Asian Fisheries Science **20**(2007):359-368
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2007.20.4.003

Asian Fisheries Society, Manila, Philippines

Shelf Life of Modified Atmosphere Packaged Fresh Grouper (*Epinephelus* sp.) Fillets

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Abstract

The effect of two modified atmosphere (MA) conditions (80% CO_2 : 20% N_2 and 60% CO_2 : 40% N_2) on the shelf life of grouper fillets (*Epinephelus* sp.) stored at refrigeration temperature (2 ± 2 ^oC) were studied. Chemical and microbiological changes, and sensory alterations were monitored. Values were compared with those obtained from 100% air-stored samples. Modified atmosphere conditions slowed down the deterioration rate of grouper fillets. Results from chemical and microbiological analyses showed that fillets under MA were significantly better than those under 100% air-stored fillets throughout all storage periods, and sensory properties scored significantly ($p<0.05$) higher. The shelf life of fillets packaged in 80% CO_2 : 20% N_2 and 60% CO_2 : 40% N_2 were 18 and 14 days respectively, and seven days for 100% air-stored fillets.

Introduction

A satisfactory method for extending the shelf life of fish and shellfish has long been sought to ensure quality, continuity of supply and minimal wastage. Fresh sea foods stored in ice undergo a series of sensory changes which will gradually reduce the quality until rejected by consumers. Since the shelf life of fresh sea foods on ice is limited, there is an urgent need to develop technologies which will prolong the shelf life of these products.

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Modified atmosphere packaging (MAP) using carbon dioxide (CO_2) to replace or partially replace the air surrounding the fresh product is used to extend the shelf life of certain animal foods. The mode of action of CO_2 in delaying the onset of spoilage in fresh muscle foods is believed to be due to the inhibition of psychrotrophic, aerobic, Gram-negative spoilage bacteria (Sutherland et al. 1977; Gill and Tan 1980).

The Malaysian aquaculture industry has experienced rapid growth and market expansion over the last decade. This industry provided about 11% of the total fish production in Malaysia and is expected to be the main potential sector to meet the increasing demand for fish. In line with the government-backed policy of increasing the consumption of freshwater fishes and considering Malaysia's distance from the major markets, there would be obvious advantages if such effort in extending the shelf life could be achieved.

Carbon dioxide has been used to extend the shelf life of fish and other seafood products (Banks et al. 1980; Brown et al. 1980; Veranth and Robe 1979). These reports described a primary system for bulk transportation or storage. Little information, however, is available on the potential use of CO_2 at the retail level. The objective of this study was to determine the potential of preserving fresh grouper fillets in modified gaseous atmospheres by monitoring the chemical, microbiological and sensory changes of this product in retail packages containing carbon dioxide and nitrogen.

Materials and Methods

Preparation of samples and packaging procedures

Fillets of fresh brackishwater cage-raised grouper were obtained immediately after processing from the Asealot Aquaculture Sdn. Bhd., Jalan Kuchai Lama, Kuala Lumpur. Iced fillets were washed and trimmed to approximately 120–150 g each upon arrival at the Food Technology Research Centre, MARDI, Serdang.

Trimmed fillets were packaged in retail-type tray package employing a polystyrene tray and three layer high barrier flexible bags (linear low density polyethylene/ ethylene vinyl alcohol/ linear low density polyethylene). Each film bag containing fillet was first evacuated and then packaged under MA of 80% CO_2 : 20% N_2, 60% CO_2 : 40% N_2 and 100% air which served as control. The CO_2 and N_2 concentrations in the headspace of

every 10th package were analysed using Mocon Pac Check (Dual Head Space Analyzer Model 650, USA) by inserting a needle connected to the outer barrier film to ensure that these packages contained the required MA. All packages were stored at 2 ± 2 ^{o}C.

Sampling

Six samples of each treatment were withdrawn from refrigerated storage for evaluation at predetermined intervals. Two samples were used for chemical measurements, two for microbiological analysis and the other two for sensory evaluations.

Chemical measurement

pH

The pH of samples were determined using a Hana Instrument pH meter on 5 g of flesh homogenized with 45 ml of CO_2 free distilled water (Lim 1987).

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 g and 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

Total aerobic counts on the flesh were determined using the pour plate method according to AOAC (1990). Duplicate samples (about 10 g) were taken from the tail-end of each fillet at predetermined intervals. Samples were placed in a sterile stomacher bag and homogenized with 90 ml Ringer's solution in the Seward Stomacher (400 Lab Blender) to give 10^{-1} dilution. Further 10-fold serial dilutions were made as required using the same diluent. One ml of appropriate dilution was pipetted into two plates and molten standard plate count agar (cooled to 42–45^{0}C) was then poured in. Plates were incubated at 37^{0}C for 48 h. Total coliform in the homogenate was determined by a pour plate method (AOAC 1990), using violet

red bile agar and incubated at 37^0C for 48 h. Plates were counted and expressed as log CFU g^{-1} sample.

