

Effects of modified atmosphere packaging on microbiological load and physico-chemical properties of barramundi (*Lates calcarifer* Bloch) fillets at 8°C

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Abstract

Different modified atmosphere conditions with various CO₂ concentrations on microbial load (psychrophile, mesophile aerobic and anaerobic bacteria) and physico-chemical properties of barramundi (*Lates calcarifer* Bloch) fillets stored at 8°C were compared to determine the best packaging conditions. The gas conditions evaluated were 100% CO₂ (M₁), 75% CO₂/25% N₂ (M₂), 50% CO₂/50% N₂ (M₃), 25% CO₂/75% N₂ (M₄) and 100% N₂ (control). High CO₂ concentration (M₁ and M₂) of fish fillets delayed the psychrophilic bacteria growth compared to low CO₂ concentration (50% and 25% CO₂) as well as the mesophilic, anaerobic and histamine forming bacteria. We concluded that the atmosphere with 75:25% and 100:0 (CO₂:N₂) had the most appropriate gas composition to inhibit the microbial growth and prolong the shelf life of barramundi fillets ($p < 0.05$).

Keywords: Barramundi, CO₂ concentration, Psychrophile bacteria, Histamine forming bacteria, MAP, *Lates calcarifer*.

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Introduction

Fish and fish products are some of the most important protein sources in human nutrition. An increasing number of seafood products are preserved by modified atmosphere packaging with refrigeration and addition of low levels of preservatives (Gram, 2009). Due to the increasing demand of consumers for fresh refrigerated fish products, a lot of research works have been conducted on preservation techniques to control the bacterial growth and the extending the shelf-life of the seafood products. Modified atmosphere packaging (MAP) and vacuum packaging (VP), 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 the consumers' demands (Brody, 1989).

Seafoods have susceptible chemical composition, which influence subsequent spoilage patterns. The presence of microorganisms on the live animal and contamination of raw materials during processing provides favorable conditions for microbial action and subsequent deterioration and spoilage of the products (Bagge-Ravn *et al.*, 2003; Gram, 2009;).

The growth of microorganisms makes food organoleptically unacceptable for consumption because of changes in colour, smell, odour and texture. To reduce the rate of these changes, the growth of spoilage microorganisms should be inhibited by

application of MAP technology and increasing the lag phase of facultative and anaerobic microorganisms, which results in extension of the potential shelf life of MAP products. Nevertheless, there is a safety concern with the extended shelf life, because anaerobic and facultative pathogens that might have shorter lag times and faster growth rates are capable of growing during the extended storage period (Arashisar *et al.*, 2004; Ravi Sankar *et al.*, 2008; Gram, 2009).

The effect of MAP on lactic acid bacteria can vary depending on the type of product package. The increased CO₂ and decreased O₂ concentrations used in MAP generally favor the growth of lactic acid bacteria. Although the effect of MAP on yeasts is negligible, molds are aerobic microorganisms and therefore CO₂ can cause growth inhibition at concentrations as low as 10% (Molin, 2000). The most common gases used in MAP are carbon dioxide, oxygen and nitrogen (Reddy *et al.*, 1994; Gimenez *et al.*, 2002). CO₂ concentration in MAP has been extended the shelf-life of foods by inhibiting the microbial growth of *Enterobacteria* and H₂S-producing bacteria (López-Caballero *et al.*, 2002), histamine forming bacteria (Özogul and Özogul, 2006), *Pseudomonas spp.* (Erkan *et al.*, 2006) and *Lactobacillus sake* (Devlieghere *et al.*, 1998). Carbon dioxide inhibits the growth of psychrophilic microorganisms during the logarithmic phase and extends the lag phase. Oxygen stimulates the

growth of aerobic bacteria, but inhibits the growth of strictly anaerobic bacteria (Arashisar *et al.*, 2004). Nitrogen delays the oxidative rancidity and inhibits the growth of aerobic microorganisms by displacing the oxygen in packs (Farber, 1991). However, the synergetic effects of all these gases need to be established for the specific food application such as seafood.

