Phillips, 1996). This can alter the cell membrane function and inhibit or

decrease enzyme activity in the microorganism. According to ICMSF (2002), precooked products were unacceptable when the total plate count value exceeded 6 log CFU/g. In the present experiment, precooked clam meat packed in air had surpassed that specified level after storage for 10 d while samples packed under 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and 60% CO₂ + 20% O₂ + 20% N₂ exceeded 6 logCFU/g after storage for 15, 21 and 24 d, respectively.

In the present study, *Clostridium botulinum* was not determined due to the aerobic conditions used in the experiment. Counts of *E. coli* and coliform were less than 2 log cfu/g in all precooked clam meat samples irrespective of the air composition and MAP conditions of the packages throughout the storage period (results not shown). According to Holzapfel (1998), members of the Enterobacteriaceae are sensitive to extrinsic factors such as heat. Therefore, heating clam meat until its internal temperature reached 73 °C for 1 min was sufficient to eliminate this microorganism. No evidence of *Vibrio* sp. was found in any of the samples (results not shown).

Physicochemical analysis

Figure 2a shows the calculation results of drip loss from each treatment. As the storage time increased, the drip loss of precooked clam

meat increased significantly. The clam meat packed under 60% CO₂ + 20% O₂ + 20% N₂ displayed the highest rate of drip loss; however there was no significant difference ($P > 0.05$) in this parameter among the samples packed under 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and under atmospheric conditions (Control) during the first 12 d of storage. Moreover, there was no significant difference in drip loss observed among samples packed under various modified atmospheric conditions from storage day 21 to the end of the experiment. This finding contrasted with some previous studies (Civera *et al.*, 1993; Penney *et al.*, 1994) which reported that the high CO₂ concentration did not produce any drip or muscle exudate in smoked fishery products. However, the moisture content of the smoked products was very low compared to precooked clam meat. In the present study, the moisture content of all precooked samples was between 81.62 and 82.23% throughout the storage period (results not shown). The high level of moisture content might have a direct consequence on the amount of CO₂ dissolving into the meat surface thus decreasing the water holding capacity of the meat.

The texture quality, as measured by the texture strength, of all precooked clam meat treatments is illustrated in Figure 2b. The

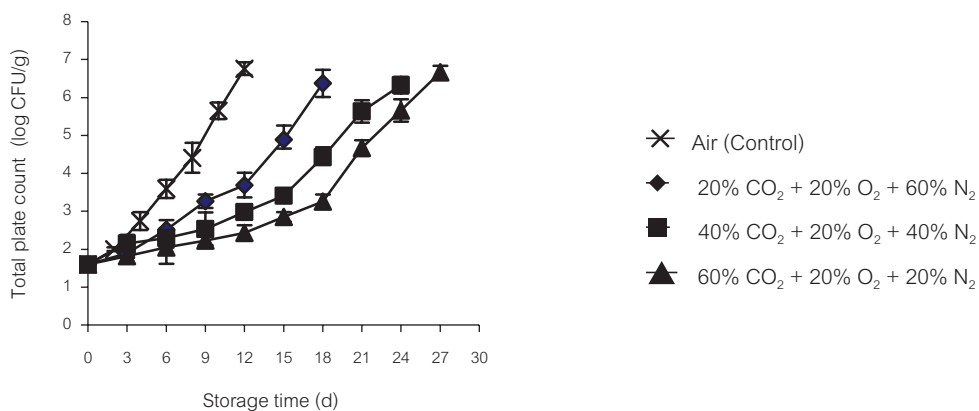


Figure 1 Changes (log cfu/g) in total plate count of precooked baby clam packed in air and under MAP conditions. The vertical bars show the SD range from the mean value.