Sensory evaluation

Sensory evaluation was performed by 15 trained panelists. They were required to evaluate the raw fillets based on the colour, odour (from no odour to strong off-odour), texture (from firm to soft) and overall acceptability using a seven-point hedonic scale. Scores below four points were considered unacceptable.

Statistical analysis

Data were analysed statistically using Analysis of Variance Method at 5% level (ANOVA). Duncan Multiple Range Test (DMRT) was used to determine significant difference between treatments and storage times. The statistical program used was Statistical Analysis System (SAS).

Results and Discussion

pH

The results of the pH measurements are presented in figure 1. Fresh grouper fillets had a pH of 6.82. After 3 days of storage, pH of the samples stored in 100% air and MA decreased to 6.76 and 6.71 to 6.73, respectively. A possible explanation for the decrease in pH value of fillets stored in 100% air is the production of CO_2 in the enclosed packages by microbial and tissue respiration. When CO_2 dissolved in the moisture at tissue surface, this resulted in the drop of pH due to the formation of carbonic acid (Lannelongue et al. 1982).

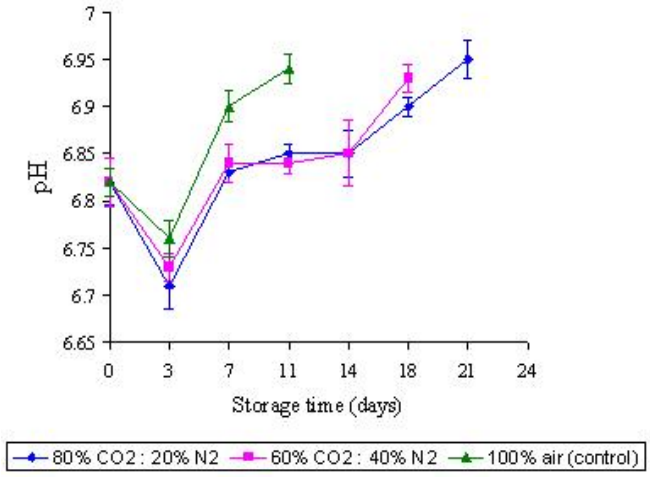

Figure 1. pH of grouper fillets

After the initial decline, pH of all samples subsequently increased. From the 7th day and onward, pH values of 100% air control fillets were significantly higher than the MA stored samples ($p<0.05$). However, percentages of CO_2 (60 and 80%) in the packages had no significant effect on the pH value of fillets. Although pH cannot be used as a reliable index of the state of freshness or onset of spoilage of seafood, increases in pH value, however, are usually observed with advanced bacterial spoilage, presumable due to the production of basic amines (Wang and Brown 1983).

K-value

The freshness indicators, namely K-value of grouper fillets were calculated from the concentration of nucleotide over the storage periods. The increases in the pattern of K-value for grouper fillets held under three storage conditions are shown in figure 2. Freshness or spoilage indicator related to the breakdown of nucleotides is 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).

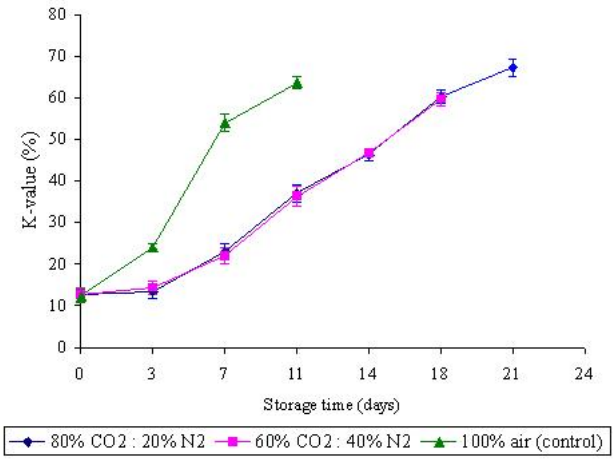

Figure 2. K-value of grouper fillets

The K-value was 60% from an initial value of 12% after 11 days of storage for 100% air control samples, 18 days for samples packaged under 60% CO_2 : 40% N_2; and 21 days for fillets with 80% CO_2 : 20% N_2. The slowest increase in K-value was observed from grouper stored in MA, which was possibly influenced by the presence of CO_2. This is in agreement with previous studies with barramundi fillets (Siah and Mohd Ariff 2002). There was a significant difference ($p<0.05$) between the treatments stored in 100% air and MA except at the initial stage. However, no signifi-

cant effects were observed on fillets with 60% and 80% CO_2. These results were in agreement with the observations of Reddy et al. (1992).