The most effective CO₂ mixtures has been applied by various researchers for specific fish species such as pearlspot (Ravi Sankar *et al.*, 2008), sardine (Özogul *et al.*, 2002; Özogul *et al.*, 2004; Özogul and Özogul, 2006), tilapia (Reddy *et al.*, 1994), sole (López-Gálvez *et al.*, 1998), trout (100% CO₂) (Arashisar *et al.*, 2004) and tuna (Emborg *et al.*, 2005). The CO₂ content is as low as 40% and as high as 75% of the total gas mixtures. This CO₂ composition reduces microbial growth and extends the shelf life of the products. Therefore, the aim of this study was to evaluate the effects of CO₂ concentration from 25-100% in combination with N₂ on microbial growth and physico-chemical properties of packed barramundi fillets. The role of microorganisms particularly total plate count (TPC), psychrophile, histamine forming bacteria and anaerobic bacteria on the spoilage process was also evaluated.

Materials and methods

Sample preparation

Whole barramundi (*L. calcarifer*) with an average weight of 0.5 kg were

bought from local fish market. The fish was transported to the laboratory on ice and upon arrival decapitated and filleted manually. All samples preparation was done according to Bakar *et al.* (2010) and Yassoralipour *et al.* (2012).

Modified atmosphere packaging of sample

Each fillet was packed individually in a pouch with Multivac packaging (GOOD-And-WELL, Taiwan) and stored at 8±1°C for 20 days. The material (220×300 mm) were made of Nylon (thickness 0.09 mm) and had an O₂, N₂, CO₂ and H₂O (at 23°C) transmission rate of 1.55, 0.465, 6.15 cm³/m²/day atm and 15 g/m²/day atm, respectively. The final gas/product ratio in all packages was adjusted to about 2:1 (v/w) by a gas mixer (PBI Dansensor MAP Mix 9001 ME, USA). The treatments were labelled as control (100% N₂), M₁ (100% CO₂), M₂ (75% CO₂/25% N₂), M₃ (50% CO₂/50% N₂), and M₄ (25% CO₂/75% N₂). All treatments were prepared in triplicates.

Mesophile, psychrophile, histamine forming and anaerobic bacteria

The enumerations of mesophile and psychrophile bacteria were carried out according to method of AOAC (2000) while the counting of histamine-forming bacteria were carried out according to the procedure of Niven's (Niven *et al.*, 1981). Anaerobic bacteria enumeration on tryptose sulphite cycloserine (TSC) agar (Oxoid, USA) was performed according to Perez-

Alonso *et al.* (2004) and Ravi Sankar *et al.* (2008) and incubated an aerobically in anaerobic jar, at 30°C for 5 days. All samples were replicated twice.

Total volatile base-nitrogen (TVB-N)

A vapour distillation method was used for total volatile bases nitrogen (TVB-N) determination (Arashisar *et al.*, 2004; Goulas and Kontominas, 2005). The results were expressed as mg N/100g of fish muscle.

pH determination

The muscle was homogenised in distilled water in the ratio of 1:10 (w/v) and the measurement was done by a digital pH-meter (DELTA 320) at room temperature (Goulas and Kontominas, 2005).

Statistical analysis

Data collected were analyzed by analysis of variance (ANOVA). Pearson

correlation for the effect of CO₂ was conducted to determine the relationship between mesophile, psychrotrophile, histamine forming and anaerobe bacteria in all treatments. The significance of results was at $p < 0.05$. The software used was SPSS for windows (SPSS Inc. 2008).

Results

Microbiological changes

The total plate count (TPC), aerobic psychrophile count (APC), histamine forming bacteria (HFB) and anaerobic bacteria counts in barramundi fillets as a function of atmosphere storage at 8°C are shown in Figs. 1, 2, 3 and 4, respectively. The initial TPC for samples were 3.54 log cfu/g (Fig. 1) and APC 4.28 log cfu/g (Fig. 2).

