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Effect of different packaging materials on the shelf life of modified atmosphere packaged red tilapia *(Oreochromis mossambica)* fillets

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Abstract: The effect of four different packaging films: (1) linear low density polyethylene/ethylene vinyl alcohol/ linear low density polyethylene (LLDPE/EVOH/LLDPE, 60 µm); (2) oriented nylon/polyethylene (ONy/ PE, 70 µm); (3) oriented polypropylene/polypropylene (OPP/PP, 60 µm); and (4) high density polyethylene (HDPE, 50 µm) on the shelf life of modified atmosphere packaged Red Tilapia *(Oreochromis mossambica)* fillets were studied. pH, K-value, microbial growth, as well as sensory alterations during storage under modified atmosphere of 80% CO_2 : 20% N_2 at 2 ± 2°C were monitored. Fillets packaged in LLDPE/EVOH/LLDPE and ONy/PE showed a further inhibition of biochemical, microbiological and sensory deterioration compared with OPP/PP and HDPE-packaged fillets. pH, K-value, and total plate counts of LLDPE/EVOH/LLDPE and ONy/ PE-packaged fillets were significantly lower (p<0.05) than OPP/PP and HDPE-packaged fillets throughout the storage periods. The shelf life of tilapia fillets packaged in LLDPE/EVOH/LLDPE and ONy/PE were 14 days, whereas fillet in OPP/PP was 9 days, and 6 days when packaged in HDPE.

Keywords: Packaging materials, shelf life, red tilapia, fillet, modified atmosphere packaging

Introduction

It is well known that packaging performs many complementary functions; protecting the product against external polluting agents, giving the product marketing value and providing the customer with information about ingredients or how to use the product. As a result, packaging has become an indispensable element in the food manufacturing process. In order to meet the huge demand of the food industry, there has been a remarkable growth in the development of food packaging in the past decades. Among the packaging technologies developed by and for the food industry, modified atmosphere packaging (MAP) has led the evolution of fresh and minimally processed food preservation. MAP refers to a condition initially produced at the time of packaging. The gases within the package are allowed to change as the physical and biological conditions dictate. Rather than preserving food through the extremes of heat (sterilization) or cold (freezing), MAP utilizes 'minimal processing' to preserve food with the absolute least amount of damage to quality, texture, taste and nutrition (Fernandez *et al*., 2009).

Modified atmosphere packaging (MAP) has gained considerable popularity over the last decades as a modern non-thermal method of food preservation. The proper combination of gases (carbon dioxide, nitrogen and oxygen) in the headspace of food packs results in suppression of the microbial flora of perishable foods developed under aerobic conditions and retention of their sensorial attributes. Other than proper gases combination in the headspace of packages, the shelf life of products in MAP also very much depends on quality of raw material, storage temperature and packaging materials used (Farber, 1991; Sivertsvik, 2007; Rotabakk *et al*., 2008; Fernandez *et al*., 2010).

Packaging materials used for MAP are a combination of different substrates. The materials can be as simple as two-ply laminations or as sophisticated as multi-layer co-extrusions, incorporating EVOH as a high barrier substrate. Film properties will greatly affect the shelf life of the product depending on film permeability and transparency. The main purpose of this investigation was to evaluate four different polymeric packaging materials for their ability to maintain a favourable storage environment for tilapia filets within the package.

Materials and Methods

Preparation of fish

Fresh skin-on red tilapia fillets were purchased from local fish company located in Jalan Kuchai Lama, Kuala Lumpur. Tilapia fillets were sealed in polyethylene bag, layered with crushed ice and transported to the Food Technology Research Centre, MARDI, Serdang. Fillets were then washed with tap water and trimmed to approximately 100 g each.

Packaging and storage of fillets

After drained for 5 min under chill condition (Protech, Model Chill-1050, Malaysia), the fillets

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were divided into four groups. Fillet (100 g) was laid on polystyrene tray and inserted in film bag before being flushed with 80% CO_2 : 20% N_2. Four types of film bags (size: 18 cm x 25 cm) were utilized: (1) linear low density polyethylene/ethylene vinyl alcohol/ linear low density polyethylene (LLDPE/EVOH/ LLDPE, 60 µm); (2) oriented nylon/polyethylene (ONy/PE, 70 µm); (3) oriented polypropylene/ polypropylene (OPP/PP, 60 µm); and (4) high density polyethylene (HDPE, 50 µm). The CO_2, O_2 and N_2 concentrations in the headspace of every 10th package were analyzed using Mocon Pac Check (Dual Head Space Analyser , Model 650, USA) to ensure that these packages contained the required gas mixtures. All samples were stored in chiller (Protech, Model Chill-1050, Malaysia) with temperature of 2 ± 2°C for 16 days.

