

Effect of potassium sorbate dips on refrigerated storage of modified atmosphere packed barramundi (*Lates calcarifer*) filets

[Kesan celupan larutan kalium sorbat terhadap penyimpanan sejuk dingin filet ikan siakap (*Lates calcarifer*) dalam atmosfera terubahsuai]

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

Key words: fillet, potassium sorbate, modified atmosphere packaging, shelf life extension, barramundi (*Lates calcarifer*)

Abstrak

Kesan pelbagai kombinasi kepekatan dan masa celupan larutan kalium sorbat terhadap filet ikan siakap yang disimpan dalam keadaan atmosfera terubahsuai (80% CO₂ : 20% N₂) pada 2 ± 2 °C, telah dikaji. Filet ikan dibungkus dalam sistem tertutup dengan menggunakan beg plastik yang bertelapan rendah. Ujian fizikal, kimia, mikrobiologi dan penilaian deria terhadap filet menunjukkan bahawa pembungkusan atmosfera terubahsuai dan celupan 1 minit di dalam larutan kalium sorbat berkepekatan 1%, paling berkesan bagi memanjangkan jangka simpan filet ikan siakap. Jangka simpan sampel kawalan iaitu filet dalam keadaan atmosfera 80% CO₂ : 20% N₂ adalah selama 14 hari, manakala yang dicelup ke dalam larutan kalium sorbat selama 24 hari.

Abstract

The effects of various concentrations of potassium sorbate and dipping time on the barramundi filets during storage under modified atmosphere conditions (80% CO₂ : 20% N₂) at 2 ± 2 °C, for a specified period under a closed system in high-barrier flexible bags were studied. Physical, chemical and microbiological measurements, and sensory observations indicated that a preservation procedure consisting of modified atmosphere plus dipping filets in a 1% potassium sorbate solution for 1 minute greatly extends the shelf life of barramundi filets. Shelf life of control sample packed under 80% CO₂ : 20% N₂ conditions was 14 days, whereas fillet with dipping treatment was 24 days.

Introduction

Fresh fish and other seafood products are perhaps the most susceptible to postmortem deterioration compared to other muscle foods consumed in the world today, thus, extension of the shelf life of fresh seafood products would be very advantageous to the industry. This could result in increasing the

marketing range of fresh products and in enabling greater amounts of seasonal surplus to be marketed fresh. Modified atmosphere packaging (MAP) using carbon dioxide (CO₂) 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 CO₂ in delaying the

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

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

E-mail: wmsiah@mardi.my

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onset of spoilage in fresh muscle food is believed to be due to the inhibition of psychrotrophic aerobic, gram-negative spoilage bacteria (Sutherland et al. 1977; Gill and Tan 1980).

The application of MAP technology to seafood has received widespread attention. In conjunction with refrigeration, MAP has doubled the shelf life of fresh or minimally processed seafood products (Statham 1984; Reddy et al. 1992) and has potential for use at the retail level. However, MA provided conditions for the growth of gram-positive bacteria and food pathogens within the package at the same time. The extension of the storage life of the refrigerated MA products may enable the slower-growing gram-positive bacteria to reach high population (Conner et al. 1989).

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), a compound generally recognized as safe (GRAS) in the United States, protects against spoilage and pathogenic organisms and inhibits the growth of trimethylamine (TMA)-producing bacteria in fresh fish. Debevere and Voets (1972) showed 0.135% of K-sorbate almost completely inhibited the spoilage of cod fillets for 6 days by slowing the growth of bacteria capable of producing TMA. Chung and Lee (1981) found the presence of 1.0% K-sorbate extends the lag phase to over 6 days at 0 °C.

The purpose of this experiment was to assess the efficacy of a K-sorbate dip when used in conjunction with 80% CO₂ : 20% N₂ atmosphere, to extend the refrigerated storage life of barramundi (*Lates calcarifer*) fillets.