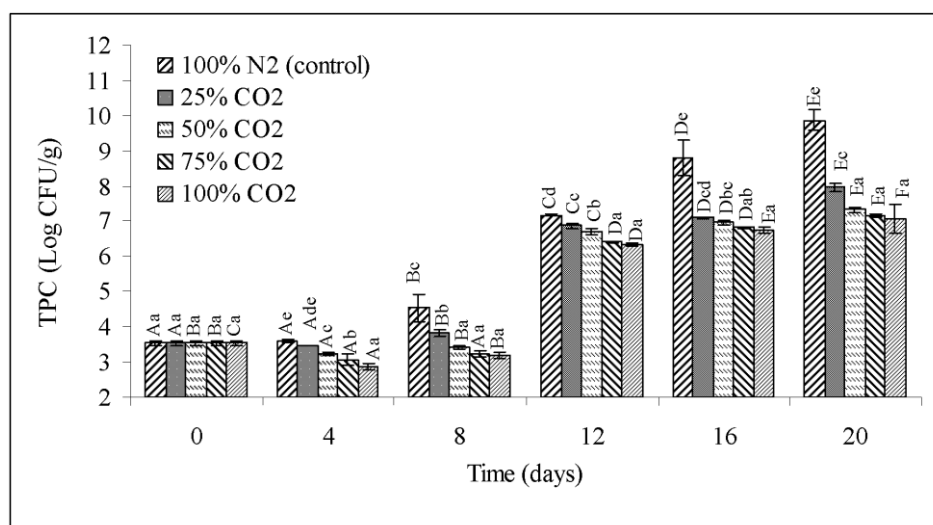


Figure 1: Effect of packaging atmosphere on growth of total plate count on barramundi fillets stored at 8 °C. ^{A, a} TPC (means±standard deviation) with different upper case (for the same MAP and different days) and different lower case (for the same days and different MAP) are significantly different ($p < 0.05$).

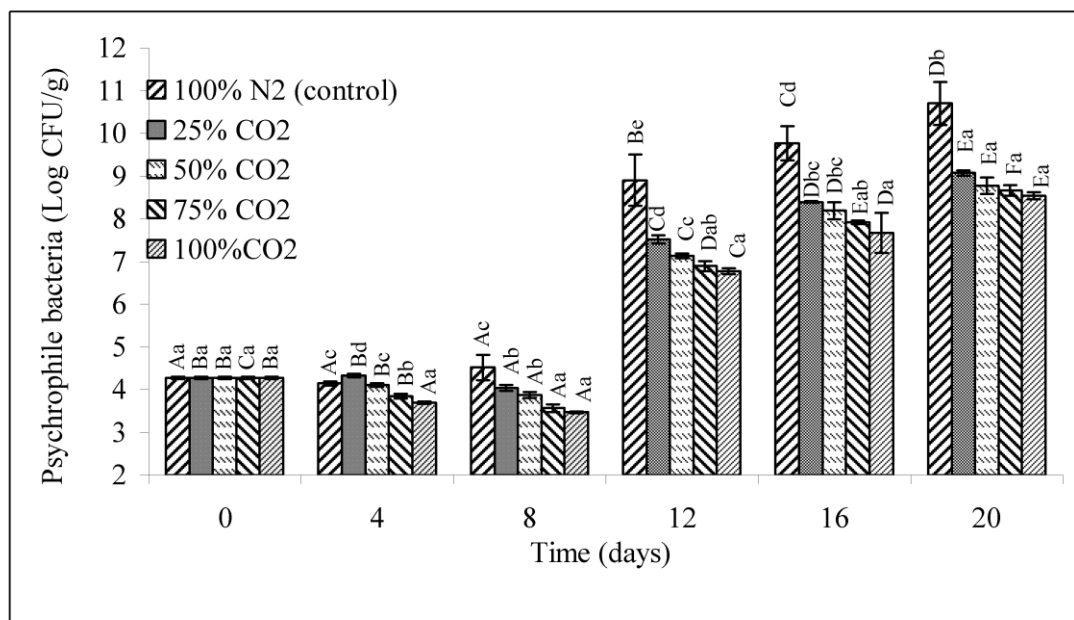


Figure 2: Effect of packaging atmosphere on growth of psychrophile aerobic bacteria on barramundi fillets stored at 8 °C.

A, a APC (means±standard deviation) with different upper case (for the same MAP and different days) and different lower case (for the same days and different MAP) are significantly different ($p < 0.05$).