Analysis of O_2 and CO_2 transmission rate of films

The O_2 transmission rate of films were measured using a Mocon Oxytran 2/20 following ASTM D 3985-02 method. Meanwhile CO_2 transmission rate of films were measured using Mocon Permatron C 200. All analysis were carried out under atmospheric conditions (21% O_2), temperature of 23°C and 0% relative humidity. Readings were recorded as cc/m^2/ day.

Sampling

Six samples of each treatment were withdrawn from refrigerated storage for evaluation at 0, 3, 6, 9, 12, 14 and 16 days. Two samples were used for chemical analyses, two for microbiological analyses, and the other two for sensory evaluations.

Chemical measurement

pH

The pH of samples were determined using a portable surface pH meter (IQ Scientific Instruments, Model IQ 150, Illinois, USA) Three readings were taken at different positions in each sample.

K-value

The K-value was determined by a colorimetric method (Fresh Test Transia) using a test strip containing two bands corresponding respectively to the evaluation of inosine (HxR) + hypoxanthine (Hx) (Band A) and inosine monophosphate (IMP)(Band B). A dorsal muscle sample (between 0.2 to 0.5 g) was homogenized in a mortar with 5 mL of buffer solution. The strip was immersed in the suspension and was then shaken so that a uniform film of liquid covered Band A and Band B. The strip was then placed in darkness at room temperature for 10-15 min. The colors of bands were then compared with those of the standard to determine the corresponding K-value (Malle and Isabelle 1992).

Microbiological analysis

Samples (10 g) of fillet were aseptically weighed, added a sterile quarter strength Ringer's solution (90 mL), and homogenized in a stomacher (Lab Blender 400, Seward Medical, London) for 60 s at room temperature. Decimal dilutions in quarter strength Ringer's solution were prepared, and 1 mL of the appropriate dilutions were pipetted into two petri dishes and poured in the following media: plate count agar (PCA) for total plate count (TPC), incubated at 7°C for 10 days (AOAC 1990); and MRS agar for lactic acid bacteria (LAB) incubated at 37°C for 3 days according to the method of Ordonez *et al*. (1991). Plates were counted and expressed as log cfu/g.

Sensory evaluation

Sensory evaluation of fillets were performed during storage by a sensory panel composed of 15 experienced members. They were required to evaluate the raw fillets based on the colour, odour, texture and overall acceptability using a 7-points hedonic scale: 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = like slightly, 6 = like moderately, and 7 = like very much.

Statistical analysis

The experiments were run in three replicate. Data obtained were subjected to the Analyses of Variance (ANOVA). The Duncan Multiple Range Test (DMRT) was used to determine significant differences between treatments and storage times. Statistical analysis was performed using the Statistical Analysis System (SAS).

Results and Discussion

O_2 and CO_2 transmission rate of films

Determining the transmission rate of packaging materials is critical to assuring package integrity. Without proper barriers, products fail to achieve desired shelf life. Major factors effecting the transmission rate of films include environmental relative humidity and temperature; partial pressure; Barometric pressure; and material thickness. Results showed in Table 1 indicated that LLDPE/EVOH/ LLDPE has the lowest transmission rate for both O_2 and CO_2, followed by ONy/PE, OPP/PP and HDPE. LLDPE/EVOH/LLDPE is three layers laminated

films where EVOH is a high barrier material; hence it has the highest barrier against O_2 and CO_2. Whereas HDPE is a single layer film which allowed O_2 and CO_2 to transmit through the film easily.