Materials and methods

Fish source

Fillets of fresh brackishwater cage-raised barramundi (1 year old) were obtained immediately after processing from Asealot

Aquaculture Sdn. Bhd., Jalan Kuchai Lama, Kuala Lumpur. Iced fillets were washed and trimmed to approximately 180–200 g each upon arrival at the Food Technology Research Centre, MARDI, Serdang,

Preparation of samples

A 0%, 1% and 2% potassium sorbate (K-sorbate) solutions were prepared by dissolving K-sorbate in distilled water. pH of the solutions were adjusted to 6.8 using acetic acid. The fillets were immersed in the solutions for 1 min and 2 min at room temperature. Dip solutions were changed after every 10 fillets. Fillets were allowed to drain for 5 min before being packaged in retail-type tray package employing a polystyrene tray and high barrier flexible bags. Each film bag containing fillets was first evacuated and then packaged under a modified atmosphere of 80% CO₂ : 20% N₂. The concentration of CO₂ and N₂ of every 10th package were determined using Mocon Pac Check (Dual Headspace Analyzer, Model 650, USA) to ensure that packages contained the required MA. All packages were stored in chiller with temperature of 2 ± 2 °C.

Sampling

Six different treatments were employed (Table 1). Six samples of each treatment were withdrawn from refrigerated storage for evaluation at predetermined intervals. Two samples were used for physical and chemical measurements, two for microbiological analysis and the other two for sensory evaluations.

Table 1. Summary of treatments

Dipping time (min)	Concentration of K-sorbate (%)	Sample code
1	0	T1C0
1	1	T1C1
1	2	T1C2
2	0	T2C0
2	1	T2C1
2	2	T2C2

Physical measurement

Weight loss Weight loss was calculated as the per cent difference between the weights of each individual fillet at the beginning of the storage period and at the time of sampling.

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 CO₂ 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 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 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 °C) was then poured in. Plates were incubated at 37 °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 °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 (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

Weight loss

The weight loss due to drip was shown in *Figure 1*. Results indicated that there were no significant differences ($p < 0.05$) between treatments in all sampling days. Less than 3% weight loss were noticed in fillets after 3 days of storage and these values were observed to slowly increase to 6.2–6.4% at the end of storage period. Fish in general seem to have high drip level, running as high as 3–8% and a longer storage period might have a more marked effect (Connell 1975). There were also no significant differences between treatments with or without K-sorbate dip.

pH

The initial pH of the barramundi fillets in all treatments were in the range of 6.80 to 6.87 (*Figure 2*). After 3 days of storage, pH values decreased to 6.70–6.75 and maintained for a few days before these values increased again. As observed in samples T1C0 and T2C0, pH values were