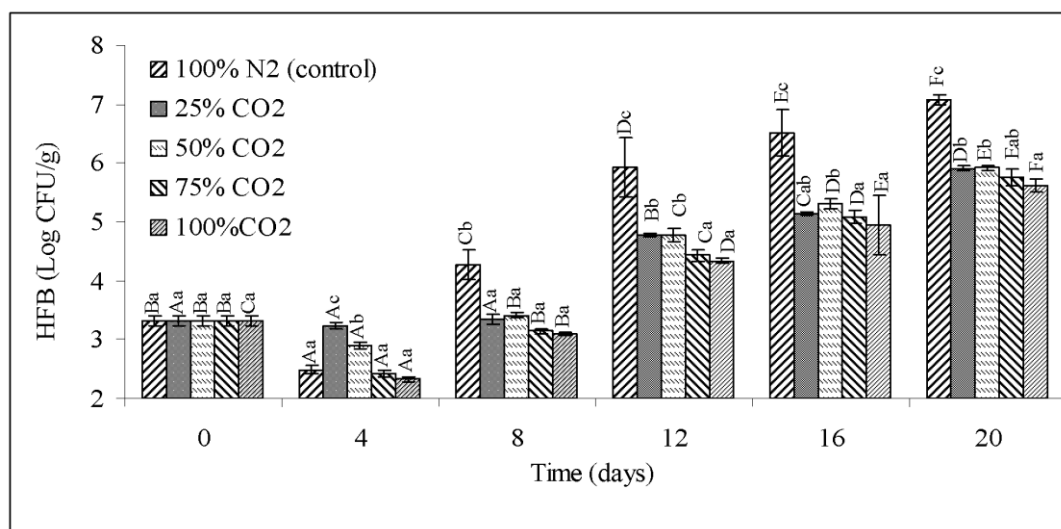


Figure 3: Effect of packaging atmosphere on growth of histamine forming bacteria on barramundi fillets stored at 8 °C.

A, a HFB (means±standard deviation) with different upper case (for the same MAP and different days) and different lower case (for the same days and different MAP) are significantly different ($p < 0.05$).

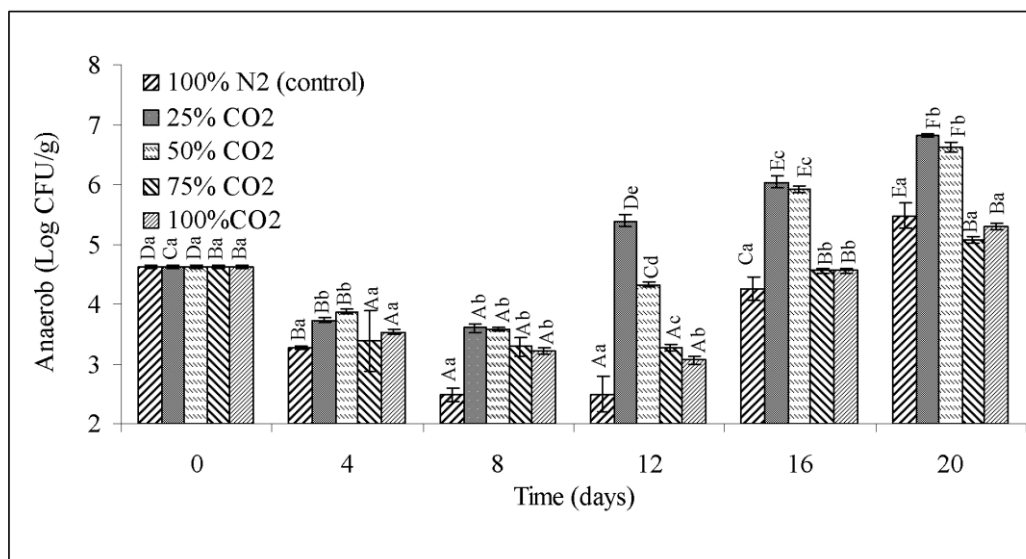


Figure 4: Effect of packaging atmosphere on growth of anaerobic bacteria on barramundi fillets stored at 8 °C.

A, a Anaerobe (means±standard deviation) with different upper case (for the same MAP and different days) and different lower case (for the same days and different MAP) are significantly different ($p<0.05$).

Total volatile base-nitrogen

Changes in TVB-N concentration for control and MAP conditions of barramundi fillet during the 20 days storage is given (Fig. 5). As expected, TVB-N levels were initially low (13.53 mg N/100g fish muscle) for fresh barramundi fillets. TVB-N increased gradually after 4 days of storage for

control barramundi fillet, reaching final (day 20) value of 111.10 mg N/100g fish muscle. TVB-N values of M₁, M₂, M₃ and M₄ compared to control also increased significantly ($p<0.05$) after 8 days of storage; however, the rate of enhancement was lower than control.