Table 1. O_2 and CO_2 transmission rate of films

Type of film	O_2 transmission rate (cc/cm^2/day)	CO_2 transmission rate (cc/cm^2/day)
LLDPE/EVOH/LLDPE, 60 µm	11.12	11.15
ONy/PE, 70 µm	58.90	319.50
OPP/PP, 60 µm	1106.00	4749.00
HDPE, 50 µm	3633.00	17469.50

Chemical measurement

pH

The results of the surface pH measurements were presented in Figure 1. From the results obtained, surface pH for fillets packaged in LLDPE/EVOH/LLDPE and ONy/PE significantly decreased (p<0.05) from 6.85 to 6.70 on the first 3 days compared to fillets packaged in OPP/PP and HDPE bags (from 6.85 to 6.77 and 6.82 respectively). The changes in surface pH will depend on the amount of CO_2 available within the package and absorbed by the fish tissue. The drop in pH can be explained by the surface reaction of CO_2 with water forming carbonic acid resulting in the acidification of the fillets (Bank *et al*., 1980). Fillets packaged in LLDPE/EVOH/LLDPE and ONy/PE with the lowest CO_2 transmission rate retained more CO_2 within packages thus lowering the surface pH of fillets significantly.

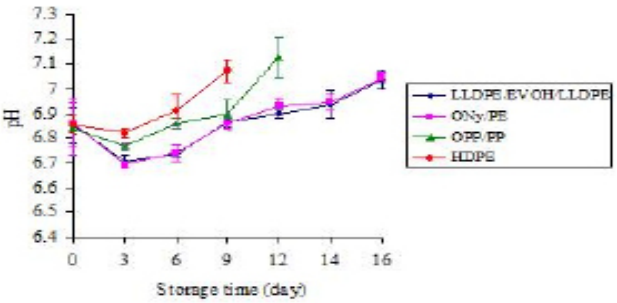

Figure 1. pH of tilapia fillets in different packaging films

After a substantial decrease over the first 3 days, the surface pH of the fillets in all treatments consistently rose from the 3rd day toward the end of the storage period. According to Wang and Brown (1983), the increase in pH at the later stage is associated with bacterial growth and is probably caused by the formation of basic amines. From Figures 3 and 4, higher counts for total microbial in the later stage explained why pH values increased in all samples. Statistically, the difference in pH values between the fillets packaged in LLDPE/EVOH/LLDPE and ONy/PE; with fillets in OPP/PP and HDPE were significantly different (p<0.05) as confirmed by analysis of variance. However, there were no significant differences (p>0.05) between fillets in LLDPE/EVOH/LLDPE and ONy/PE.

K-value

The freshness indicators, namely K-value of tilapia fillets were calculated from the concentration of nucleotide over the storage periods. The increases in the pattern of K-value for tilapia fillets held under four different packaging films are shown in Figure 2. Freshness or spoilage indicator related to the breakdown of nucleotides was based on the autolysis of adenosine triphosphate (ATP) in the muscle. The rapid rise of the K-value is entirely due to the sharp decline of inosine phosphate (IMP) in the fish flesh. The loss of IMP through degradation to inosine (HxR) and hypoxanthine (Hx) would cause a loss of fresh fish desirable compounds (Ozogul *et al*., 2004).

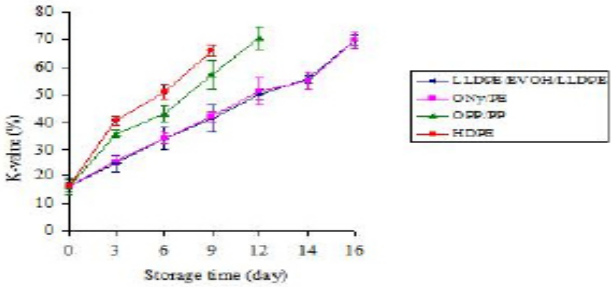

Figure 2. K-value of tilapia fillets in different packaging films

The K-value rose at a fairy rapid rate reaching over 66% from an initial value of 16% after 9 days of storage for HDPE-packaged fillets. For OPP/PP-packaged fillets, maximum K-value reached on the 12th day of storage. The slowest increase in K-value were observed on fillets packaged in LLDPE/EVOH/LLDPE and ONy/PE, which was possibly due to ability of these films to maintain higher quantity of CO_2 within the packages. There was a significant difference (p<0.05) between the treatments stored in HDPE and OPP/PP with those in LLDPE/EVOH/LLDPE and ONy/PE except at the initial stage. However, no significant (p>0.05) effect was observed in LLDPE/EVOH/LLDPE-packaged fillets with ONy/PE-packaged fillets.

