

The effects of modified atmosphere packaging on chemical, sensory and microbiological changes in black tiger prawn (*Penaeus monodon*)

[Kesan pembungkusan atmosfera terubah suai terhadap perubahan kimia, sensori dan mikrobiologi pada udang harimau (*Penaeus monodon*)]

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Key words: modified atmosphere packaging, tiger prawn, sensory, microbiological changes, chemical changes

Abstract

Chemical, sensory and microbiological changes in black tiger prawn packed in modified atmosphere (40% CO₂: 30% N₂: 30% O₂) packaging (MAP) and air-packed packaging (AP) at 1 ± 2 °C were investigated. Quality assessment was done by monitoring the changes in moisture, pH, bacterial counts, trimethylamine (TMA), total volatile bases nitrogen (TVN) and sensory quality. No significant change ($p > 0.05$) in moisture content was observed under both storage conditions. The pH and TVN increased significantly ($p < 0.01$) with time of storage in both AP and MAP prawns but TMA increased significantly only in AP prawns. Total plate count (TPC) and psychrotrophic bacteria count increased significantly ($p < 0.01$) during storage under both storage conditions. Bacteria grew faster in prawns stored in AP compared to prawns in MAP. All samples were rejected when TPC exceeded 10⁷ cfu/g with the TVN content at approximately 25 mg/100 g, pH >7.5, and mean odour and acceptability scores ≤5.0 for all storage conditions. MAP had extended the shelf life of black tiger prawn stored at 1 ± 2 °C by 30–45% longer as compared to AP. Based on sensory and microbiological results, the shelf life of tiger prawn in AP and MAP was 14 and 19 days, respectively.

Introduction

Fish is one of the most highly perishable food products. The shelf life of such products is limited in the presence of oxygen due to the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms. Modification of the atmosphere within the package by decreasing the oxygen concentration, while

increasing the content of carbon dioxide and/or nitrogen, has significantly prolonged the shelf life of perishable food products at chill temperatures (Parry 1993). Modified atmosphere packaging (MAP) and vacuum packaging (VP), along with refrigeration have become increasingly popular preservation techniques, which have brought major changes in storage, distribution, and

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marketing of raw and processed products to meet consumer demands.

The growth of microorganisms affects the food quality making it unacceptable for consumption because of changes in colour, odour and texture. MAP and VP systems could provide improvement in seafood shelf life, organoleptic quality, and product range. However, undoubtedly the single most important concern with such products is the potential for the outgrowth and toxin production by the non-proteolytic *Clostridium botulinum* type E which can grow at temperatures as low as 3.3 °C. In addition to this, pack collapse, increased exudates or drip loss, discoloration and histamine production are major potential problems during the storage of fish and shellfish products in MAP (Church 1998).

The shelf life of fish products in MAP can be extended, depending on raw materials, temperature, gas mixtures and packaging materials (Farber 1991). The percentage increase of shelf life in MAP ranges from 0 to 280%, compared with aerobic storage (Reddy et al. 1992). Inhibition of the growth of these microorganisms and increase in the lag phase of facultative and anaerobic microorganisms result in an increase in the potential shelf life of MAP products. Nevertheless, there is a safety concern with extended shelf life because of the growth of pathogenic organisms in these packaging systems especially at temperatures above 4 °C (Farber 1991). Fagan et al. (2004) found freeze-chilling combined with MAP performed very well in extending the shelf life of raw whiting, mackerel and salmon fillets.

Trimethylamine (TMA) content (Tozawa et al. 1971) and total volatile bases nitrogen (TVN) (Antonacopoulos and Vyncke 1989) have been proposed as indices of deterioration of fish quality. TMA is derived from trimethylamine-oxide (TMA-O), which is reported to be present only in marine fish and shellfish, hence TMA is not produced by the spoiling

freshwater fishes. Levels of biogenic amines such as histamine, putrescine and cadaverine, can also be useful in estimating freshness or degree of spoilage of certain species of fish since their formation is associated with bacterial spoilage (Mackie et al. 1997). Histamine has been reported to increase significantly during chilled storage of tuna and sardines in air, VP and MAP (Ozogul et al. 2004; Emborg et al. 2005). *Morganella morganii*-like bacteria dominated the spoilage microbiota of MAP tuna under 60% CO₂/40% N₂ at 1.7 °C (Emborg et al. 2005).

