MICROBIOLOGICAL, CHEMICAL AND SENSORY ASSESSMENT OF AREOLATED GROUPER (*Epinephelus areolatus*) FILLETS STORED UNDER MODIFIED ATMOSPHERE PACKAGING

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***Abstract*__** Fresh fish fillets (*Epinephelus areolatus*) were packed in different modified atmospheres; 80% CO_2 + 20% N_2; 80% CO_2 + 10% O_2+ 10% N_2 and 60% CO_2 + 20% O_2+ 20% N_2. Control samples were packed in air. All samples were stored at 5ºC. Total viable count; *Enterobacteriaceae;* lactic acid bacteria and H_2S-producing bacteria were inhibited compared to the control ($P<0.05$). The highest inhibition were observed with packaging fish fillets in 80% CO_2 + 20% N_2 and 80% CO_2 + 10% O_2+ 10% N_2, with no differences between them in microbiological quality. There was significant difference ($P<0.05$) in pH values between all samples packed in modified atmospheres and samples packed in air (control). This mainly due to the production of carbonic acid by the dissolved CO_2 in the aqueous phase of the fish fillets. Total volatile bases nitrogen (TVB-N) values were also decreased ($P<0.05$) by packaging fish fillets in modified atmospheres after 3 and 6 days of storage at 5ºC as compared with the control. The sensory evaluation of fish fillets revealed that color of fish fillets was improved by packaging in 80% CO_2 + 10% O_2+ 10% N_2 and 60% CO_2 + 20% O_2+ 20% N_2. In contrast the color of samples packed in 80% CO_2 + 20% N_2 was poor after 3 days of storage. However, the color of control samples were still acceptable after 6 days of storage. The 80% CO_2 + 10% O_2+ 10% N_2 gas mixture was the most effective for the color stability of the fish fillets. Fish fillets packed in 60% CO_2 + 20% O_2+ 20% N_2; 80% CO_2 + 20% N_2 and 80% CO_2 + 10% O_2+ 10% N_2 have a better shelf life at 5ºC.

Key Words: Fish Fillets, Meat Quality, MAP, Shelf life.

INTRODUCTION

Fresh fish are highly perishable products due to their biological composition. Microbial growth in fresh fish is the main factor associated with quality deterioration, spoilage and economic loss (Ashie *et al*., 1996; Sivertsvik *et al*., 2002). Gram negative aerobic bacteria are mainly responsible for spoilage of fish muscle. Thus, lead to a short shelf life in fish and seafood products (Ashie *et al*., 1996; Gram and Huss 1996). The current status of modified atmosphere packaging (MAP) of fishery products was recently reviewed (Gimenez *et al*., 2002; Sivertsvik *et al*., 2002). Successful MAP is conditioned by low storage temperature, high quality raw materials and availability of carbon dioxide (CO_2) (Gimenez *et al*., 2002; Sivertsvik *et al*., 2002).

Oxygen, nitrogen and carbon dioxide are the most usual gases used in MAP (Randell *et al*., 1997; Sivertsvik *et al*., 2002). Oxygen is used to enhance color by maintaining the oxygenated form of myoglobin and to inhibits growth of strictly anaerobic bacteria (Farber, 1991; Sivertsvik *et al*., 2002). Nitrogen delays oxidative rancidity and inhibits the growth of aerobic microorganisms by displacing the oxygen in packs (Farber, 1991; Philips, 1996). Carbon dioxide is the most important antimicrobial gas in practical food preservation (Farber, 1991; Sivertsvik *et al*., 2002), it inhibits the growth of microorganisms during the logarithmic phase and extends the lag phase, which delay the overall increase of bacterial population (Church, 1994; Ohlsson, 1994; Sivertsvik *et al*., 2004).

The aim of this study was to evaluate the effect of modified atmospheres packaging with various gas mixtures (80% CO_2 + 20% N_2; 80% CO_2 + 10% O_2+ 10% N_2 and 60% CO_2 + 20% O_2+ 20% N_2) on microbiological, chemical and sensory characteristics of Areolated grouper (*Epinephelus areolatus*) fillets stored at 5ºC.