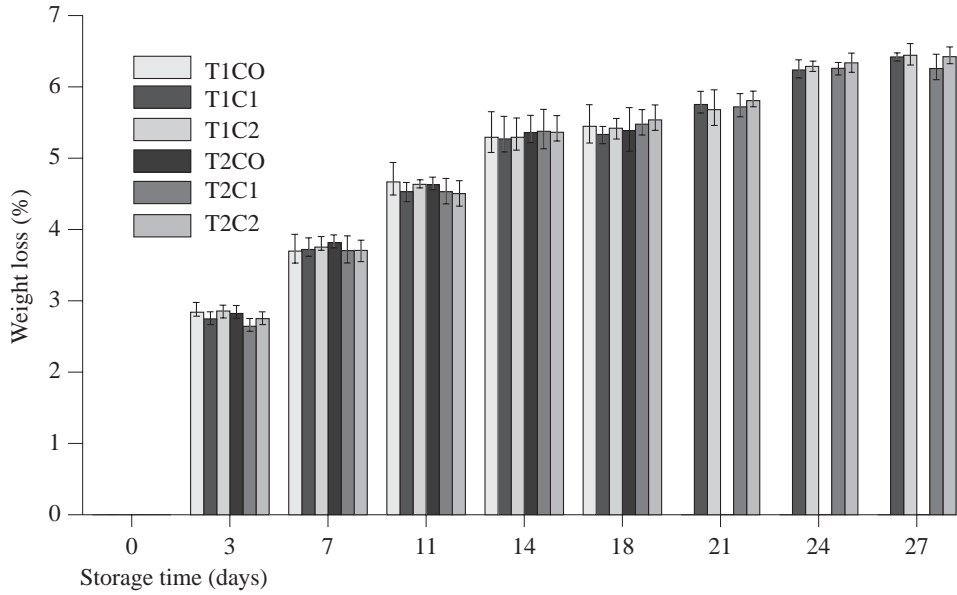


Figure 1. Changes in weight loss of barramundi fillets during storage

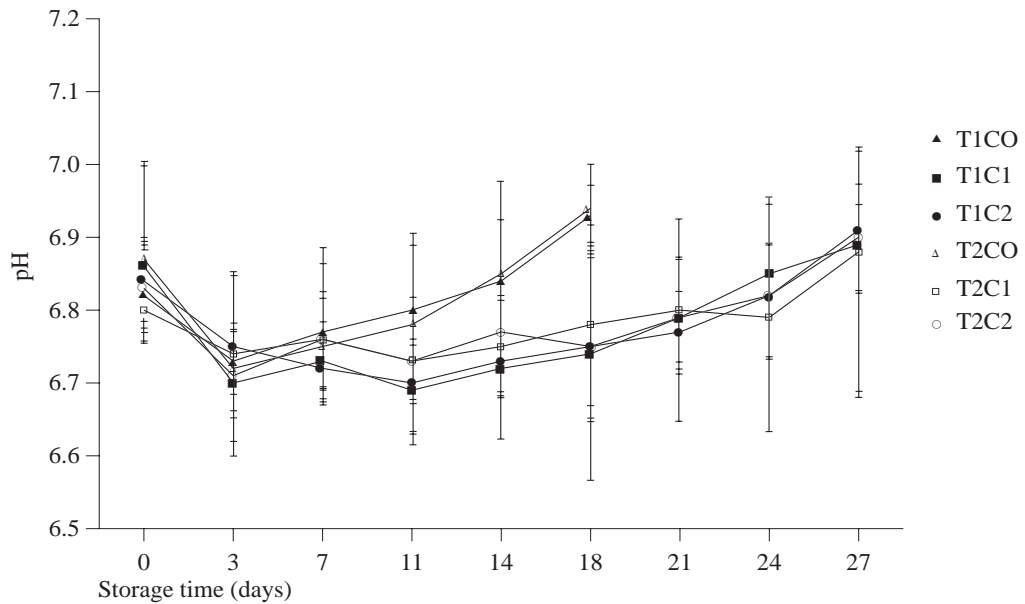


Figure 2. Changes in pH of barramundi fillets during storage

slowly increased from 6.75 to 6.94 from day 7 to the end of the storage period. However, in samples T1C1, T1C2, T2C1 and T2C2, pH values subsequently increased from day 14 to day 27. pH of fillets in either treatment decreased in early storage and increased during later storage. The drop in pH can be explained by the surface reaction of CO₂ with water forming carbonic acid (Banks et al. 1980):



resulting in the acidification of the medium. Even though most bacteria are sensitive to acidic media, the decrease in pH is not entirely responsible for the reduction of bacterial group. 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. There was a high count for total microbial in the later stage and this explained why the pH values increased in all samples.

There were no significant differences between pH of either K-sorbate treated or without treated samples in all sampling days showing that potassium sorbate dip did not affect pH value of fillets in this study.