The effects of MAP on seafood have been reviewed extensively (Farber 1991) but little information is available on storage of black tiger prawn (*Penaeus monodon*) in MAP. Black tiger prawn is an important aquacultured species in Malaysia which is cultured mainly in brackish water ponds. Therefore, the main objective of this study was to investigate the effects of MAP and AP on the quality and safety of black tiger prawn. Quality attributes were assessed by different methods including chemical, microbiological and sensory evaluation.

Materials and methods

Packaging and storage of tiger prawn

Black tiger prawn (*Penaeus monodon*) was purchased live from local farms in Selangor and Terengganu. The prawn was immediately washed in potable water and transported in ice (2 parts ice: 1 part prawn) to the fish processing laboratory at MARDI Kuala Terengganu. The prawn was then head removed, cleaned in potable water with 3% salt and packed in high barrier film bags, 200 g prawn for each bag, and divided into two lots for air packaging (AP) and modified atmosphere packaging (MAP). Each lot consisted of 60 bags. From time of harvest to sample preparation and final packing for storage took less than 48 h.

One lot was packed in 100% air using horizontal impulse sealer and the other lot was packed in modified atmosphere using a Multivac model A300 vacuum-packaging

machine (Bury, Lancs., UK) equipped with gas flushing and monitoring devices. The packaging material used was high barrier film (LLDPE/EVOH/LLDPE), 0.08 mm thick and the size of the bag was 18 cm x 25 cm. The gas composition for MAP was 40% CO₂, 30% O₂ and 30% N₂, as recommended for packing shellfish in MAP (Cann et al. 1985). The final gas/sample ratio in all bags was about 2:1 (v/w) for MAP conditions.

All samples were stored for 22 days in a refrigerator under controlled temperature (1 ± 2 °C). Samples were evaluated for microbial, chemical and sensory quality changes on every three days interval. Six bags from each treatment, two each for chemical, microbiological analysis and sensory evaluation, were taken out at each sampling period. The experiment was replicated three times.

Chemical analysis

Moisture, protein content and pH of prawn were determined by the AOAC methods (AOAC 1990). The total volatile bases nitrogen (TVN) content was measured according to the modified Conway micro-diffusion method. Trichloroacetic acid (TCA) extract of prawn meat is placed in the outer ring of the Conway dish together with sufficient saturated alkali solution (K₂CO₃) to liberate the amines which will be trapped in the boric acid placed in the central wall of the dish (Obrink 1955). The TVN is expressed as mg-N/100 g prawn meat.

The trimethylamine (TMA) was determined using the modified method of Dyer's (Tozawa et al. 1971; Cobb et al. 1973) using trichloroacetic acid extract of prawn meat. The method consists of treating the prawn extract with formaldehyde to fix any other amines present and adding an alkali, 45% potassium hydroxide to liberate the TMA before extracting it into toluene. A dried aliquot of toluene was reacted with picric acid reagent and the subsequent colour

developed was estimated colorimetrically at 410 nm. TMA was expressed as mg TMA-N/100 g prawn meat.

Microbiological analysis

Standard microbiological method was used for evaluation of the product (Harrigan 1998). Duplicate samples from both storage conditions were taken for the evaluation to estimate total plate count (TPC) and psychrotrophic bacteria count. Prawn sample (10 g) was aseptically cut into small pieces using a knife and mixed with 90 ml of sterile Ringer solution and then stomached for 3 min.

Further decimal dilutions were made. For the TPC, 1 ml of each dilution was aseptically inoculated onto the surface of plate count agar (Oxoid) plates in triplicate and incubated at 37 °C for two days. For psychrotrophiles, 1 ml of each of the three selected dilutions were aseptically inoculated onto the surface of plate count agar (Oxoid) plates in duplicate and incubated for 14 days at 4 °C. After incubation all plates were examined for the formation of colonies. Isolated colonies between 30 and 300 were counted, averaged and reported as log cfu/g.

Sensory evaluation

Two pieces of shell-on prawn from each treatment (AP and MAP) were carefully removed from the experimental bag and transferred into a covered glass dish before being presented for sensory evaluation by the taste panel. The 12 panellists were staff of the Food Technology Research Centre, MARDI Kuala Terengganu who were trained to evaluate fish and fishery products. Each panellist was asked to sniff the odour of the prawn upon opening the covered glass dish before evaluating other attributes namely texture, colour and overall acceptability. Scores were based on a nine-point hedonic scale (Larmond 1970) of 1 to 9 (1 being the lowest score). Panellists were asked to state whether or not the prawn samples were acceptable to determine the

shelf life of the prawn. The acceptable shelf life was found to correspond to an average score of not less than 5.0.