Microbiological analysis

Figures 3 and 4 show aerobic plate and coliform counts in grouper fillets stored in 100% air, 60% CO_2 : 40% N_2 and 80% CO_2 : 20% N_2, at 2 ± 2 ^{0}C. Microorganisms grew most quickly in grouper fillets stored in 100% air, followed by those in 60% CO_2 : 40% N_2, and the lowest counts were with 80% CO_2 : 20% N_2 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).

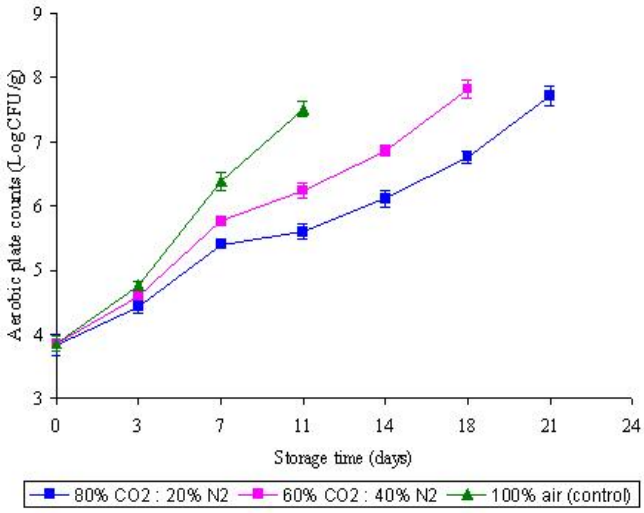

Figure 3. Aerobic plate counts of grouper fillets

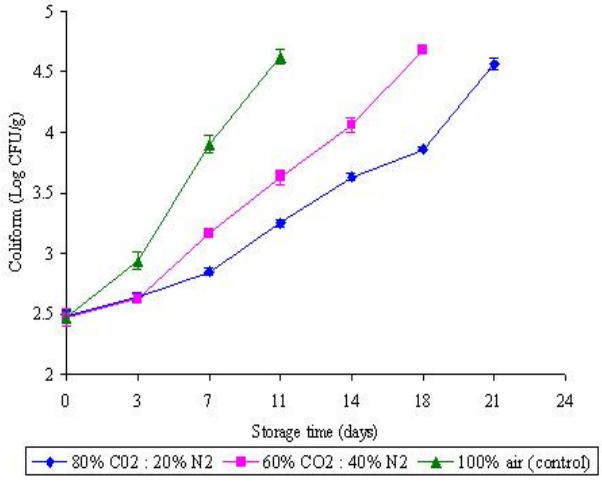

Figure 4. Coliform counts of grouper fillets

Pastroriza et al. (1996) reported that significant differences were found between control (air) and MAP stored samples in terms of bacterial counts. In the present study, significant differences ($p<0.05$) were also observed between samples kept in 100% air, samples in 60% CO_2 : 40% N_2, and samples in 80% CO_2 : 20% N_2. Microbial counts in fillets packaged under 100% air remained consistently higher than those under various MA during storage, reaching a maximum on day 11. However, the fillets appeared spoiled before the 11th day of storage, based on a strong off-flavor and soft texture and presence of thick slime on the fillet surface. Scores from sensory evaluations also indicated that these fillets were accepted up to 7 days only. Fillets packaged under 60% CO_2 : 40% N_2 and 80% CO_2 : 20% N_2 reached the maximum microbial loads at the 18th and 21st days respectively.

Sensory evaluation

Fresh raw fillets had a firm texture, pink color and had no off-odor. The colour, odour, texture and overall acceptability of control and all MA fillets changed with length of storage. Fillets packaged under 100% air were spoiled at 11 days as indicated by the sensory characteristics (Figs. 5 to 8*)*. The strong off-flavour associated with spoiled fishery products result when metabolites are released by bacterial activities (Lannelongue et al. 1982; Reddy et al. 1992). Increased levels of CO_2 from 60 to 80% delayed spoilage of MA fillets from 14 to 18 days (i.e. the shelf life was 7 – 11 days longer than that of controls (Table 1).