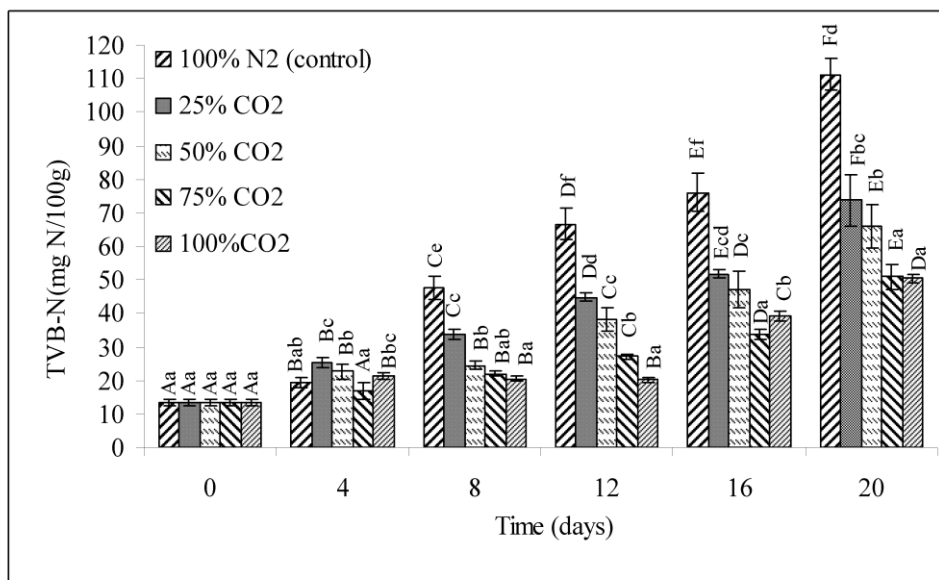


Figure 5: Effect of packaging atmosphere on TVB-N value of barramundi fillets stored at 8 °C. ^{A, a} TVB-V (means±standard deviation) with different upper case (for the same MAP and different days) and different lower case (for the same days and different MAP) are significantly different ($p<0.05$).

pH

pH values of the MAP samples were significantly ($p<0.05$) different throughout storage period and the initial value which was 6.42, reached to 6.49,

6.55, 6.65, 6.67 and 6.92 for 100% CO₂, 75% CO₂, 50% CO₂ 25% CO₂ and control, respectively (Fig. 6).