Statistical analysis

An analysis of covariance was carried out on data for all parameters over days of storage (SAS Inst. 1999). Covariance analysis compares the regression of MAP and AP storage conditions.

Results and discussion

The tiger prawn used in this study contained $77.6 \pm 0.5\%$ moisture, $20.5 \pm 0.4\%$ protein, $1.5 \pm 0.04\%$ ash and $0.6 \pm 0.1\%$ fat.

Figure 1 shows the changes in moisture content and pH during chilled storage of black tiger prawn stored in AP and MAP. The moisture content remained relatively stable at 77–78% with no significant changes ($p > 0.05$) throughout the storage period for both storage conditions. The pH

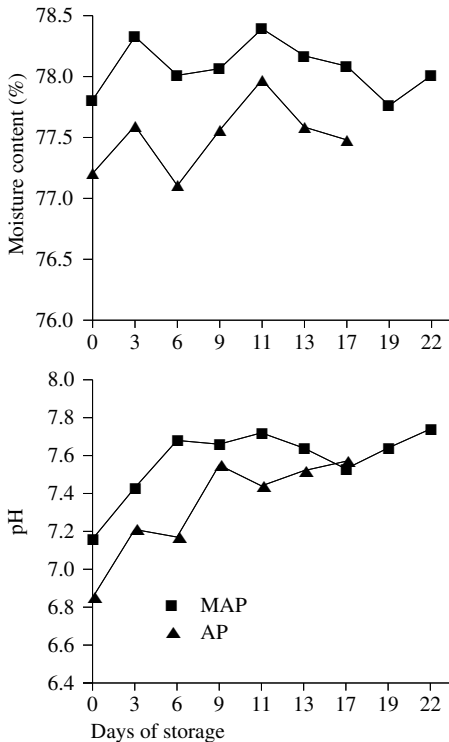


Figure 1. Changes in moisture content and pH in tiger prawns during storage under air (AP) and modified atmosphere packaging (MAP)

increased significantly ($p < 0.01$) from 6.8 to 7.5 in AP prawn and from 7.1 to 7.7 in MAP prawn, indicating some protein breakdown and amines formation during storage.

The changes in concentrations of total volatile bases nitrogen (TVN) and trimethylamine (TMA) in the prawn meat are shown in Figure 2. TVN increased significantly ($p < 0.01$) under both storage conditions. The highest increase was obtained in prawn stored in air. The TVN in AP samples increased from 12.9 at 0 day to 40.2 mg-N/100 g after 17 days of storage. The concentrations in MAP samples increased from 12.7 at 0 day to 22.3 after 17 days and to 25.1 mg-N/100 g after 22 days of storage. The results indicated that MAP had a limited inhibitory effect on the growth of bacteria that is responsible for the formation of the volatile amines.

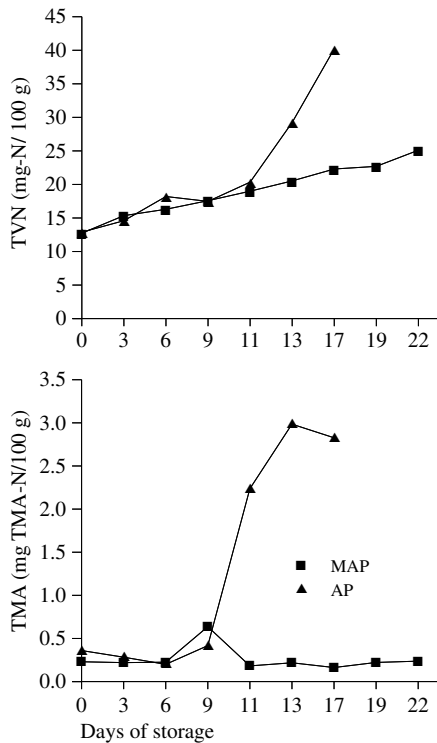


Figure 2. Changes in total volatile nitrogen (TVN) and trimethylamine (TMA) in tiger prawns stored in air (AP) and modified atmosphere packaging (MAP)

Similar observation was reported by Debevere and Boskou (1996) in cod fillets under MAP where all samples were considered spoiled after 4 days of storage at 6 °C due to high level of TVN production. TVN is one of the most widely used measurements of seafood quality which measures the volatile nitrogenous compounds. These compounds include trimethylamine (TMA), produced by specific spoilage bacteria action on TMAO which is present in marine fish and shellfish, and ammonia produced by bacteria action on proteins, peptides and amino acids. The European Communities Directives (95/149/EC) (1995) has specified the TVN contents exceeding 25–35 mg-N/100 g flesh of certain species of fish as unfit for human consumption. However, the limit for shellfish has not been specified.

