

## **Effect of modified atmosphere packaging and potassium sorbate on chemical, microbiological and sensorial properties of grouper (*Epinephelus* sp.) fillets**

[Kesan pembungkusan atmosfera terubah suai dan kalium sorbat terhadap ciri kimia, mikrobiologi dan penilaian deria filet ikan kerapu (*Epinephelus* sp.)]

W.M. Siah\* and W. Mohd. Ariff\*

Key words: fillet, potassium sorbate, modified atmosphere packaging, refrigerated storage

### **Abstract**

The effect of an optimum gas mixture (80% CO<sub>2</sub> : 20% N<sub>2</sub>) on grouper fillets (*Epinephelus* sp.) with combination of potassium sorbate (K-sorbate) dip (1 min in 1% K-sorbate solution) were studied. pH, K-value, microbial growth, as well as sensory alterations during storage at 2 ± 2 °C were monitored. Values were compared with those obtained from the control (100% air-stored) and modified atmosphere packed (MAP) samples. Potassium sorbate dips showed a further inhibition of biochemical, microbiological and sensory deterioration of MAP-stored grouper fillet. pH, K-value, and total plate counts of MAP + K-sorbate treated fillets were significantly lower ( $p < 0.05$ ) than MAP and 100% air-stored fillets throughout the storage periods. Consequently, the shelf life of grouper fillets under 100% air was 7 days, extended to 18 days when stored under MAP conditions, and 24 days when dipped in potassium sorbate solutions prior to MAP storage.

### **Introduction**

Fish is one of the most highly perishable food products and the shelf life of such products is limited in the presence of normal air 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 nitrogen, has been shown to significantly prolong the shelf life of perishable food products at chill temperatures (Parry 1993; Dhananjaya and Stroud 1994; Reddy et al. 1995; Siah and Mohd. Ariff 2002). 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). Modified atmosphere packaging (MAP), along with refrigeration, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution, and marketing of raw and processed products to meet consumer demands.

The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in colour,

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\*Food Technology Research Centre, MARDI Headquarters, Serdang, P.O. Box 12301, 50774 Kuala Lumpur, Malaysia

Authors' full names: Siah Watt Moeey and Mohd. Ariff Wahid

E-mail: wmsiah@mardi.my

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odour and texture. Inhibition of the growth of these microorganisms and increase in the lag phase of facultative and aerobic microorganisms results in an increase in the potential shelf life of MAP products which is one of the main benefits of MAP technology. Nevertheless, there is a safety concern with extended shelf life because of the growth of pathogenic organisms in this packaging system (Farber 1991; Church and Parsons 1995). Pretreatment before MA storage has been studied to minimize the risk of foodborne illness and at the same time improve the keeping quality and shelf life of fresh seafood products. Potassium sorbate (K-sorbate) protects against spoilage and pathogenic organisms and inhibits the growth of trimethylamine producing bacteria in fresh fish.

The purpose of this experiment is to assess the effect of MAP and the efficacy of a potassium sorbate dip, when used in conjunction with 80% CO<sub>2</sub> : 20% N<sub>2</sub> atmosphere to extend the refrigerated-storage life of grouper fillets. Quality attributes were assessed by different methods including chemical, microbiological and sensory evaluation.

## **Materials and methods**

### ***Fish source***

Fillets of fresh brackishwater cage-raised grouper were obtained from 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.

### ***Preparation of samples and packaging procedures***

For allocation to treatments, the fillets were divided into three lots. Each treatment consisted of 162 bags. The three treatments included: 1) MAP + K-sorbate dip: 1% potassium sorbate solutions were prepared by dissolving K-sorbate in distilled water. The pH of the solutions was adjusted to 6.8 using acetic acid. The fillets were immersed

in the solutions for 1 min. Dip solutions were changed after every 10 fillets. Fillets were allowed to drain for 5 min before being packaged (Siah and Mohd. Ariff 2003). 2) MAP, and 3) 100% air (control). Each fillet was placed on a polystyrene foam tray (12 cm x 20 cm) and inside a three layer high barrier film bag (linear low density polyethylene/ethylene vinyl alcohol/ linear low density polyethylene, 20 cm x 30 cm). Each film bag containing fillet for first and second treatments was first evacuated and then packaged under MA of 80% CO<sub>2</sub> : 20% N<sub>2</sub>. The CO<sub>2</sub> and N<sub>2</sub> concentration in the headspace of every 10th package was 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. Whereas for the third treatment (control), bags were sealed without any treatment. All packages were stored at 2 ± 2 °C.