K-value

Adenosine triphosphate (ATP) is an essential molecule for all living cells and it is rapidly degraded after death. ATP degradation produces a successive sequence of compounds :

Adenosine triphosphate (ATP) → Adenosine diphosphate (ADP) → Adenosine monophosphate (AMP) → Inosine phosphate (IMP) → Inosine (HxR) → Hypoxanthine (Hx)

These metabolites from ATP are considered excellent indicators for measuring food freshness, especially in fish. The K-value represents the amount of inosine and hypoxanthine compared with all ATP-degradation products:

$$\text{K-value (\%)} = \frac{\text{HxR} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx}} \times 100$$

The lower the K-value, the fresher the fish. The K-value enables observation of the effect of autolysis occurring before bacterial growth commences (Malle and Isabelle 1992). The results presented in *Figure 3* expressed the K-value of muscle tissue of fillets as a function of the number of storage in 2 ± 2 °C. Fresh barramundi fillets had a K-value of 12%. The K-value of the fillets in all treatments increased significantly with length of storage, reaching a maximum of 60–62% on day 18 for samples T1C0 and T2C0, whereas samples T1C1, T1C2, T2C1 and T2C2 reached a maximum reading of 63–65% on day 27. Generally, K-values for fillets treated with K-sorbate (T1C1, T1C2, T2C1 and T2C2) were significantly lower than samples without K-sorbate treatment (T1C0 and T2C0) after the 7th day and onward. Thus K-sorbate dip seemed to improve the freshness of the fillets. However, longer dipping time and higher concentration of K-sorbate did not have any additional effect in lowering the K-values.

A relationship between K-value and sensory scores for odour could not be observed until day 14. On the 11th day, K-values for T1C0 and T2C0 were significantly higher than potassium treated samples (T1C1, T1C2, T2C1 and T2C2). However panellists could not detect any differences. From day 14 onward, panellists gave higher rating for K-sorbate-treated samples which had lower K-values than untreated samples.

Microbiological analysis

Bacterial numbers in treatments T1C0 and T2C0 increased rapidly during the first 7 days of storage with no evident lag phase (*Figure 4*). Counts from fillets to which MA and K-sorbate were added (T1C1, T1C2, T2C1 and T2C2) decreased from log 4.4 CFU/g sample to log 3.4 CFU/g sample after 3 days. This suggested a bactericidal