MATERIALS AND METHODS

Freshly caught Areolated grouper (*Epinephelus areolatus*) was obtained from Al-Katteif market, Saudi Arabia, with average weight of 350 g. Fish were immediately placed in ice-boxes and transported to the laboratory within 2 hour, and decapitated and filleted. Two fillets were obtained from each fish. Control samples were packed with air in polyethylene bags (70µm thickness, with oxygen permeability of 750 ml/m^2/24 hr. at 1 atmosphere and 23 ºC).

Gas packaging: The fish fillets were packed separately in Sidamil plastic bags (permeability : 6ml O_2 /m^2 /24 hr., 15 ml CO_2 /m^2 /24 hr., 2 ml N_2 /m^2 /24 hr., at 1 atmosphere and 23 ºC). The bags were filled with the appropriate gas mixture: 80% CO_2 + 20% N_2; 80% CO_2 + 10% O_2+ 10% N_2 and 60% CO_2 + 20% O_2+ 20% N_2 and stored at 5 ºC. The ratio fish : gas was 1:2 (v/v). Samples was analyzed for microbial contents, sensory qualities and chemical properties after 0, 3, 6, 8, 10 and 12 days of storage at 5 °C.

Microbiological analysis: At each sampling time, three samples were aseptically taken by means of 25 g of fish fillet (each) and added to 225 ml of sterile physiological saline supplemented by 0.1% (w/v) peptone, and homogenized in a stomacher (Lab Blender 400, Seward Medical, London) for 60 s at room temperature. From this homogenate decimal dilutions were made in duplicate in sterile physiological saline containing 0.1% peptone. Total viable count (TVC) were determined in plate count agar according to Kyrana and Lougovois (2002). *Enterobacteriaceae* were determined as colony forming units on Violet Red Bile Glucose Agar (VRBG) according to Gram and Huss (1996). Lactic acid

bacteria were determined on MRS agar according to Gram and Huss (1996). H_2S-producing bacteria were determined according to Gram *et al*.,(1987).

Sensory analysis: Sensory evaluation was carried out according to Metin *et al*., (2002) using five trained panelists.

Chemical analysis: The total volatile bases nitrogen (TVB-N) values were determined according to Vyncke *et al*., (1987). The pH values were determined recorded to (AOAC, 2002).

Statistical analysis: The data for total viable count, lactic acid bacteria, H_2S-producing bacteria, *Enterobacteriaceae*, pH, sensory and total volatile bases nitrogen (TVB-N) were analyzed using analysis of variances in two ways and subjected least significant difference (LSD) at 0.05% level of significant to compare the treatment means SAS (2001).

RESULTS AND DISCUSSION

The total viable count increased rapidly on control samples (Table 1). In contrast a marked increase in the lag phase for all samples packed in modified atmospheres was evident. No significant differences were observed between samples packed in 80% CO_2 + 10% O_2+ 10% N_2 and samples packed in 80% CO_2 + 20% N_2 with respect to total viable count. The 80% CO_2 was more effective than 60% CO_2 for the inhibition of total viable count. This fact may be attributed to its bacteriostatic effect, which inhibits the growth of aerobic Gram-negative bacteria, as a result of an extension of lag phase of growth and a decrease in the growth rate during the logarithmic phase (Farber, 1991; Gram and Huss 1996; Sivertsvik *et al*., 2002). Similar effects of MAP have been reported for various marine species (Gimenez *et al*., 2002; Ozogul *et al*., 2004).

Table (1): Changes in total viable count on fish fillets stored in different modified atmospheres at 5ºC.

Gas atmosphere	Log_{10} CFU of total viable count at n days of storage.					
	0	3	6	8	10	12
Air (control)	3.42^{Ca}	5.10^{Ba}	6.78^{Aa}	n.d.	n.d.	n.d.
60% CO_2 + 20% O_2+20%N_2	3.42^{Ea}	3.92^{Db}	4.75^{Cb}	5.48^{Ba}	6.80^{Aa}	n.d.
80% CO_2 + 10% O_2+10%N_2	3.42^{Ea}	3.60^{Ec}	3.96^{Dc}	4.67^{Cb}	5.55^{Bb}	6.59^{Aa}
80% CO_2 + 20%N_2	3.42^{Ea}	3.57^{Ec}	4.02^{Dc}	4.75^{Cb}	5.67^{Bb}	6.64^{Aa}

1. Values with the same superscripts in the same horizontal row (A-E) or vertical column (a-d) is not significantly different (P≥ 0.05). 2. The log colony forming units (C.F.U) values stated refer to three samples. 3. n.d. = not determined because of spoilage.