Comparing the results of TVN and TMA in *Figure 2*, it would appear that the increase in TVN was mainly due to the formation of ammonia. There was almost no formation of TMA under both storage conditions during the first 9 days of storage where the values remained at less than 1 mg TMA-N/100 g. The TMA levels in AP samples increased significantly ($p < 0.01$) from 0.41 mg TMA-N/100 g after 9 days of storage to 2.23 mg TMA-N/100 g after 11 days of storage. This value was far below the suggested upper limit of 5–10 mg TMA-N/100 g for sardines (Ababouch et al. 1996), therefore TMA is not a useful indicator of black tiger prawn freshness.

There was no significant ($p > 0.05$) increase in TMA level in MAP prawns throughout the storage period. The result shows that MAP (40% CO₂: 30% O₂: 30% N₂) had reduced the TMA production in brackish water tiger prawn. Boskou and Debevere (1997) showed that a combination of 60–70% CO₂ and 30–40% O₂ was effective to inhibit the growth and TMAO-reducing activity of *Shewanella* spp., the bacteria responsible for TMA production in marine fish.

Figure 3 shows the changes in total plate counts (TPC) and psychrotrophic bacteria counts during storage of black tiger prawn. Both TPC and psychrotrophic bacteria counts increased significantly ($p < 0.01$) with time of storage under both storage conditions. The initial TPC was around 4.0 log cfu/g, higher by about 1 log number than the psychrotrophic counts in both samples but the psychrotrophiles started to increase slightly faster, 1–2 log numbers, than the TPC after a lag phase period of about 6 days in AP samples and 9 days in MAP samples. However, the spoilage was observed to be related more to the TPC where the formation of off-odour and slimes was detected after the TPC exceeded 7 log cfu/g. Similar observation on the faster growth of psychrotrophic bacteria was reported by Arashisar et al. (2004). They reported that the mesophilic bacteria count on trout fillets remained below 1×10^6 cfu/g after 14 days of storage in 100% CO₂, but psychrotrophic bacteria grew to above 1×10^7 cfu/g on the 12th day of storage.

The initial microbial load in raw fish and shellfish varies depending on many factors especially water conditions and temperature. Therefore, TPC can be considered as indicative of hygiene conditions. The initial TPC found in the present study is in correlation with

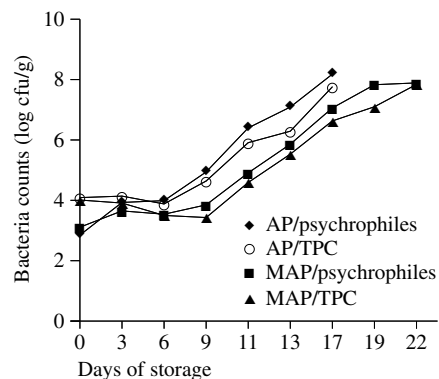


Figure 3. Changes in total plate counts and psychotropic bacteria during chilled storage of black tiger prawn in air (AP) and modified atmosphere packaging (MAP)

reported initial counts of 10^2 – 10^6 cfu/g raw fish reported by many researchers (Chytiri et al. 2004; Ozogul et al. 2004). The MAP containing 40% CO₂: 30% N₂: 30% O₂ slowed down the bacterial growth as compared to normal air as shown in Figure 3. The psychrotrophic bacteria counts reached 7 log cfu/g after 13 days under AP and 17 days under MAP. The TPC took slightly longer to reach the proposed microbiological upper limit of 7 log cfu/g for fresh fish (ICSMF 1986). Similar inhibitory effect of MAP on bacterial growth which resulted in extended shelf life of fish had been reported by many researchers (Cann et al. 1985; Ozogul et al. 2004; Emborg et al. 2005) but no study on MAP of tiger prawn had been reported.