Effect of potassium sorbate on shelf life of barramundi fillets

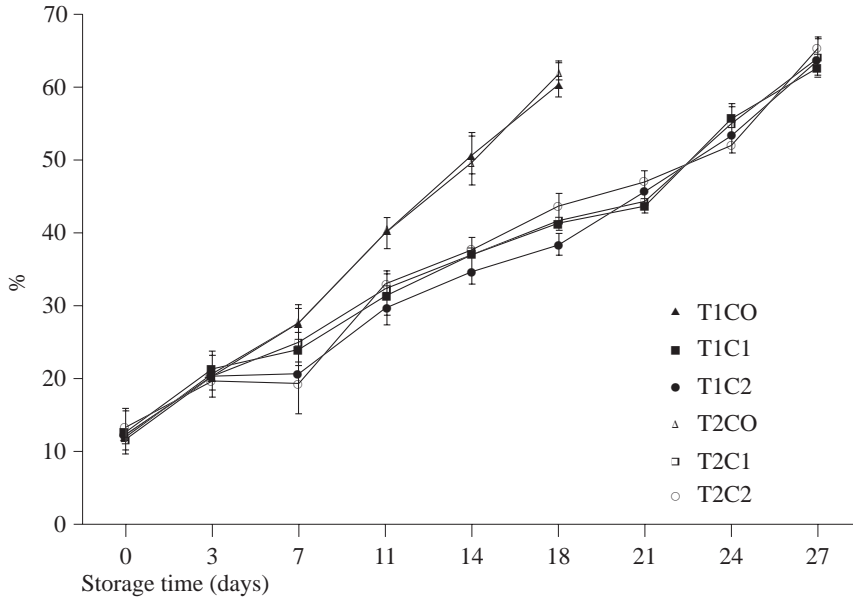


Figure 3. Changes in K-value of barramundi fillets during storage

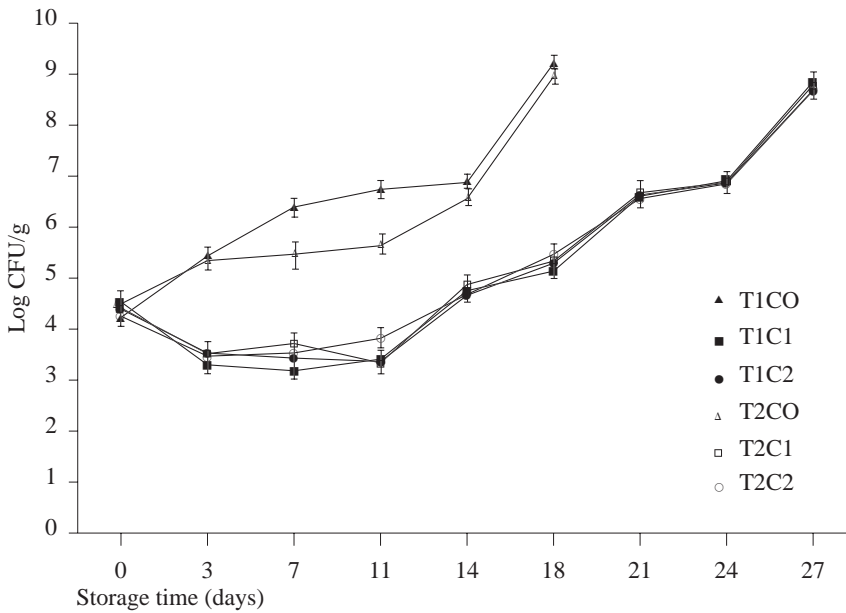


Figure 4. Changes in aerobic plate counts of barramundi fillet during storage

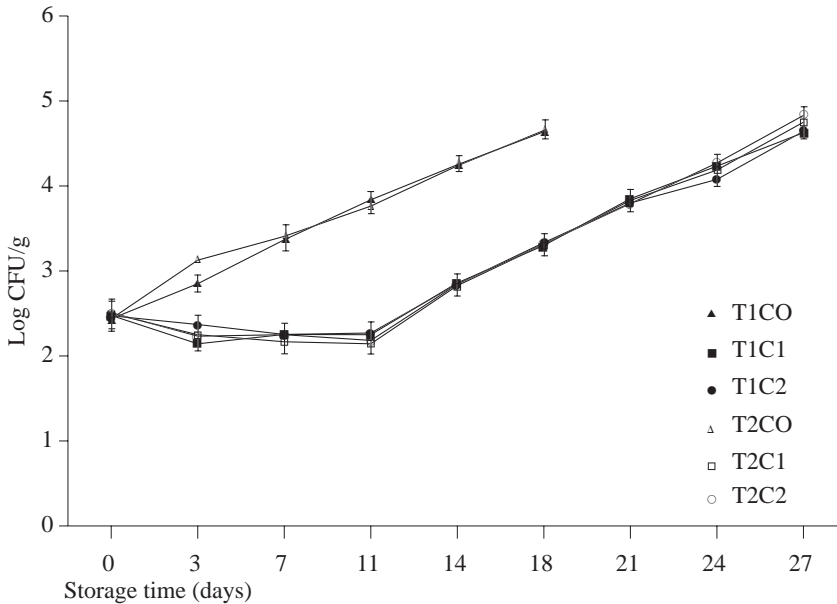


Figure 5. Changes in coliform counts of barramundi fillets during storage

effect found on the initial flora. An extended lag phase lasted for 11 days, after which bacterial numbers increased to a maximum of log 8.7 CFU/g sample. Bacterial numbers in MA + K-sorbate treated samples did not differ significantly ($p < 0.05$), showing that dipping time (1 min or 2 min) and concentration of K-sorbate at 1% and 2% applied in this experiment had no significant effect on the bacterial numbers. Spoilage characteristics in barramundi fillets generally observed when bacterial counts reached log 7.0 CFU/g sample or higher (Siah and Mohd. Ariff 2001), thus fillets without K-sorbate dips (T1C0 and T2C0) lasted for 14 days whereas with K-sorbate dips samples lasted up to 24 days.