### ***Sampling***

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

### ***Chemical measurement***

**pH** The pH of samples was determined using a Hana Instrument pH meter on 5 g of flesh homogenised with 45 ml of CO<sub>2</sub> 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 and 0.5 g) was homogenised 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 colours 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 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 dilutions was pipetted into two plates and molten standard plate count agar (cooled to 42–45 °C) was then poured in. Plates were incubated at 37 °C for 48 h. Plates were counted and expressed as log CFU/g sample.

#### ***Sensory evaluation***

Sensory evaluation was performed by 15 trained panellists. 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 7-point hedonic scale. Scores below 4 points were considered unacceptable.

#### ***Statistical analysis***

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

## **Results and discussion**

### ***Chemical measurement***

**pH** Fresh grouper fillet had a pH of 6.81. A significant decrease ( $p < 0.05$ ) of pH was observed in all treatments after 3 days of storage (*Figure 1*). A decrease of pH in fillets under MAP + K-sorbate and MAP treatments was due to the absorption and dissolution of filled CO<sub>2</sub> in the moisture at tissue surface resulted in the formation of carbonic acid.

A possible explanation for the decrease in pH of fillets stored in 100% air (control) is the production of CO<sub>2</sub> by microbial and tissue respiration in the enclosed packages. Subsequently, this gave similar effect as in the MAP stored sample of brown shrimp (Lannelongue et al. 1982). However, the drop in pH value in the control sample was not as great as the MAP + K-sorbate and MAP samples. After the initial decline, pH of all samples subsequently increased. pH values of fillets that were stored under MAP + K-sorbate and MAP conditions were significantly lower ( $p < 0.05$ ) than the control throughout the whole storage period.

There were no significant difference between pH values of MAP + K-sorbate samples and MAP samples up to 11 days of storage. However, from 14 days onward, the pH of fillets with potassium sorbate dips reduced significantly ( $p < 0.05$ ). Results showed that the treatment of fish fillet in K-sorbate prior to refrigerated storage acts synergistically with MAP on pH of fish muscle. This is thought to be a result of the effect of both factors on microbial growth. 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.

**K-value** Increase in the pattern of freshness indicators, namely K-value is shown in *Figure 1*. Freshness or spoilage indicators related to the breakdown of nucleotides are based on the autolysis of adenosine triphosphate (ATP) in the muscle. The rapid rise of the K-value is