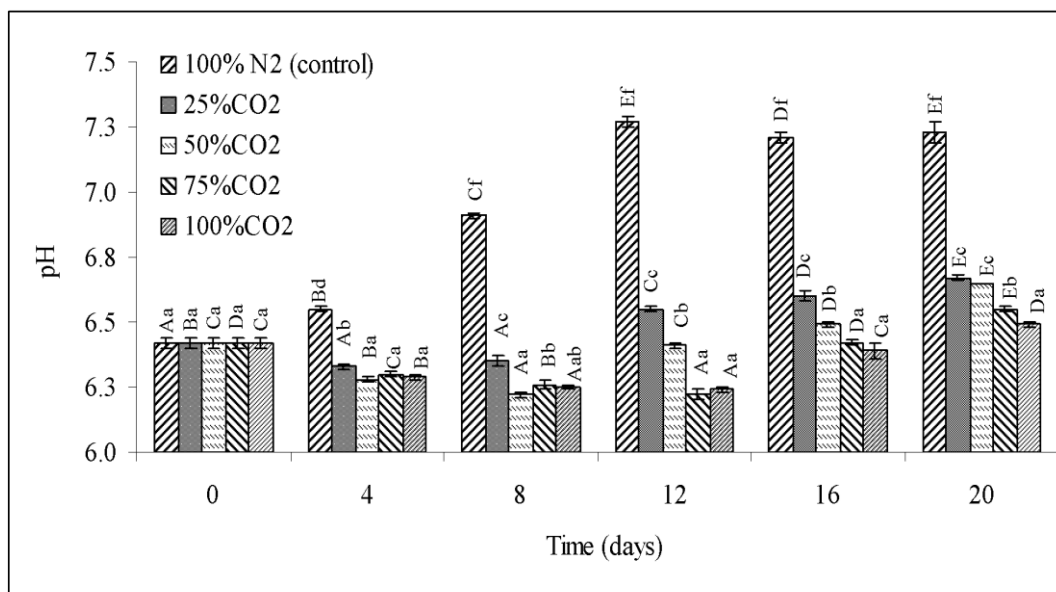


Figure 6: Effect of packaging atmosphere on pH value of barramundi fillets stored at 8 °C. ^{A, a} pH (means±standard deviation) with different upper case (for the same MAP and different days) and different lower case (for the same days and different MAP) are significantly different ($p<0.05$).

Discussion

Microbiological changes

The total microbiological limit for human consumption in fresh fish, proposed by ICMSF (2002) is 7 log cfu/g, while other authors recommend 6 log cfu/g (Ababouch *et al.*, 1991; Arashisar *et al.*, 2004; Scherer *et al.*, 2006). Psychrophile counts exceeded mesophilic counts in all the samples after 4 days. Similar observations were reported using the gas composition of 90:2.5:7.5% (CO₂:O₂:N₂) and 40:30:30% (CO₂:O₂:N₂) for rainbow trout by Arashisar *et al.* (2004) and Ravi Sankar *et al.* (2008) stored at 4±1°C.

Erkan *et al.* (2006) reported that mesophilic aerobic counts showed a slight increase for sardines (*Sardina pilchardus*) during storage 70:5:25% (CO₂:O₂:N₂) at 4° C, which reached a value of 4 log cfu/g (day 7). Total mesophilic count exceeded 6 log cfu/g after 12 days in barramundi stored in M₃ and M₄, and after 16 days for samples stored in M₁ and M₂. In sample stored in M₄ (100% CO₂ concentration), the growth was slower by 1-2 log cfu/g. These results were similar to research of Arashisar *et al.* (2004), Lalitha *et al.* (2005) and Ravi Sankar *et al.* (2008), who kept their samples in MAP. They mentioned that the CO₂ concentration prolongs lag phase of bacteria growth,

increases generation time and delaying bacteria growth in fish filets. The HFB are usually considered part of the normal microflora of the gut, skin, or gills of fish (Huss, 1994). The histamine-forming bacteria grew faster in barramundi fillets kept in M₃ and M₄ sample, as compared to M₁ and M₂ (Fig. 3). However, the different concentration of CO₂ in the MAP sample might have influenced the growth of HFB (Özogul *et al.*, 2004; Özogul and Özogul, 2006). Results of ANOVA (Table 1) indicated that MAP and storage days significantly ($p<0.05$) changed the pH, TVB-N and microbial load of fish fillet. As illustrated by Montgomery (2008), the corresponding variable will be more significant ($p<0.05$) if the absolute F ratio became larger and P value became smaller.

Reddy *et al.* (1994) found that initial aerobic bacteria counts and anaerobic bacteria count in CO₂:N₂ (75:25%) for tilapia stored at 4°C was 4.3 and 3.2 log cfu/g, respectively. This count reached a 4.6 log cfu/g after 10 days of storage in packed tilapia. Significant reduction of 1 to 2 log cfu/g was noticed in the anaerobic count in all samples until the 12 day of storage (Fig. 4). Reddy *et al.* (1994) and Ravi Sankar *et al.* (2008) also reported the same reduction for CO₂:O₂ (60:40%) of pearlspot (*Etroplus suratensis*) stored at 0-2°C and CO₂:N₂ (75:25%) tilapia stored at 4°C, respectively.

Table 1: ANOVA results for the effect of MAP and storage days for all parameters evaluated for barramundi fillets.