Average total coliform counts of fresh barramundi fillets were log 2.4 CFU/g sample (Figure 5). Growth trend of coliform in all treatments were similar to that of aerobic bacterial. Rapid increase in coliforms counts occurred in treatments T1C0 and T2C0 reaching around log 4.6 CFU/g sample after 18 days. Total coliform for T1C1, T1C2, T2C1 and T2C2 declined to log 2.2 CFU/g sample after 3 days and

gave a lag period of 11 days, followed by a rise to around log 4.7 CFU/g sample after 27 days.

K-sorbate is a generally recognized as safe (GRAS) food preservative and it is effective against bacteria, mould and yeast (Pedrosa and Regenstein 1990). K-sorbate protects against spoilage and pathogenic organisms and inhibits the growth of trimethylamine-producing bacteria in fresh fish. Its typical usage levels in products range from 0.025% to 1%. Higher concentration is used in dips (Pedrosa and Regenstein 1990). Its antimicrobial activity is in the undissociated form of sorbic acid and is pH dependent. As pH is lowered (between 8.5 and 4.5), the amount of undissociated sorbic acid increased. According to Khuntia et al. (1993), K-sorbate retards microbial activity by inhibiting various enzymes of the microbial cell, specifically those in the citric acid cycle. Its application in extending the shelf life of fish and fishery products has been well studied. Fey and Regenstein (1982) reported that a combination of 1% K-sorbate ice and MA of 60% CO₂, 20% O₂ and 20% N₂ at 1 °C

extends the shelf life at least 28 days for fresh redhake and salmon packaged in gas-impermeable bags compared with that of these products packaged without K-sorbate. Bremmer and Statham (1983) found significant improvement in the shelf life of vacuum-packaged scallops treated with 0.1% K-sorbate dip. The K-sorbate treated scallops stored at 4 °C were acceptable for up to 28 days, i.e., an extension of 22 days beyond that for untreated scallops.

Sensory evaluation

For all attributes evaluated, there were no significant differences between treatments for the first 11 days. However, from the 14th day and onward, scores for all sensory attributes were significantly lower for treatments T1C0 and T2C0 as compared to the other four treatments. From the results obtained, it was concluded that panellists could not detect any differences among the K-sorbate treated fillets (T1C1, T1C2, T2C1 and T2C2) right from the initial stage to the end of storage study.

There was a significant effect of storage time on the sensory qualities of barramundi fillets. Very high scores were given to the colour of barramundi fillets on day 0, ranging from 6.67 to 6.83 (*Table 2*). There were no significant differences for the first 7 days for treatments T1C0 and T2C0, however after 11 days, significant decrease was noticed and panellists rated the sample as unacceptable at the 18th day for both samples. For K-sorbate treated samples (T1C1, T1C2, T2C1 and T2C2), significant decrease in scores for colour was noticed from day 14. At the end of storage studies, the colour score for these K-sorbate treated samples still at the acceptable level (>4.0 points) even though scores for odour, texture and overall acceptability were below acceptable levels.

In the first 3 days of storage, scores for odour in all treatments were higher than 6.5 (*Table 2*). However 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 in treatments T1C0 and T2C0 on the 18th day and in T1C1, T1C2, T2C1 and T2C2 on the 27th day. From *Figure 4*, very high aerobic plate 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 barramundi fillets (*Table 2*). Higher scores were given to all treatments in the first few days of storage and when stored longer, scores given were subsequently lower. Treatments T1C0 and T2C0 showed the most marked changes. Fillets became more 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, from the results of sensory evaluation, treatments T1C0 and T2C0 were acceptable up to 14 days whereas samples T1C1, T1C2, T2C1 and T2C2 were acceptable up to 24 days.