The initial pH value was 6.54.After 3 and 6 days of storage at 5ºC, there was significant difference (P<0.05) between samples packed in MAP and control samples (Table 2). This mainly due to the production of carbonic acid by the dissolved CO_2 in the aqueous phase of the fish fillets (Devlieghere *et al*., 1998; Sivertsvik *et al*., 2004).

Table (2): Changes in pH of fish fillets packed in different modified atmospheres and stored at 5ºC.

Gas atmosphere	The pH values at n days of storage.					
	0	3	6	8	10	12
Air (control)	6.54^{Ca}	6.78^{Ba}	7.12^{Aa}*	n.d.	n.d.	n.d.
60% CO_2 + 20% O_2+ 20%N_2	6.54^{Ca}	6.45^{Db}	6.49^{CDb}	6.62^{Ba}	6.81^{Aa}*	n.d.
80% CO_2 + 10% O_2+ 10%N_2	6.54^{BCa}	6.38^{Ec}	6.40^{EDb}	6.48^{CDb}	6.57^{Bb}	6.70^{Aa}*
80% CO_2 + 20%N_2	6.54^{BCa}	6.40^{Dbc}	6.43^{Db}	6.52^{Cb}	6.60^{Bb}	6.75^{Aa}*

1. Values with the same superscripts in the same horizontal row (A-E) or vertical column (a-c) are not significantly different (P≥ 0.05). 2. n.d. =not determined because of spoilage. 3. The pH values stated refer to three samples. 4. * =Typical off odors (spoilage) on the next day.

The growth of *Enterobacteriaceae* on all samples packed in modified atmospheres was strongly inhibited (Table 3). On the other hand, the log_{10} CFU/g of *Enterobacteriaceae* on samples packed in air (control) increased rapidly and was approximately 3 log_{10} units higher than that on fillets packed in modified atmospheres after 6 days of storage at 5ºC. After 6, 8 and 12 days of storage, there were significant differences between count on fillets packed in 80% CO_2 + 10% O_2+ 10% N_2; 80% CO_2 + 20% N_2 and count on samples packed in 60% CO_2 + 20% O_2+ 20% N_2. It seems that *Enterobacteriaceae* were sensitive to an increase in CO_2 from 60 to 80 %. Several investigator found that *Enterobacteriaceae* were sensitive to increase concentration of CO_2 (Gimenez *et al*., 2002; Metin *et al*., 2002).

Table (3): Changes in *Enterobacteriaceae* count on fish fillets stored in different modified atmospheres at 5ºC.

Gas atmosphere	Log_{10} CFU of *Enterobacteriaceae* bacteria count at n days of storage.					
	0	3	6	8	10	12
Air (control)	1.85^{Ca}	3.12^{Ba}	5.95^{Aa}	n.d.	n.d.	n.d.
60% CO_2+20% O_2+ 20%N_2	1.85^{Ea}	2.10^{Db}	3.08^{Cb}	4.12^{Ba}	5.28^{Aa}	n.d.
80% CO_2+10% O_2+ 10%N_2	1.85^{Ea}	1.65^{Ec}	2.12^{Dc}	2.84^{Cb}	3.55^{Bb}	4.40^{Aa}

80% CO_2 + 20%N_2	1.85^{Ea}	1.89^{Ec}	2.25^{Dc}	2.72^{Cb}	3.67^{Bb}	4.58^{Aa}

1. Values with the same superscripts in the same horizontal row (A-E) or vertical column (a-d) is not significantly different (P≥ 0.05). 2. The log colony forming units (C.F.U) values stated refer to three samples. 3. n.d. = not determined because of spoilage.

The initial number of lactic acid bacteria on the fish fillets was 1.94 $\log_{10}$ CFU/g (Table 4). The number of lactic acid bacteria increased rapidly on samples packed in air (control). On day 3 and 6, their were significant difference (P< 0.05) for the number of lactic acid bacteria between samples packed in MAP and control samples. After a lag period of 3 days, the number of lactic acid bacteria on samples packed in MAP started to increase at a slower rate. On the day of spoilage on all samples held in MAP lactic acid bacteria were found to be the predominating flora. These results are contradictory to those obtained by other investigators (Gimenez *et al*., 2002; Sivertsvik *et al*., 2002).