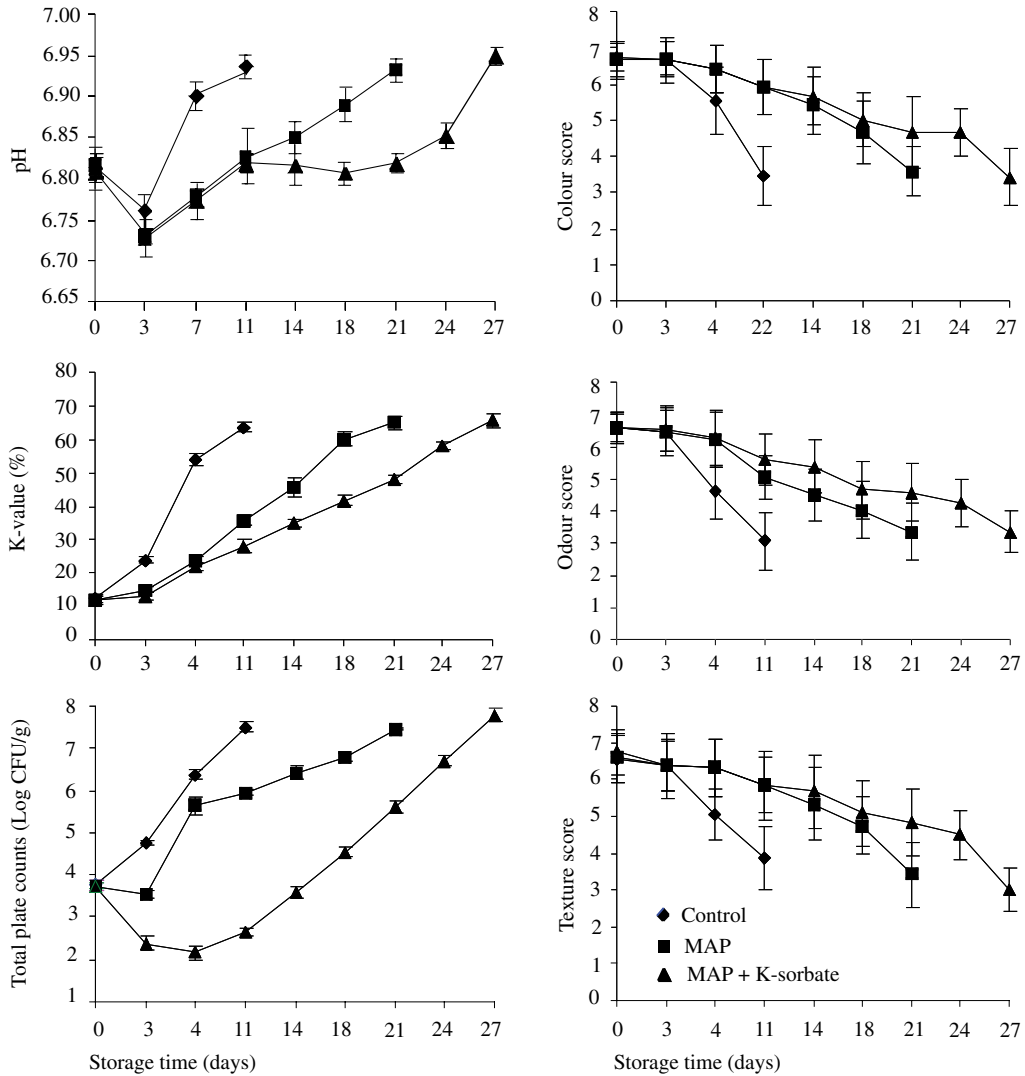


Figure 1. pH, K-value, total plate counts, colour score, odour score and texture score of grouper fillets

entirely due to the sharp decline of inosine monophosphate (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).

The K-value rose at a fairly moderate rate reaching over 60% from an initial value of 12% after 11 days of storage for control samples, 18 days for MAP samples, and 27 days for fillets with MAP + K-sorbate. Generally, K-value for fillets treated with K-sorbate were significantly lower ( $p < 0.05$ )

than samples without K-sorbate from 11 days and onward. Thus, K-sorbate dip seemed to improve the freshness of the grouper fillets. This is in agreement with previous studies with barramundi fillets (Siah and Mohd. Ariff 2003).

**Microbiological analysis**

Bacteria grew most quickly in grouper fillets stored in air (control), followed by those in MAP (Figure 1). The lowest counts were with MAP + K-sorbate where the lag phase was apparently extended. One of the major

mechanism of MAP techniques is to change the level of oxygen in a food environment so as to have an effect on the growth of different groups of microorganisms. Aerobic microorganisms are generally sensitive to CO<sub>2</sub>; therefore, MAP delays the spoilage of fish.

Huss (1972) indicated that CO<sub>2</sub> has an important effect on microbial growth, exerting a selective inhibitory action. To achieve microbiological benefits, the storage temperature of MAP products should be as low as possible, since solubility of CO<sub>2</sub> decreases with an increase in temperature (Daniels et al. 1985). K-sorbate protects against spoilage and pathogenic organisms and inhibits the growth of trimethylamine-producing bacteria in fresh fish (Pedrosa and Regenstein 1990). Its antimicrobial activity is in the undissociated form of sorbic acid and is pH dependent. According to Khuntia et al. (1993), K-sorbate retards microbial activity by inhibiting various enzymes of the microbial cell.

When the TPC reaches log 6–7 CFU (Colony Forming Units) per gramme in food products, it is assumed to be at or near spoilage. Siah and Mohd. Ariff (2002) reported that initial TPC of MAP barramundi fillet was log 4.5 CFU/g, reaching the limit counts of log 7.0 CFU/g at day 14. In this study, the limit of acceptability (log 6–7 CFU/g) in terms of TPC was 7 days for grouper fillet stored in 100% air, 18 days for MAP, and 24 days for MAP + K-sorbate.

Microbial counts in fillets packaged under 100% air remained consistently higher than that under MAP and MAP + K-sorbate during storage reaching a maximum on day 11. However, the fillets appeared spoiled before the 11th day of storage, based on a strong off-flavour and soft texture and presence of thick slime on the fillet surface. Score from sensory evaluations also indicated that these fillets were accepted up to 7 days only.