Conclusion

The combined action of MA + K-sorbate give rise to additive or synergistic effects, providing greater assurance of product quality and stability. MA + K-sorbate effectively extended the shelf life of barramundi fillets to 24 days at 2 ± 2 °C. From the economic point of view, 1% K-sorbate and 1 minute dip is thought to be the best combination as other combinations did not show any additional effect in extending the shelf life of barramundi fillets.

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Table 2. Colour, odour, texture and overall acceptability of barramundi fillets

Storage time (days)	T1C0	T1C1	T1C2	T2C0	T2C1	T2C2
Colour acceptability						
0	6.750a A	6.667a A	6.667a A	6.833a A	6.667a A	6.750a A
3	6.667a A	6.583a A	6.750a A	6.583a A	6.667a A	6.667a A
7	6.417a A	6.667a A	6.583a A	6.500a A	6.500a A	6.667a A
11	5.500b A	6.417a A	6.417a A	5.500b A	6.333a A	6.250a A
14	4.750c B	5.583b A	5.583b A	4.667c B	5.583b A	5.583b A
18	4.083d B	5.750b A	5.500b A	3.917d B	5.417b A	5.500b A
21	–	5.250b A	5.167b A	–	5.167b A	5.250b A
24	–	4.417c A	4.500c A	–	4.417c A	4.667c A
27	–	4.250c A	4.333c A	–	4.083c A	4.083d A
Odour acceptability						
0	6.583a A	6.583a A	6.833a A	6.917a A	6.750a A	6.833a A
3	6.667a A	6.667a A	6.583a A	6.667a A	6.500a A	6.750a A
7	5.417b A	5.833b A	5.917b A	5.417b A	5.667b A	5.833b A
11	5.167b A	5.417bc A	5.500bc A	4.917bc A	5.417bc A	5.167bc A
14	4.583c B	5.333bc A	5.333cd A	4.583c B	5.250bc A	5.250bc A
18	3.250d B	4.833cd A	4.833de A	3.000d B	4.833cd A	4.750cd A
21	–	4.833cd A	4.667e A	–	4.833cd A	4.917c A
24	–	4.583d A	4.333e A	–	4.417d A	4.167d A
27	–	3.083e A	3.500f A	–	3.167e A	3.250e A
Texture acceptability						
0	6.833a A	6.750a A	6.917a A	6.833a A	6.750a A	6.833a A
3	6.667a A	6.750a A	6.833a A	6.667a A	6.853a A	6.750ab A
7	6.333a A	6.250ab A	6.250bc A	6.167a A	6.250ab A	6.167bc A
11	5.083b AB	5.667bc A	5.667cd A	4.833b B	5.750bc A	5.667cd A
14	4.833b B	5.500cd A	5.417de A	4.583b B	5.333cd A	5.250de A
18	3.583c B	4.917de A	5.000ef A	3.333c B	5.000d A	5.083de A
21	–	5.167cd A	5.083def A	–	5.167cd A	5.000e A
24	–	4.417e A	4.667f A	–	4.667d A	4.333f A
27	–	3.667f A	3.500g A	–	3.583e A	3.167g A
Overall acceptability						
0	6.833a A	6.750a A	6.917a A	6.833a A	6.667a A	6.750a A
3	6.583a A	6.750a A	6.833a A	6.750a A	6.750a A	6.750a A
7	6.417a A	6.417ab A	6.333ab A	6.500a A	6.333ab A	6.167ab A
11	5.833b A	5.833bc A	6.000bc A	5.833b A	6.167ab A	5.917bc A
14	4.083c C	5.500c AB	5.583cd A	4.667c C	5.750bc A	5.250cd AB
18	3.417d B	5.167cd A	5.083d A	3.500d B	5.167c A	5.083d A
21	–	5.250c A	5.167d A	–	5.250c A	5.333cd A
24	–	4.500d A	4.333e A	–	4.250d A	4.167e A
27	–	4.250e A	3.417f A	–	3.250e A	3.147f A

a – g: Means bearing the same letter within the same column are not significantly different at 5% level

A – B: Means bearing the same letter within the same row are not significantly different at 5% level

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