Table (4): The growth of lactic acid bacteria on fish fillets stored in different modified atmospheres at 5ºC.

Gas atmosphere	Log_{10} CFU of lactic acid bacteria count at n days of storage.					
	0	3	6	8	10	12
Air (control)	1.94^{Ca}	3.40^{Ba}	5.11^{Aa}	n.d.	n.d.	n.d.
60% CO_2+20% O_2+20%N_2	1.94^{Ea}	2.38^{Db}	3.94^{Cb}	5.17^{Ba}	6.25^{Aa}	n.d.
80%CO_2+10% O_2+10%N_2	1.94^{Ea}	2.09^{Ec}	2.82^{Dc}	4.14^{Cb}	4.99^{Bb}	6.22^{Aa}
80% CO_2+ 20%N_2	1.94^{Ea}	2.15^{Ec}	3.06^{Dc}	4.05^{Cb}	5.17^{Bb}	6.37^{Aa}

1. Values with the same superscripts in the same horizontal row (A-E) or vertical column (a-d) is not significantly different (P≥ 0.05). 2. The log colony forming units (C.F.U) values stated refer to three samples. 3. n.d. = not determined because of spoilage.

A strong inhibition was evidence in number of H_2S-producing bacteria for all samples packed in modified atmospheres (Table 5). In contrast the number of H_2S-producing bacteria on samples packed in air (control) increased rapidly and was approximately 3.15 log10 units higher than that on fillets packed in MAP after 6 days of storage. A similar trend was obtained for the *Enterobacteriaceae* (Table 3).

Table (5): The inhibition of H_2S-producing bacteria count on fish fillets stored in different modified atmospheres at 5ºC.

Gas atmosphere	Log_{10} CFU of H_2S-producing bacteria count at n days of storage.					
	0	3	6	8	10	12
Air (control)	1.32^{Ca}	4.18^{Ba}	6.02^{Aa}	n.d.	n.d.	n.d.
60% CO_2+ 20% O_2+ 20%N_2	1.32^{Fa}	1.95^{Db}	2.85^{Cb}	4.12^{Ba}	5.18^{Aa}	n.d.
80% CO_2 + 10% O_2+10%N_2	1.32^{Ea}	1.43^{Ec}	2.12^{Dc}	2.90^{Cb}	3.66^{Bb}	4.52^{Aa}
80% CO_2 + 20%N_2	1.32^{Ea}	1.50^{Ec}	2.30^{Dc}	3.18^{Cb}	3.84^{Bb}	4.66^{Aa}

1. Values with the same superscripts in the same horizontal row (A-E) or vertical column (a-d) is not significantly different (P≥ 0.05). 2. The log colony forming units (C.F.U) values stated refer to three samples. 3. n.d. = not determined because of spoilage.

The initial total volatile bases nitrogen (TVB-N) value of fish was 4.82 mg/100g of fish (Table 6). The TVB-N continuously increased during storage for all samples. The initial TVB-N value of samples packed in air (4.82mg/100g), increased rapidly to 23.64 mg/100g, while the TVB-N of samples packed in 80% CO_2 + 10% O_2+ 10% N_2 increased from 4.82mg/100g to 8.87 after 6 days of storage at 5ºC. However, the rates of increase for all samples packed in MAP were significantly slower (P<0.05) than control samples.

Table (6): Changes in total volatile bases nitrogen (TVB-N) of fish fillets stored in different modified atmospheres at 5ºC.

Gas atmosphere	The TVB-N mg/100g at n days of storage.					
	0	3	6	8	10	12
Air (control)	4.82^{Ca}	12.25^{Ba}	23.64^{Aa} *	n.d.	n.d.	n.d.
60% CO_2+ 20% O_2+20%N_2	4.82^{Ea}	6.72^{Db}	11.67^{Cb}	16.43^{Ba}	22.85^{Aa}*	n.d.
80% CO_2+ 10% O_2+10%N_2	4.82^{Fa}	6.18^{Eb}	8.87^{Dc}	12.75^{Cb}	17.16^{Bb}	21.78^{Aa}*
80% CO_2+ 20%N_2	4.82^{Fa}	6.35^{Eb}	9.32^{Dc}	13.18^{Cb}	17.84^{Bb}	*22.13^{Aa}

1. Values with the same superscripts in the same horizontal row (A-F) or vertical column (a-c) are not significantly different (P≥ 0.05). 2. n.d. =not determined because of spoilage. 3. The total volatile bases nitrogen values stated refer to three samples. 4. *=Typical off odors (spoilage) on the next day.