Bacterial counts of MAP stored fillets increased significantly ( $p < 0.05$ ) after 7 days of storage, but this was avoided if

fillets were previously dipped in K-sorbate solutions. K-sorbate further inhibited bacterial growth with no significant increases of TPC during the first 2 weeks of storage.

### **Sensory evaluation**

There was a significant effect of storage time on the sensory qualities of grouper fillets. High scores (>6.66) were given to the colour of fillets on day 0 (*Figure 1*). There were no significant differences ( $p < 0.05$ ) for the first 3 days for the 100% air-stored samples. From the 7th day, colour score decreased significantly and to the unacceptable levels at the 11th day (<4.0 point). As for MAP and MAP + K-sorbate samples, no significant difference were noticed up to 7 days of storage. Panellists rejected the fillets in terms of colour at the 21st day for MAP and at the 27th day for MAP + K-sorbate samples.

There were no significant changes in terms of odour for 100% air-stored samples for the first 3 days of storage, and for MAP and MAP + K-sorbate samples up to 7 days (*Figure 1*). However, when they were stored longer, an odour developed and the scores decreased significantly ( $p < 0.05$ ). The products continued to deteriorate ultimately having what is often described as an intense and putrid odour and this could be noticed on the 11th day for 100% air-stored samples, 21st day for MAP samples, and 27th day for the MAP + K-sorbate samples. 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.

Similar trends were also observed in texture and overall acceptability of grouper fillets (*Figures 1 and 2*). Higher scores were given to all treatments in the first few days of storage and when stored longer, scores given were subsequently lower. 100% air-stored samples showed the most marked changes. Fillets became more tender, less succulent, less firm, less springy,

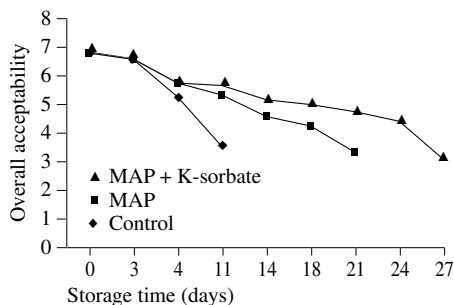


Figure 2. Overall acceptability of grouper fillets

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, 100% air-stored fish were rejected by sensory evaluation at 11 days of storage, 21 days for MAP samples, and up to 27 days for samples dipped in K-sorbate solutions prior to MAP storage.

### Conclusion

When K-sorbate was combined with MAP, a delay in chemical, microbiological and sensorial alterations was observed. Chemical and microbiological levels were significantly lower when MAP stored samples had been previously dipped in K-sorbate. K-sorbate dipping had significantly increased the time of storage as MAP stored samples were rejected due to the development of off-odours. 100% air stored samples had a shelf life of 7 days, 18 days for MAP samples, and 24 days for MAP + K-sorbate samples.

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### Abstrak

Kesan campuran gas optimum (80% CO<sub>2</sub> : 20% N<sub>2</sub>) dan celupan ke dalam larutan kalium sorbat (selama 1 minit di dalam 1% larutan kalium sorbat) terhadap filet ikan kerapu (*Epinephelus* sp.) telah dikaji. Perubahan dari segi nilai pH, K-value, pertumbuhan mikroorganisma dan penilaian deria dikaji semasa penyimpanan pada suhu 2 ± 2 °C. Nilai yang diperoleh dibandingkan dengan yang diperoleh daripada filet kawalan (100% udara) dan filet yang disimpan dalam atmosfera terubah suai (MAP). Rawatan dengan kalium sorbat menghalang perubahan yang ketara dari segi ciri kimia, mikrobiologi dan penilaian deria bagi filet dalam MAP. Nilai pH, K-value, Jumlah kiraan plat dalam filet MAP + K-sorbat adalah lebih rendah secara bererti ( $p < 0.05$ ) berbanding dengan filet di dalam 100% udara dan filet MAP semasa penyimpanan. Manakala untuk penilaian deria, filet dalam MAP + K-sorbat memperoleh skor yang lebih tinggi berbanding dengan filet di dalam MAP dan 100% udara. Secara kesimpulan, jangka hayat di dalam 100% udara ialah 7 hari, dilanjutkan 18 hari apabila disimpan dalam keadaan MAP, dan selama 24 hari apabila filet tersebut dicelup ke dalam larutan K-sorbat sebelum disimpan secara MAP.