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Effect of modified atmosphere and vacuum packaging on quality changes of refrigerated tilapia (*Oreochromis niloticus*) fillets

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Abstract

The assessment effect of modified atmosphere packaging (60% CO_2, 10% O_2, 30% N_2; MAP) and vacuum packaging on the quality of tilapia (*Oreochromis niloticus*) fillets stored at 4°C was investigated. Maximum inhibition of the bacteria mesophilic and psychrotrophic growth was achieved with stored under MAP. Moreover, tilapia kept under CO_2-enriched atmosphere had lower total volatile base (TVB-N), trimethylamine (TMA), trichloroacetic acid soluble peptide contents than those stored in air (control) ($P < 0.05$). However, the increase in exudate loss was observed for sample packaged in modified atmosphere packaging, suggesting that denaturation of muscle proteins by carbonic acid formed. Thiobarbituric acid-reactive substances (TBARS) of samples kept under vacuum packing were lower than those stored under other conditions throughout the storage of fifteen days. The odour and flavour acceptability of MAP and vacuum packaged samples was accepted throughout the stored of fifteen and twelve days, respectively. However, the samples stored in air had the acceptability only six days of storage. Therefore, MAP was chosen as the optimum condition for extending the shelf-life of tilapia fillets.

Introduction

Fish and fishery products have been recognized as a nutrition source due to their high protein and unsaturated fatty acid content. Tilapia (*Oreochromis niloticus*) is a popular food fish and a highly nutrient source (Garduno-Lugo *et al*., 2003). It is cultured in tropical areas of South-East Asia, especially in Thailand (Suanyuk *et al*., 2010). It is commonly sold as whole fish or as fillets. Since ready-to-cook or fresh fish have become increasingly popular, they are available in the market. However, the short shelf-life is a limiting factor for these perishable products. Immediately after death, several biochemical and microbiological changes are triggered in fish and fishery products, especially with improper handling. Therefore, certain techniques have been applied to extend the shelf-life of fish and fishery products (Duan *et al*., 2011).

Over the last years, modified atmosphere packaging (MAP) and vacuum packaging, with refrigeration, have received increasing attention as method of food preservation (Masniyom, 2011; Noseda *et al*., 2012). MAP is defined as the enclosure of food products in gas-barrier materials, in which the gaseous environment has been changed (Sivertsvik *et al*., 2002; Kykkidou *et al*., 2009). Shelf-life of iced or refrigerated fish could be extended by using MAP, specifically elevated CO_2 levels, which has shown to retard the growth of spoilage and pathogenic bacteria. Refrigerated fish including catfish and sea bass packed with CO_2 had 40-100% increase in stability, mainly due to an extension in the lag phase of organisms and their reduced growth rate in the logarithmic phase (Maqsood and Benjakul, 2010; Provincial *et al*., 2010). Vacuum packaging is used for long-term storage of dry foods and the shelf-life extension of seafood. The product is packed in a vacuum package which has good barrier properties towards oxygen and water and is easily sealed. Air is removed under vacuum and the package is sealed. The products kept under the lower O_2 less than 1% had the exhibited growth of aerobic spoilage microorganisms, particularly *Pseudomonas* spp., *Shewanella putrefaciens* and *Enterobacteriaceae* compared with the atmosphere packaging (Arashisar *et al*., 2004; Stamatis and Arkoudelos, 2007; Mastromatteo *et al*., 2010). It was reported that psychrophilic bacterial counts were reduced in *Rutilus frisii kutum* fillets packaged under vacuum packaging (Etemadian *et al*., 2012). Furthermore, vacuum packaging could prevent oxidative rancidity and improve organoleptic quality in seafood (Arkoudelos *et al*., 2007). Although, MAP and vacuum packaging could prevent microbial and

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chemical changes of seafood, including fish and fishery products but the information is limited on the quality changes of tilapia fillets packed under MAP and vacuum packaging. Thus, the present work was undertaken to study the shelf-life of tilapia packed under MAP and vacuum packaging during refrigerated storage.