Parameters	100% CO ₂	75% CO ₂	50% CO ₂	25%CO ₂	100% N ₂	Adjusted <i>p</i> -value	MAP effect	Day (MAP) effect
pH								
							*	*
100% CO ₂		0.03610	0.00000	0.00000	0.00000	<i>p</i> -value	0.0000	0.0000
75% CO ₂	0.03610		0.00000	0.00000	0.00000	F-ratio	3953.44	459.99
50% CO ₂	0.00000	0.00000		0.00000	0.00000			
25% CO ₂	0.00000	0.00000	0.00000		0.00000			
100%N ₂	0.00000	0.00000	0.00000	0.00000				
TVB-N								
							*	*
100% CO ₂		0.99900	0.00000	0.00000	0.00000	<i>p</i> -value	0.0000	0.0000
75% CO ₂	0.99900		0.00000	0.00000	0.00000	F-ratio	377.57	233.36
50% CO ₂	0.00000	0.00000		0.00000	0.00000			
25% CO ₂	0.00000	0.00000	0.00000		0.00000			
100%N ₂	0.00000	0.00000	0.00000	0.00000				
TPC								
							*	*
100% CO ₂		0.17560	0.00000	0.00000	0.00000	<i>p</i> -value	0.0000	0.0000
75% CO ₂	0.17560		0.00020	0.00000	0.00000	F-ratio	480.27	1362.25
50% CO ₂	0.00000	0.00020		0.00000	0.00000			
25% CO ₂	0.00000	0.00000	0.00000		0.00000			
100%N ₂	0.00000	0.00000	0.00000	0.00000				
APC								
							*	*
100% CO ₂		0.05860	0.00000	0.00000	0.00000	<i>p</i> -value	0.0000	0.0000
75% CO ₂	0.05860		0.00040	0.00000	0.00000	F-ratio	270.56	980.18
50% CO ₂	0.00000	0.00040		0.00010				
25% CO ₂	0.00000	0.00000	0.00010		0.00000			
100%N ₂	0.00000	0.00000	0.00000	0.00000				
HFB								
							*	*
100% CO ₂		0.01510	0.00000	0.00000	0.00000	<i>p</i> -value	0.0000	0.0000
75% CO ₂	0.01510		0.00000	0.00000	0.00000	F-ratio	403.22	833.82
50% CO ₂	0.00000	0.00000		0.99610	0.00000			
25% CO ₂	0.00000	0.00000	0.99610		0.00000			
100%N ₂								
Anaerobe								
							*	*
100% CO ₂		0.99900	0.00000	0.00000	0.00000	<i>p</i> -value	0.0000	0.0000
75% CO ₂	0.99900		0.00000	0.00000	0.00000	F-ratio	258.84	97.63
50% CO ₂	0.00000	0.00000		0.01460	0.00000			
25% CO ₂	0.00000	0.00000	0.01460		0.00000			
100%N ₂	0.00000	0.00000	0.00000	0.00000				

TVB-N: Total volatile base nitrogen; TPC: Total plate count; APC: Aerobic psychrophilic count; HFB: Histamine forming bacteria. * $p < 0.05$ is significant difference

Total volatile base-nitrogen

The levels of 25 mg N/100g fish muscle is thought to indicate incipient spoilage in fish (Lannelongue *et al.*, 1982) but a higher TVB-N level of 30-35 mg N/100g fish muscle depending on fish species, has been suggested as the limit of acceptability for fish spoilage (Connell, 1990). In the current study, barramundi samples stored as control and M₄ exceeded after 4 and 12 days of storage, respectively.

pH

A significant increase ($p < 0.05$) of pH was observed in control samples during the storage period, and reached to pH 7.23 after 20 days of air storage. Increases in pH indicate the accumulation of alkaline compounds, such as ammonia mainly derived from microbial action (Hebard *et al.*, 1982).

Pastoriza *et al.* (1998) found similar results for pH values in control samples of ice-stored hake (*Merluccius merluccius*). The pH is lower for MAP-stored fish since carbon dioxide can be absorbed into fish muscle surface, acidifying it via the formation of carbonic acid (Banks *et al.*, 1980).

There were no significant differences ($p > 0.05$) among the total plate count and psychrophile aerobic bacteria in M₁ as compared to M₂; however, there was significant difference ($p < 0.05$) with the other treatments (M₃, M₄ and control) in 8 days of storage and after that significantly increased. Among four treatments, M₁ and M₂ had the least

changes in the all parameters evaluated. 75 and 100 % CO₂ concentration caused significant reductions ($p > 0.05$) in the contents of TVB-N, pH and microbial counts in barramundi fillets, which has been stored at 8°C for 20 days. The atmosphere with 75:25% and 100:0 (CO₂:N₂) had least significant ($p < 0.05$) changes and the most appropriate gas composition to reduce the microbial load that resulted in prolonging the shelf life of barramundi fillets.

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