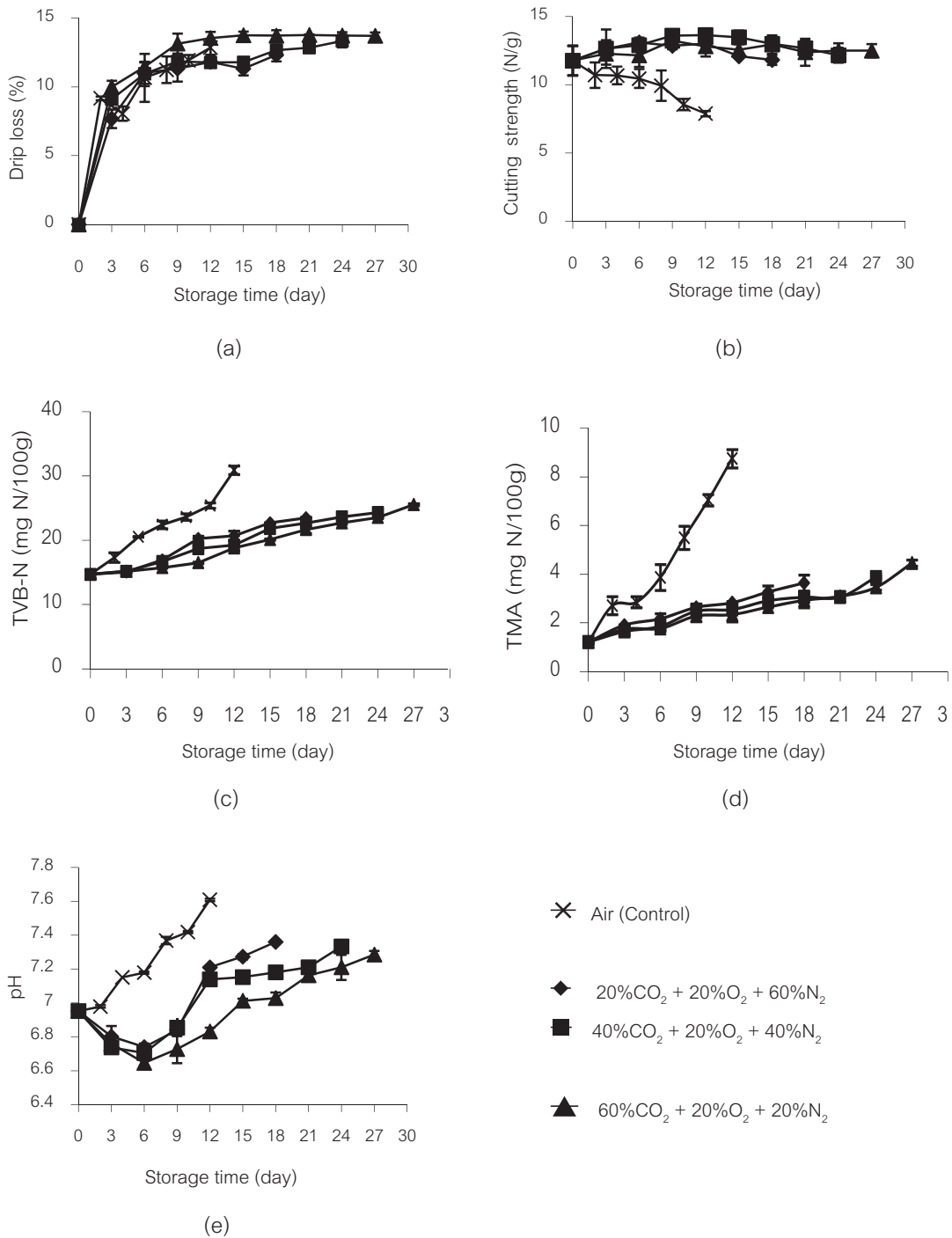


Figure 2 Changes in: (a) % drip loss; (b) cutting strength; (c) TVB-N; (d) TMA; and (e) pH of precooked baby clam packed in air and under MAP conditions. The vertical bars show the SD range from the mean value.

precooked samples packed under MAP conditions, regardless of the CO₂ concentration, maintained their texture quality during storage with cutting strength values in the range 11.78–13.64 N/g. The cutting strength of precooked clam meat in air packaging significantly decreased from 11.78 ± 1.09 N/g to 7.88 ± 0.23 N/g after storage for 12 d. This decrease correlated with the development of microbial growth (Figure 1); therefore, softening of the precooked samples in air packaging might have resulted from the aerobic microorganisms.