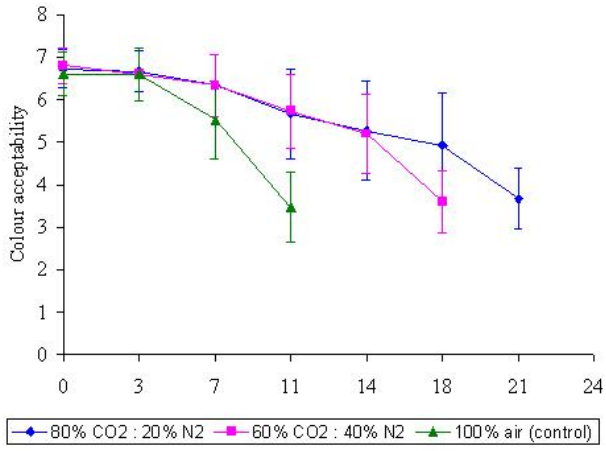

Figure 5. Colour acceptability of grouper fillets

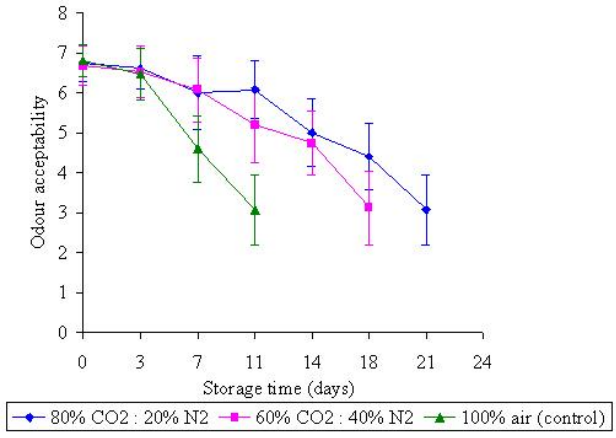

Figure 6. Odour acceptability of grouper fillets

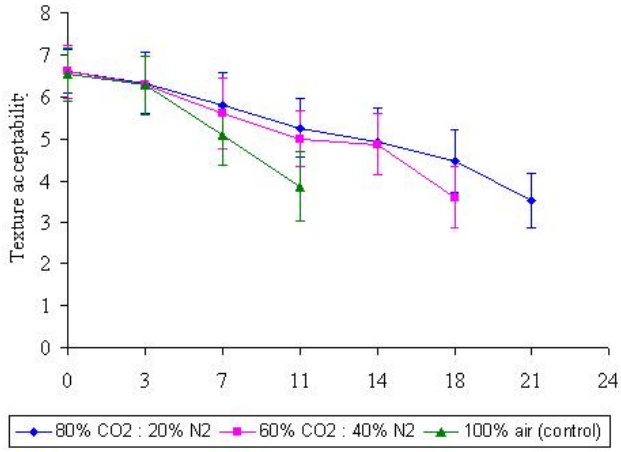

Figure 7. Texture acceptability of grouper fillets

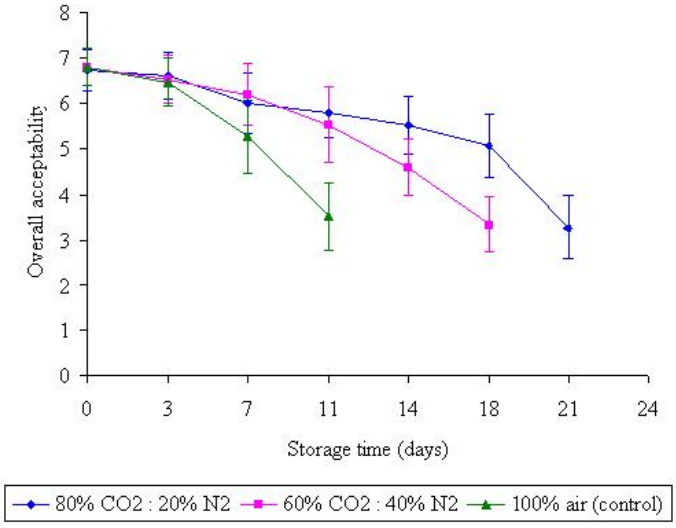

Figure 8. Overall acceptability of grouper fillets

Table 1. Shelf life of grouper fillets

Treatment	Shelf life (day)	Shelf life extension (%)
Control	7	-
60% CO_2 : 40% N_2	14	100
80% CO_2 : 20% N_2	18	157

Conclusions

Modified atmosphere packaging systems have been shown to provide a technological avenue worth pursuing in their application to the handling of fresh seafood products. As a result of this and previous studies, dramatic extensions of shelf life have been observed for fillets held under such system. Packaging of fresh grouper fillets in high barrier film bags under MA of 60% CO_2 : 40% N_2 and 80% CO_2 : 20% N_2, and storing them at refrigeration temperature could extend their shelf life to >100% compared to air packaged fillets.

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Received July 7, 2006; Accepted February 10, 2007