Microbiological analysis

Figures 3 and 4 showed aerobic plate and lactic acid bacteria counts in tilapia fillets stored in LLDPE/EVOH/LLDPE, ONy/PE, OPP/PP, and HDPE at 2 ± 2^{0}C. Microorganisms grew most quickly in tilapia fillets stored in HDPE bags, followed by those in

OPP/PP, and the lowest counts were with LLDPE/EVOH/LLDPE and ONy/PE where the lag phase was apparently extended. One of the major mechanisms of MAP technique is to change the level of oxygen in the food environment so as to have an effect on the growth of different groups of microorganisms. Aerobic microorganisms are generally sensitive to CO_2; therefore, MAP delays the spoilage of fish. To minimize spoilage, the storage temperature of MAP products should be as low as possible since solubility of CO_2 decrease with an increase in temperature (Daniels *et al*., 1985). In the present study, significant differences ($p<0.05$) were observed between samples kept in HDPE and OPP/PP with those kept in LLDPE/EVOH/LLDPE and ONy/PE. Microbial counts in HDPE-packaged fillets remained consistently higher than that under various packaging films during storage, reaching a maximum on day 9. However, the fillets appeared spoiled before the 9th day of storage, based on a strong off-flavour and soft texture and presence of thick slime on the fillet surface. This finding was in accordance with previous investigations showing that bacterial growth can lead to the production of slim and changes in texture (Schirmer *et al*., 2009; Gram *et al*., 2002). Score from sensory evaluations also indicated that these fillets were accepted up to 6 days only. Fillets packaged in OPP/PP were acceptable up to 9 days, and 14 days for fillets packaged in LLDPE/EVOH/LLDPE and ONy/PE.

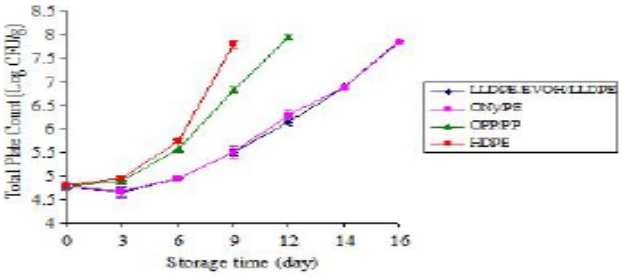

Figure 3. Total Plate Counts of tilapia fillets in different packaging films

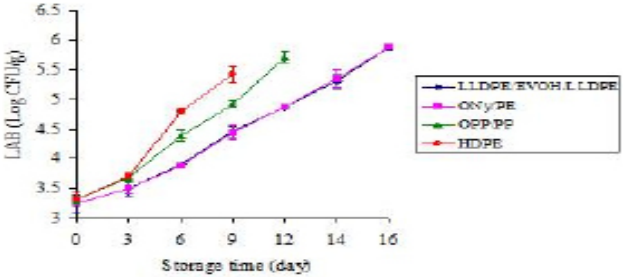

Figure 4. Lactic acid bacteria of tilapia fillets in different packaging films

Sensory evaluation

There was a significant effect of storage time on the sensory qualities of tilapia fillets packaged in four different types of films. The highest colour scores (>6.40) were given to the fillets at the 0 day (Figure 5) as compared to others. There were no significant changes ($p>0.05$) in colour on the first 3 days for the all samples evaluated. However, from the 6th day, colour score decreased significantly for HDPE and OPP/PP-packaged fillets to the unacceptable levels at the 9th and 12th day respectively (<4.0 point). As for LLDPE/EVOH/LLDPE and ONy/PE-packages samples, no significant differences were noticed up to 6 days of storage until panelists rejected the fillets at the 16th day.