Materials and Methods

Chemicals

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

Sample preparation

Tilapia *(Oreochromis niloticus*) with an average size of 5-7 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 Industry, Prince of Songkla University, within 2 h after harvesting. Upon arrival, they were then washed with tap water, filleted and deskinned. Fillets weighing approximately 90-100 g were placed 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). Three packaging systems were prepared: (1) packaged in air; (2) packaged under vacuum and (3) packed under MAP with a mixture of 60% CO_2, 10% O_2 and 30% N_2. Bags were sealed using a Henkovac type H 1502 (Netherlands). All samples were stored at 4°C for 18 days and were removed for microbiological, chemical, physical and sensory analysis every 3 days.

Microbiological analysis

Tilapia flesh samples (25 g) were aseptically collected in a stomacher bag and ten volumes of sterile saline solution (0.85%) were added. After homogenizing in a Stomacher M400 (Seward, Worthing, UK), a series of ten-fold dilutions was made using saline solution. Mesophilic and psychrotrophic bacterial counts were determined by plate count agar (Merck, Darmstadt, Germany) with the incubation at 35°C for 2 days and 7°C for 7 days, respectively (Arashisar *et al*., 2004). Microbial counts were expressed as log colony-forming unit (CFU)/g.

Chemical analysis

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% TCA. The homogenate was filtered through a Whatman No.1 filter paper and the filtrate was used for analysis. 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 sample extract to tie up ammonia. The results were expressed as mg TVB/g muscle and mg TMA/g muscle.

Determination of trichloroacetic acid- 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, Staufen, Germany) at speed of 13500 rpm for 1 min. 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 per gram muscle.

Determination of thiobarbituric acid-reactive substances

Thiobarbituric acid-reactive substances (TBARS) were determined according to the method of Buege and Aust (1978). Chopped samples (0.5 g) were homogenized in 2.5 mL of the mixture containing 0.375% TBA, 15% TCA and 0.25M 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, USA) and the absorbance was measured at 532 nm using UV 1601 spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). TBARS were calculated from the standard curve of malondialdehyde and expressed as milligram malondialdehyde/kg muscle.

Physical analysis

Determination of exudate loss

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

Sensory evaluation

The sensory evaluation was performed by thirty trained panelists (male:female, 15:15) from the Graduate School of Fishery Technology, Prince of Songkla University. The assessment was conducted for the odour of raw fish samples using a nine-point hedonic scale (Mailgaad *et al*., 1999): 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike ; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. The evaluation of odour was carried out at the moment of opening the pack. For cooked samples, the samples were wrapped with aluminium foil, cooked in steaming pot until the core temperature of each sample reached 70°C. Stick water was drained and allowed to cool to room temperature (25-28°C). The flavour likeness of cooked samples was evaluated using a 9-point hedonic scale.

Statistical analysis

All experiments were run in triplicate. Data were subjected to analyze using analysis of variance (ANOVA). The least significant difference 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, USA).