Figure 4 shows the changes in sensory attributes of raw black tiger prawn stored

under AP and MAP. The mean scores for odour, colour, texture and overall acceptability decreased significantly ($p < 0.01$) during storage. The decrease was faster for AP prawn than MAP prawn. The decrease in the mean scores for sensory attributes was faster after 9 days of storage and seemed to be related to the increase in microbial counts and TVN contents after this period of storage. Spoilage of the black tiger prawn was observed to occur after this period. The odour and overall acceptability scores in AP-prawns decreased to less than 5.0 when the TVN content was 29.24 mg-N/100 g and the psychrotrophic bacteria count was 7.09 log cfu/g after 13 days of storage. The blackening due to melanosis occurred in both AP and MAP prawns, especially after 9 days of storage, hence resulted in the decrease in colour score.

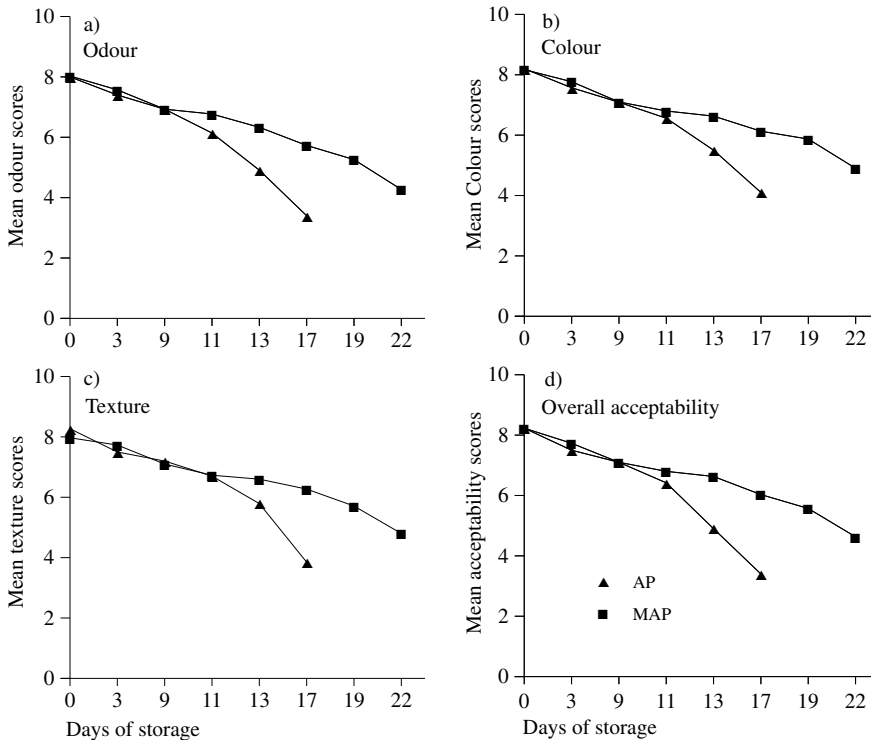


Figure 4. Changes in sensory attributes during storage of tiger prawns in air (AP) and modified atmosphere packaging (MAP) at 1 °C (each point is a mean value of 45 determinations for each sampling point)

The changes in sensory attributes followed similar linear pattern as the changes in chemical and microbiological quality. Analysis of covariance was used to understand the correlation between quality changes and days of storage of black tiger prawn at 1 ± 2 °C under AP and MAP. The regression equations and correlation coefficients, *r*, of the selected chemical, microbiological and sensory quality parameters are tabulated in *Table 1*. Regression analysis of the TPC data gave a shelf life of 15 days and 19 days for AP-prawns and MAP-prawns respectively, using the proposed upper limit of 7 log cfu/g for TPC in marine fish. If the calculation is based on the regression equation for psychrotrophic bacteria counts, then the shelf life for AP-prawns and MAP-prawns was calculated to be 13 days and 17.6 days, respectively.

However, regression analysis of the data for TVN and sensory attributes mean scores gave a longer shelf life for MAP prawns. Using the TVN limit of 25 mg-N/100 g, the lowest level specified by European Communities Directives (95/149/EC) for fish species, the calculated shelf life for AP-prawns and MAP-prawns would be 10 days and 22 days, respectively. Based on sensory scores, the shelf life would be 14 days for AP-prawns and 22 days for MAP-prawns. Again the result suggests that MAP had inhibitory effect on the spoilage organisms in black tiger prawn, hence resulted in longer shelf life.