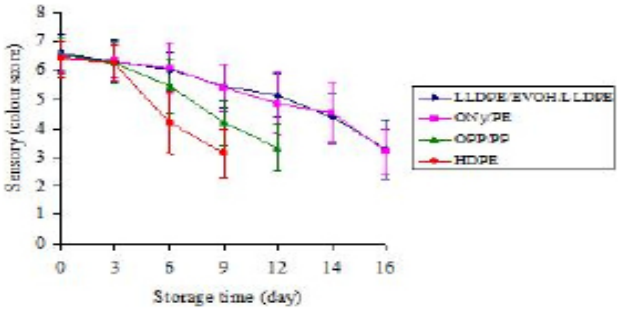

Figure 5. Colour score of tilapia fillets in different packaging films

There were significant changes in term of odour for all samples during storage period (Figure 6). When stored longer, an odour developed and the scores decreased significantly, the products continued to deteriorate ultimately having what is often described as an intense and putrid odour and this could be noticed at the 9th day in HDPE-packaged samples, at the 12th day for OPP/PP-packaged samples, and at the 16th day for the LLDPE/EVOH/LLDPE and ONy/PE-packaged samples. From Figure 3 and 4, very high microbial counts were noticed at the later stage of storage days and these could be due to the production of ammonia compounds from spoilage bacteria, resulting in the unacceptable odour.

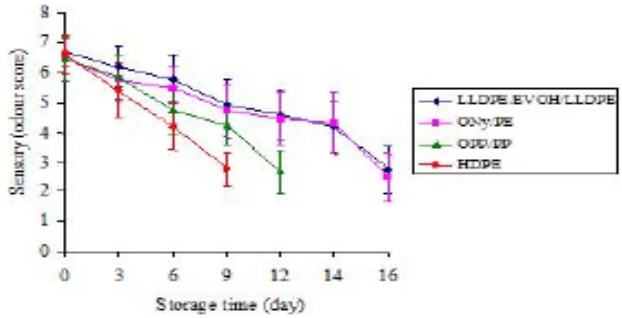

Figure 6. Odour score of tilapia fillets in different packaging films

Similar trends were also observed in texture and overall acceptability of tilapia fillets (Figure 7 and 8). Higher scores were given to all the treatments in the first few days of storage and when stored longer, scores given were subsequently lower. HDPE-packaged

samples showed the most marked changes. Fillets became tender; less succulent; less firm; less springy; less fibrous; stale, dull in appearance and produced unpleasant odour. These changes may have resulted from the effect of increasing pH on protein structure (Love *et al*., 1979) or from bacterial proteolysis (Shewan, 1974). Generally, HDPE-packaged fillets were accepted up to 6 days, 9 days for OPP/PP-packaged samples, and 14 days for LLDPE/EVOH/LLDPE and ONy/PE-packaged fillets.

Conclusions

Different packaging materials have different gas transmission rate, selection of the right packaging material in order to achieve desirable shelf life and to prevent two costly errors: under-packaging and over packaging; becomes the primary concern. From this study, LLDPE/EVOH/LLDPE and ONy/PE were found to be equally good in maintaining CO_2 within the packages and subsequently doubled the shelf life of MAP tilapia fillets. On the economy point of view, ONy/PE was recommended for uses in MAP of fishery produces since the cost of this material was lower as compared to LLDPE/EVOH/LLDPE.

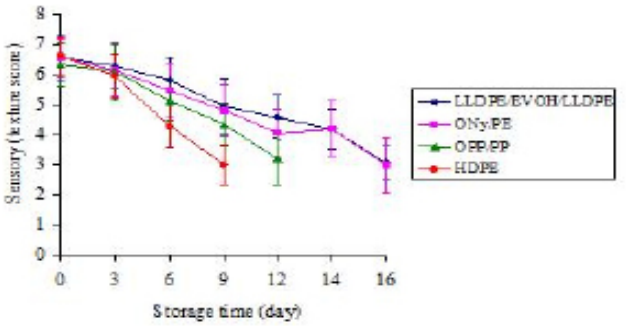

Figure 7. Texture score of tilapia fillets in different packaging films

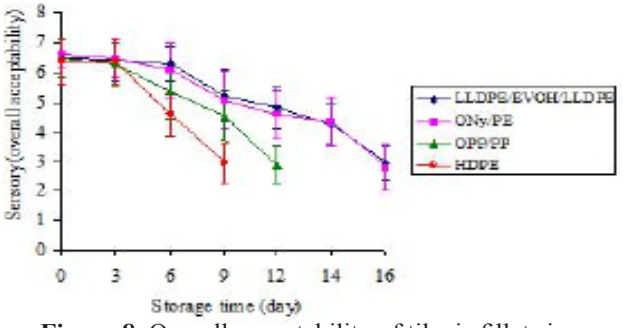

Figure 8. Overall acceptability of tilapia fillets in different packaging films

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