Results and Discussion

Microbiological analysis

Mesophilic bacterial counts of all samples increased with increasing storage time at 4°C ($P < 0.05$) (Figure 1a). Mesophilic bacterial counts of tilapia fillets in air rapidly increased from an initial value of 4 to 7 Log CFU/g within nine days and were generally higher than that of samples kept under MAP and vacuum packaging ($P < 0.05$). In contrast with the samples kept in air, viable counts of CO_2-enriched samples were typically below 7 Log CFU/g and remained at this level throughout the storage of fifteen days. ICMSF (1986) suggested that TVC of fresh water and marine species exceeded the value of 7 Log CFU/g, which is recommended as the upper acceptability limit. The storage time of samples kept in air was estimated to be six days. However, the shelf-life of tilapia was extended to twelve and fifteen days when packed under vacuum packaging and 60% CO_2 atmosphere, respectively. Lower TVC of sample kept under MAP indicated that CO_2 at a concentration 60% effectively inhibited the microbial growth. CO_2 commonly become 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 its growth is inhibited by higher carbon dioxide concentration (Sivertsvik *et al.*, 2002). Thus, CO_2-enriched atmosphere has been used in the preservation for fresh fishery products. This was probably because CO_2 entered into mass action equilibrium for enzymatic decarboxylation, leading to inhibition of the metabolic activity of microbial flora as result of an extension in lag phase and a reduction in logarithmic phase of spoilage bacteria (Lalitha *et al*., 2005; Masniyom *et al*., 2011). Our result was in agreement with Ozogul *et al*. (2004) who reported that TVC was retarded when sardine (*Sardina pilchardus*) were kept in 60% CO_2-enriched atmospheres. It has been reported that 50% CO_2 inhibited the microbial growth in chub mackerel (*Scomber colias japonicus*) during storage (Stamatis and Arkoudelos, 2007), 40%CO_2/30%N_2/30%O_2 in Mediterranean swordfish (Pantazi *et al.*, 2008) and 50%CO_2/50%N_2 in Vietnamese *Pangasius hypophthalmus* fillets (Noseda *et al*., 2012). Lower mesophilic bacterial counts of samples kept under vacuum packaging in compared with samples kept in air showed that O_2 absence could retard the growth of aerobic spoilage microorganisms, particularly *Pseudomonas* spp. and *Aeromonas* spp. (Masniyom, 2011). Manju *et al*. (2007) found that pearlspot (*Etroplus suratensis*) kept under vacuum condition had an overall increase of shelf-life of ten days, longer than that of eight days in aerobic packing. However, the growth rate of microorganisms in samples kept CO_2-enriched atmosphere was lower than that of samples kept under vacuum packaging. Our results showed that 60% CO_2-enriched atmospheres was effective in achieving of shelf-life extension for tilapia.

Higher counts of psychrotrophic bacteria were also observed in samples kept in air, compared with those packed with MAP and vacuum packaging ($P < 0.05$) (Figure 1b). However, psychrotrophic bacterial counts in sample kept under MAP were lower than those of other samples. The results indicated that an atmosphere of 60% CO_2 inhibited the growth of spoilage bacteria. It has been reported that 60%CO_2/40%N_2 inhibited the psychrotolerant growth in tuna (*Thunnus albacares*) at 2°C (Emborg *et al.*, 2005) and 60%CO_2/30%N_2/10%O_2 in sea bass (*Dicentrarchus labrax*) (Kostaki *et al*., 2009). For samples stored under vacuum packaging, psychrotrophic bacterial counts increased lower than those of samples kept in air. Etemadian *et al.* (2012) reported that *Rutilus frisii kutum* fillets packaged under vacuum packaging reduced the final aerobic

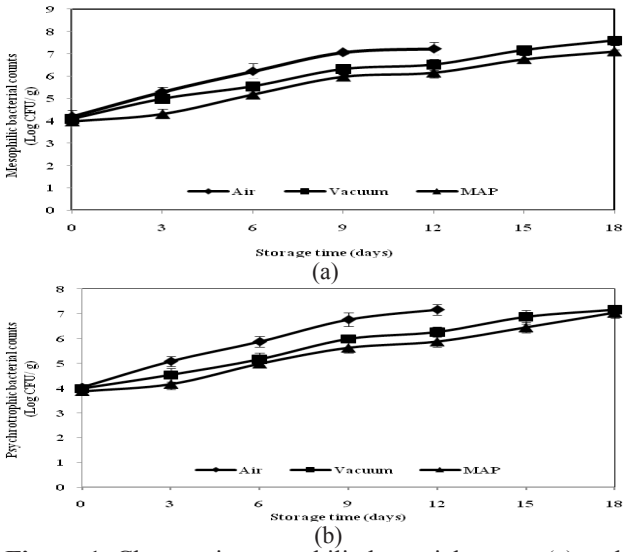

Figure 1. Changes in mesophilic bacterial counts (a) and psychrotrophic bacterial counts (b) in tilapia fillets kept with different condition during storage at 4°C: air (♦), vacuum packaging (■) and MAP (▲). Bars represent the standard deviation from triplicate determinations.

plate, compared with the samples packed in air. The results indicated that MAP in tilapia prior to storage was more effective in reducing microbial numbers on the fish.