The total volatile base nitrogen (TVB-N) and trimethylamine (TMA) for all treatments are shown in Figures 2c and 2d. Both parameters are considered as quality indices for fish and fishery product (Goulas and Kontominas, 2007). According to some researchers, increments in TVB-N and TMA are related to the activity of spoilage bacteria and endogenous enzymes (Kyrana *et al.*, 1997; Ruiz-Capillas and Moral, 2005). In the present experiment, results indicated that TVB-N and TMA values increased progressively with the time of storage for all treatments. Both these parameters of precooked clam meat in air packaging were significantly higher than those packed under MAP conditions throughout the entire storage period. Such differences may be attributed to the anti-microbial property of CO₂. The CO₂ contained in the modified atmosphere packaging inhibited bacterial growth which reduced the formation of TVB-N and TMA. However, the differences in the TVB-N and TMA values were not significant among the clam meat samples treated with different gas treatments. The TVB-N values of precooked clam meat in air packaging reached 30.91 ± 0.64 mg N/100 g after storage for 12 d, whereas those samples packed under MAP conditions had values of 23.48 ± 0.12 , 24.29 ± 0.65 and 25.54 ± 0.18 mg N/100 g after storage for 12, 24 and 27 d, for 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and 60% CO₂ + 20% O₂ + 20% N₂, respectively. According to the

European Commission standard (EEC, 1995), the TVB-N value of pre-cooked product should be less than 35 mg N/100 g. Based on this standard, all samples in the present study were recognized as good quality. For TMA, the values of all treatments were related to the TVB-N values. The TMA values of precooked clam meat in air packaging, 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and 60% CO₂ + 20% O₂ + 20% N₂ were 8.76 ± 0.37 , 3.64 ± 0.33 , 3.89 ± 0.06 and 4.47 ± 0.13 mg N/100 g after storage for 12, 18, 24 and 27 d, respectively. However, the acceptability limit for this parameter varies with fish species. For example, it is 1 mg N/100 g for sea bream (Kyrana *et al.*, 1997), 5–10 mg N/100 g for sardine (Ozogul *et al.*, 2004) and 12 mg N/100 g for bigeye-tuna (Ruiz-Capillas and Moral, 2005). According to the TMA values reported in Figure 2d, as well as the flavor scores of the precooked clam meat samples in Figure 3b, a TMA limit for this product of about 8 mg N/100 g may be appropriate.

Gas mixture conditions and storage time had significant effects on the pH of the precooked baby clam (Figure 2e). The pH of precooked baby clam in air packaging was 6.95 ± 0.03 at the beginning of the study and increased progressively to 7.86 ± 0.01 at the end of storage. The increase was due to the formation of TVB-N and TMA (Figures 2c and 2d). Conversely, the pH of the samples receiving gas treatments decreased from 6.95 ± 0.03 to 6.65–6.74 during 6 d of storage and after that the pH increased. The initial decrement of pH in the precooked samples packed under MAP conditions was due to CO₂ dissolving into the meat surface while the increment of pH after day 6 resulted from the accumulation of nitrogenous compounds (Figures 2c and 2d).

Sensory evaluation

The sensory qualities of precooked baby clam are shown in Figure 3. The acceptable samples were described as having good

appearance, firm texture and a sweet or natural flavor without any sign of putrefaction. The results showed that all precooked products gradually developed black discoloration, a fishy odor and loss of firmness. In comparison with the Control, all MAP samples showed significantly delayed reductions in their sensory scores in terms of color, flavor and texture (Figure 3) and none of them reached the critical acceptability limit throughout the entire storage period. The precooked clam meat had an orange-yellow color, an elastic and slightly

stiff texture and no exotic flavor. On the other hand, the precooked clam meat in air packaging quickly lost its qualities after storage for 12 d; its meat was dark-yellow, soft to slightly firm and had a moderate fishy odor.

The sensory data were in agreement with the results from the physicochemical changes (Figure 2). Enzymatic browning might be responsible for color discoloration, as the high pH of the precooked products and the O_2 in the packages facilitated the activity of