The sensory evaluation of color of fish fillets was improved by packaging in 80% CO_2 + 10% O_2+ 10% N_2 and 60% CO_2 + 20% O_2+ 20% N_2 (Table 7). In contrast the color of samples packed in 80% CO_2 + 20% N_2 was poor

without off odor after 3 days of storage. However, the color of control samples were still acceptable after 6 days of storage. The 80% CO_2 + 10% O_2+ 10% N_2 gas mixture was the most effective for the color stability of the fish fillets. The changes in odor followed closely the changes in bacterial counts, which agreed with those of Kyrana and Lougovois, (2002). The odor of all fish fillets packed in MAP showed improvement ($P< 0.05$) as compared with control after 3 and 6 days of storage at 5ºC. Control samples were spoiled with putrid odor after 6 days of storage.

Table (7): Sensory evaluation of fish fillets held in different MAP and stored at 5ºC. (A=Color, B=Odor).

A: Color.

Gas atmosphere	Days of storage at 5 °C					
	0	3	6	8	10	12
Air (control)	8.90^{Aa}	7.22^{Bb}	5.18^{Cc*}	n.d.	n.d.	n.d.
60% CO_2 + 20% O_2+ 20%N_2	8.90^{Aa}	8.64^{Aa}	8.52^{Ab}	7.12^{Bb}	5.74^{Cb}*	n.d.
80% CO_2 + 10% O_2+ 10%N_2	8.90^{Aa}	8.86^{Aa}	8.88^{Aa}	8.36^{Aa}	7.62^{Ba}	*6.84^{Ca}
80% CO_2 + 20%N_2	8.90^{Aa}	4.68^{Bc}	4.45^{Bd}	3.86^{Cc}	2.68^{Dc}	2.35^{Db}*

B: Odor.

Air (control)	8.84^{Aa}	7.94^{Bc}	*5.34^{Cc}	n.d.	n.d.	n.d.
60% CO_2 +20% O_2+20%N_2	8.84^{Aa}	8.51^{Ab}	7.48^{Bb}	6.65^{Cc}	*5.73^{Dc}	n.d.
80% CO_2 +10% O_2+10%N_2	8.84^{Aa}	8.87^{Aa}	8.45^{Aa}	7.72^{Ba}	7.02^{Ca}	*6.42^{Da}
80% CO_2 + 20%N_2	8.84^{Aa}	8.73^{Aa}	8.22^{Aa}	7.35^{Bb}	6.74^{Cb}	*5.98^{Db}

1. Values with the same superscripts in the same horizontal row (A-D) or vertical column (a-d) are not significantly different ($P\geq 0.05$). 2. n.d. =not determined because of spoilage. 3. 9 =extremely good 5 =marginally acceptable 1 = extremely poor. 4. *=Typical off odors (spoilage) on the next day.

The level of total volatile bases nitrogen (TVB-N) 30 mg/100g of fish has been considered to be the upper limit above which fish are considered unfit for human consumption (Vyncke *et al*., 1987; EEC, 1995; Ashie *et al*., 1996) and also, critical spoilage level of $\log_{10}$ CFU/g 7-8 of total viable count followed by typical off odor on the next day (Ashie *et al*., 1996; Gram and Huss 1996). All samples at the end of storage periods were below the critical marginal quality, followed by off odor next day. According to that limit and sensory quality, samples packed in 60% CO_2 + 20% O_2+ 20% N_2; 80% CO_2 + 20% N_2 and 80% CO_2 + 10% O_2+ 10% N_2 have a shelf life at 5ºC of 10, 12 and 12 days respectively. This signifies a prolongation of shelf life at 5ºC of 4, 6 and 6 days respectively, as compared to (control).

CONCLUSION

Packaging Areolated grouper (*Epinephelus areolatus*) fillets in 80% CO_2 + 10% O2+ 10% N_2 will improve the microbiological, chemical and sensory quality and prolong shelf life of the fish during storage at 5ºC.

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