Chemical analysis

Total volatile bases (TVB) are products of bacteria spoilage and their contents are often used as index to assess the keeping quality and shelf-life of fish and seafood products (Connell, 1990). TVB contents of sample kept under different condition are depicted in Figure 2a. Generally, the higher TVB content was found in samples kept in air, compared with samples packed under MAP and vacuum packaging throughout the storage ($P < 0.05$). Samples packed MAP tended to have lower TVB content, compared with other samples during storage. While samples kept under vacuum packaging had lower levels of TVB than samples packed in air. It was reported that TVB contents in sea bass (*Dicentrarchus labrax*) kept under MAP (60%CO_2) was increased lower than those of fillets kept under air condition (Kostaki e*t al*., 2009). For the samples kept in air, TVB content increased rapidly and reached 0.27 mg/g after six days of storage ($P < 0.05$). On the other hand, samples packed under MAP, had TVB contents less than 0.25 mg/g within fifteen days of storage. For samples kept under vacuum packaging, TVB content reached 0.25 mg/g after twelve days of storage. TVB values of fresh and good quality fish are less than 0.12 mg/g. TVB value above 0.25 mg/g indicates that fish are slightly decomposed and inedible (Lannelongue *et al.*, 1982). TVB usually includes trimethylamine (TMA), dimethylamine, ammonia and other volatile basic nitrogenous compounds associated with fishery product spoilage Thus, an increase in TVB content indicated the stage of substantial spoilage of the muscle.

TMA is generally produced by the reduction of trimethylamine oxide (TMAO), possibly by endogenous enzymes in fish, but mainly by the enzyme activity of certain bacteria. TMA is associated with the odour of the spoiled fish and is used as an indicator of bacterial activity causing the deterioration (Huss, 1995). The TMA content in samples kept in air sharply increased after six days of storage ($P < 0.05$), while it slightly increased in the samples kept under 60% CO_2 atmosphere and vacuum packaging (Figure 2b). In general, MAP led to the higher retardation of TMA formation. Since the reduction of TMAO to TMA is a property of Gram-negative microorganisms, the slow rate of TMA production in MAP stored samples was most likely due to an inhibition of aerobic, including TMA-producing microorganisms, by CO_2-enriched atmosphere (Ruiz-Capillas and Moral, 2001). Our result was in agreement with Ravi-Sankar et al. (2008) who reported that TMA was retarded when pearlspot (*Etroplus suratensis* Bloch) were kept under 60% CO_2. It is reported that TMA in swordfish under vacuum packaging was lower than those for air kept fish at the end of storage (Pantazi *et al.*, 2008). Lannelongue *et al.* (1982) shown that a TMA content of 0.05 mg/g was the limit for acceptability of fish. This presumably resulted in the poor sensory quality of fish. From the results, TMA content of tilapia kept in air reached 0.071 mg/g after six days, while samples kept under vacuum packaging and MAP, TMA content reached 0.05 mg/g after twelve and fifteen days of storage, respectively.

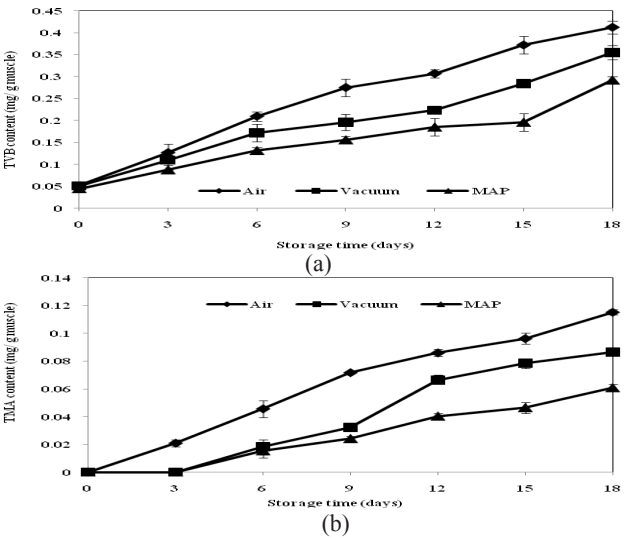

Figure 2. Changes in TVB (a) and TMA (b) contents in tilapia fillets kept with different condition during storage at 4°C: air (♦), vacuum packaging (■) and MAP (▲). Bars represent the standard deviation from triplicate determinations.