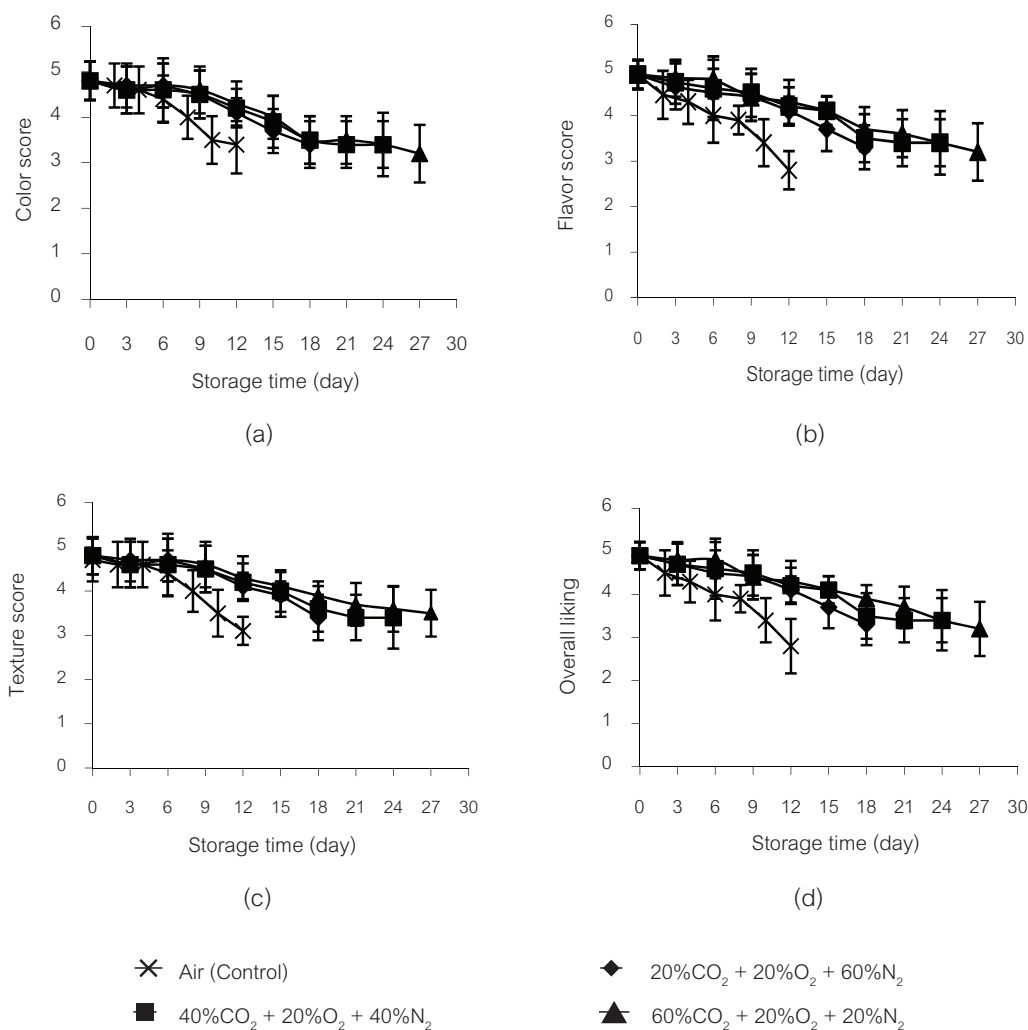


Figure 3 Changes in (a) color score; (b) flavor score; (c) texture score; and (d) overall liking of precooked baby clam packed in air and under MAP conditions. The vertical bars show the SD range from the mean value.

polyphenoloxidase remaining in the clam and microbial enzymes. Texture scores were consistent with cutting strength. The lowering in flavor scores was due to the increase amounts of volatile nitrogen compounds.

Considering all the results, the limit of shelf life for precooked clam meat was determined by microbial proliferation. The precooked clam meat packed under atmospheric, 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and 60% CO₂ + 20% O₂ + 20% N₂ conditions had a shelf life at 4 ± 2 °C of 10, 15, 21 and 24 d, respectively. From a financial viewpoint, the costs of 20% CO₂ + 20% O₂ + 60% N₂, 40% CO₂ + 20% O₂ + 40% N₂ and 60% CO₂ + 20% O₂ + 20% N₂ were 0.679, 0.543 and 0.365 baht/100 pack, respectively. Therefore, 60% CO₂ + 20% O₂ + 20% N₂ was the most suitable condition for packaging of refrigerated precooked clam products.

CONCLUSION

Precooked baby clam in modified atmosphere packages had better general qualities and longer shelf life compared with that stored in air packaging. Regarding physicochemical and sensory properties, the CO₂ concentration did not produce any clear differences in improved quality. However, microbial growth was significantly depressed by high CO₂ concentration. Based on the experimental results and the costs of the gases, the best option to preserve the refrigerated precooked product was 60% CO₂ + 20% O₂ + 20% N₂.

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