Based on these results, it is advisable not to use a single quality indicator to determine the shelf life of unprocessed seafood product. Bacteria counts together with sensory evaluation were found to be useful in determining the shelf life of black

Table 1. Regression models describing the relationship between quality parameters and days of storage of black tiger prawn in air (AP) and modified atmosphere (MAP) packaging at 1 °C

Packaging methods/quality parameters	Regression Equations	<i>r</i>
Chemical changes		
Air packaging (AP)	TVN = 9.2115 + 1.5419DOS	0.8706**
	TMA = -0.1476 + 0.1873DOS	0.7806**
Modified atmosphere (MAP)	TVN = 13.1489 + 0.5328DOS	0.8773**
	TMA = 0.2971 + 0.0031DOS	0.1170ns
Microbiological		
Air packaging (AP)	TPC = 3.4920 + 0.2227DOS	0.8702**
	Psychrotroph = 2.7387 + 0.3199DOS	0.9218**
Modified atmosphere (MAP)	TPC = 3.2190 + 0.1917DOS	0.9088**
	Psychrotroph = 2.7544 + 0.2407DOS	0.9478**
Sensory changes		
Air packaging (AP)	Odour = 8.4901 - 0.2542DOS	0.9239**
	Colour = 8.5795 - 0.2228DOS	0.8863**
	Texture = 8.6400 - 0.2227DOS	0.9106**
	Overall acceptability = 8.7384 - 0.2659DOS	0.9161**
Modified atmosphere (MAP)	Odour = 8.2255 - 0.1586DOS	0.9058**
	Colour = 8.2525 - 0.1347DOS	0.9373**
	Texture = 8.1685 - 0.1322DOS	0.9452**
	Overall acceptability = 8.3429 - 0.1491DOS	0.9456**

DOS = Days of storage; TVN = Total volatile bases nitrogen; TMA = Trimethylamine;
 TPC = Total plate count; NS = Not significant

tiger prawn under chilled storage in this study. Chytiri et al. (2004) also used similar quality indices to determine the shelf life of iced whole and filleted trout.

Therefore, based on these two quality indices, it can be concluded that the MAP conditions used in this study had extended the shelf life of black tiger prawn to a maximum of 30–45% longer compared to AP. If the calculation is based on sensory evaluation and total plate counts (TPC), the shelf life of black tiger prawn stored at 1 ± 2 °C would then be extended from 14 days in AP to 19 days in MAP.

Conclusion

MAP had extended the shelf life of black tiger prawn stored at 1 ± 2 °C by 30–45% longer as compared to AP. Results of sensory evaluation showed a good relation with the TPC. The increase in TVN was closely related with the decrease in odour and overall acceptability mean scores especially for MAP samples. However, TMA was not a good indicator of freshness for black tiger prawn. Therefore, based on sensory evaluation and total plate counts, the shelf life of black tiger prawn stored at 1 ± 2 °C would then be extended from 14 days in AP to 19 days in MAP.

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Abstrak

Perubahan kimia, sensori dan mikrobiologi pada udang harimau yang disimpan di dalam pembungkusan udara (AP) dan atmosfera terubah suai (40% CO₂: 30% N₂: 30% O₂) (MAP) pada suhu 1 ± 2 °C telah dikaji. Penilaian mutu diukur melalui perubahan kandungan lembapan, pH, bilangan bakteria, trimetilamina (TMA), jumlah nitrogen meruap (TVN) dan kualiti sensori. Tiada perubahan yang nyata ($p > 0.05$) bagi kandungan lembapan untuk kedua-dua kaedah penyimpanan. Pembentukan TVN dan pH meningkat dengan nyata ($p < 0.01$) semasa penyimpanan bagi kedua-dua kaedah tetapi TMA meningkat secara nyata bagi udang harimau di dalam MAP. Bilangan TPC dan bakteria psychrotrophic meningkat dengan ketara ($p < 0.01$) bagi udang yang disimpan di dalam AP dan MAP. Pertumbuhan bakteria lebih cepat pada udang yang disimpan di dalam AP berbanding dengan MAP. Kesemua sampel ditolak apabila TPC melebihi 10⁷ cfu/g dengan kandungan TVN sekitar 25 mg/100 g, pH >7.5, dan skor purata bagi bau dan penerimaan keseluruhan ialah ≤5.0 bagi kedua-dua kaedah penyimpanan. Kaedah MAP dapat melanjutkan jangka hayat udang harimau pada suhu 1 ± 2 °C sebanyak 30–45% lebih lama berbanding dengan udang yang disimpan di dalam AP. Jangka hayat udang harimau yang disimpan di bawah AP dan MAP masing-masing ialah 14 dan 19 hari.