A sharp increase in TCA-soluble peptides in the sample kept in air was observed after six days of storage ($P < 0.05$) (Figure 3). However, a slight increase in TCA-soluble peptides in the samples packed under vacuum and MAP was observed throughout the storage. TCA-soluble peptides of samples kept MAP were slightly lower than those packed with vacuum packaging throughout the storage. Degradation of muscle protein might be caused by either endogenous or microbial proteinases during refrigerated storage. TCA-soluble peptide has been used as the index for the protein degradation of fish muscle (Benjakul *et al*., 1997). Venugopal *et al*. (1983) found that protease from *Pseudomonas marinoglutinosa* hydrolyzed actomyosin at 0-2°C. Microorganisms being responsible for fish spoilage was dominated by Gram-negative, such as *Pseudomonas*, *Shewanella* spp. (Hobbs, 1991). It has been reported that CO_2-enriched atmospheres inhibited the actomyosin degradation in fish during refrigerated storage (Masniyom, 2011). MAP used might retard the growth of microorganisms producing proteolytic enzymes. As a consequence, a lower degradation was obtained as evidenced by the lower rate of TCA-soluble peptide formation. Therefore, MAP was shown to be the promising means to prevent the degradation of muscle proteins during prolonged storage.

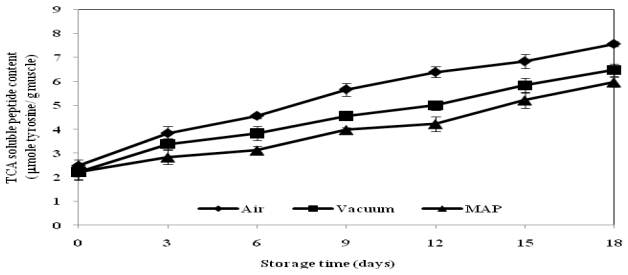

Figure 3. Changes in trichloroacetic acid soluble peptide contents in tilapia fillets kept with different condition during storage at 4°C: air (♦), vacuum packaging (■) and MAP (▲). Bars represent the standard deviation from triplicate determinations.

TBARS values in tilapia packed with different conditions are shown in Figure 4. An increase in TBARS was observed in all samples when the storage time increased ($P < 0.05$), indicating that lipid oxidation took place during storage. Lipid in muscle has typically a high percentage of polyunsaturated fatty acids and is consequently prone to oxidative reaction (Budge and Parrish, 2003). However, sample kept under vacuum packaging showed the lower TBARS, compared with samples kept under MAP throughout the storage. This indicated that vacuum packaging could prevent oxidative rancidity and improve organoleptic quality in seafood. The result was in agreement with by Gimenez *et al.* (2002) who found that vacuum packed rainbow trout had the lower malondialdehyde values, compared with that kept under MAP, as a consequence of the absence of oxygen in the package. However, TBARS in samples packed CO_2-enriched atmosphere was lower than that of samples kept in air. This result indicated that O_2 concentration caused on the oxidation of lipid in fishery products.

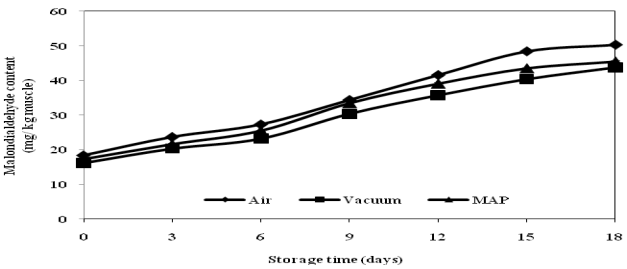

Figure 4. Changes in thiobarbituric acid-reactive substances value (measured as malondialdehyde) in tilapia fillets kept with different condition during storage at 4°C: air (♦), vacuum packaging (■) and MAP (▲). Bars represent the standard deviation from triplicate determinations.

Physical analysis

An increase in exudate loss were observed in all samples when the storage time increased ($P < 0.05$) in Figure 5. The exudate loss of tilapia kept under MAP was higher than that of other samples throughout the storage time. This might be due to a greater loss of water holding capacity of the muscle protein at lower pH values, in which higher dissolution of CO_2 in the aqueous phase of fish muscle took place (Masniyom *et al*., 2011). CO_2-enriched packaging effectively inhibited the spoilage caused by microorganisms, but it could not prevent the chemical deterioration, especially lipid oxidation and physical changes. To maximize the use of MAP, techniques should be applied to minimize these problems.

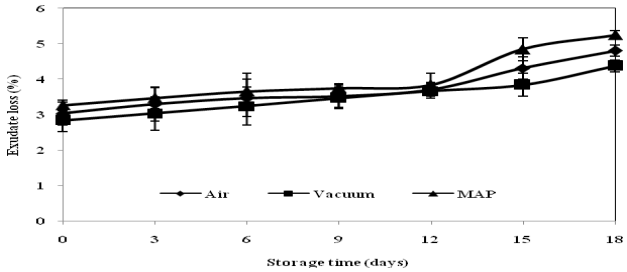

Figure 5. Changes in exudates loss in tilapia fillets kept with different condition during storage at 4°C: air (♦), vacuum packaging (■) and MAP (▲). Bars represent the standard deviation from triplicate determinations.

Sensory evaluation

The results of the sensory property (odour and flavour) of tilapia are shown in Figure 6a, 6b. Fresh

tilapia was generally considered to possess very high acceptability. Samples kept under MAP exhibited the higher likeness score for odour and flavour, compared to the other samples throughout the storage of fifteen days ($P < 0.05$). MAP used could retard the growth of microorganisms resulted in the odour and flavour acceptability of tilapia. From the result, samples packed in air were rejected after six days of storage but those kept under MAP could be accepted within fifteen days of storage. While under vacuum packaging, samples were rejected after twelve days of storage. The overall acceptability in odour and flavour of all samples decreased with increasing storage time. Generally, sensory evaluation is frequently applied in estimating the quality of fish and corrected with microbiological and chemical analysis (Karungi *et al*., 2004). Our results indicated that keeping the tilapia under 60% CO_2-enriched atmospheres effectively extended the shelf-life of fish with high acceptability; however it caused a changes in weight loss. Therefore, it suggested that the use of MAP might prevent bacteria growth and maintain quality, leading to the safety and prolonged shelf-life of tilapia fillets.

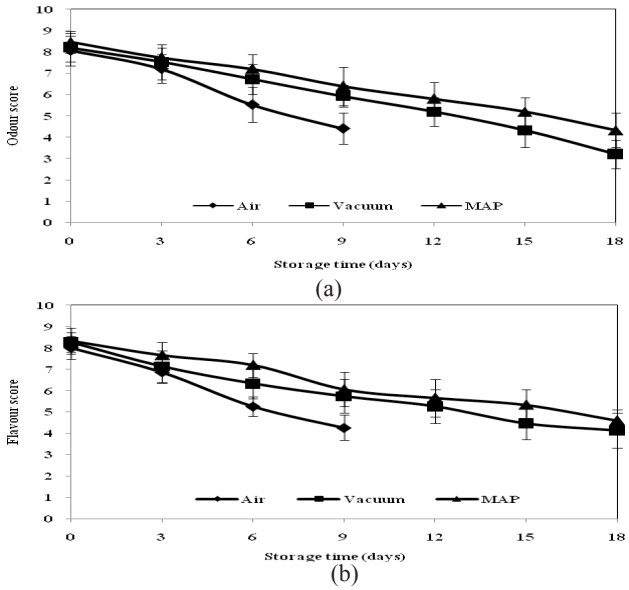

Figure 6. Changes in odour (a) and flavour (b) score in tilapia fillets kept with different condition during storage at 4°C: air (♦), vacuum packaging (■) and MAP (▲). Bars represent the standard deviation from triplicate determinations.

Conclusions

Self-life of tilapia fillets could be extended by 60% CO_2-enriched atmosphere. Microbial and chemical changes in tilapia were retarded, leading to decrease spoilage as well as delay deteriorative compounds, however, exudate loss still occurred.

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