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# 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*) of economical importance 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. To determine the microbiological, chemical and sensory qualities of fish fillets during storage in different atmospheres at 5°C. Results revealed that total viable count; *Enterobacteriaceae;* lactic acid bacteria and $H_2S$-producing bacteria were inhibited by modified atmospheres as compared with the fillets packed in air (control).The differences were significant ($P<0.05$).The highest inhibition were observed with packaging fish fillets in 80% $CO_2$ + 20% $N_2$ and 80% $CO_2$ + 10% $O_2$+ 10% $N_2$ . 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 microbiological quality. There was significant difference ($P<0.05$) in pH values between 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 significantly ($P<0.05$) decreased by packaging fish fillets in modified atmospheres after 3 and 6 days of storage at 5°C as compared with the samples packed in air (control). The sensory evaluation of fish fillets revealed that colour 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 colour of samples packed in 80% $CO_2$ + 20% $N_2$ was evaluated as poor after 3 days of storage. However the, colour of samples packed in air 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 colour stability of the fish fillets. The changes in odour followed closely the changes in bacterial counts. Microbiological and chemical changes supported the sensory results. 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 shelf life at 5°C of 10, 12 and 12 days respectively. This signifies a prolongation of shelf life at 5°C of 4, 6 and 6 days respectively, as compared with samples packed in air (control). The best results were obtained by packaging fish fillets in 80% $CO_2$ + 10% $O_2$+ 10% $N_2$.

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 5°C.

## 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 that are mainly responsible for spoilage of fish muscle (Genigeorgis, 1985; Ashie *et al.*, 1996; Gram and Huss 1996). These activities lead to a short shelf life in fish and other 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). MAP offers multiple advantages to the fish industry and the consumer (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). Various atmospheres have been examined in fish packaging (Reddy *et al.*, 1994; Randell *et al.*, 1995; Church, 1998; Gimenez *et al.*, 2002; Sivertsvik *et al.*, 2004).

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 colour 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 (Stammen *et al.*, 1990; 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 (Genigeorgis, 1985; 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 5°C.

## MATERIALS AND METHODS

Freshly caught Areolated grouper (*Epinephelus areolatus*) of commercial importance was obtained from Al-Katteif market (central market, in Al-Katteif, Saudi Arabia), with average weight of 350 g. Fish were immediately placed in ice-boxes and transported to the laboratory of college of Agricultural and Food Sciences., King Faisal University of Saudi Arabia, within 2 hour, and decapitated and filleted. Two fillets were obtained from each fish by removing the head and bone of fish. Control samples were packed (separately) with air in polyethylene bags (70µm thickness, with oxygen permeability of 750 ml/$m^2$/24 hr. at 1 atmosphere and 23 °C) and stored at 5 °C.

**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 °C). 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 °C. 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 (PCA; Oxoid CM 325), incubated at 25 °C for 72 h (Kyrana and Lougovois, 2002). *Enterobacteriaceae* were determined as colony forming units on Violet Red Bile Glucose Agar (VRBG) (Oxoid CM485) with an overlay of the same agar, incubated for 24 h at 37 °C (Gram and Huss 1996). Lactic acid bacteria colony forming units were determined on MRS agar (Oxoid CM 361) covered with an overlay of the same agar incubated for 3 days at 30 °C (Gram and Huss 1996). $H_2S$-producing bacteria were determined on iron agar, supplemented with 0.04% L-cysteine (w/v) and covered with an overlay of the same agar, incubated for 3 days at 25 °C (Gram *et al.*, 1987).Black colonies were recorded as sulphide producers.

**Sensory analysis:** Sensory evaluation was carried out using five trained panelists. Samples were judged for colour and odour. A hedonic scale was used between 9 (extremely good) and 1 (extremely poor) (Metin *et al.*, 2002). The score of each parameter was calculated in terms of average score points given by panel of judges to each sample A score of 5 was taken as the average score for minimum acceptability.

**Chemical analysis:** The total volatile bases nitrogen (TVB-N) values of the fish fillets were determined according to Vyncke *et al.*, (1987).The results were expressed as mg TVB-N/100g.
The pH values were recorded by using a digital pH meter (Thermo Orion, model 260A) (USA) after homogenization of 10g fish muscle sample in 100 ml distilled water.

**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 (ANOVA) and subjected least significant difference (LSD) at 0.05% level of significant was used to compare the treatment means (Waller and Duncan, 1969). Computations were done using SAS (1996).

## RESULTS AND DISCUSSION

Modified atmospheres packaging (MAP) foods have become increasingly more available, as food manufacturers have attempted to meet

consumer demands for fresh, refrigerated foods with extended shelf life (Gimenez *et al.*, 2002; Sivertsvik *et al.*, 2002). The use of an MAP with an enhanced carbon dioxide level has been shown to extend the shelf-life of fish (Philips, 1996; Sivertsvik *et al.*, 2002). The effect of modified atmospheres packaging on total viable count (TVC) of fish fillets stored at 5 °C are presented in Table 1. The initial total viable count on fish fillets was 3.42 $log_{10}$ CFU/g. The total viable count increased rapidly on samples packed in air (control). 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 the inhibitory effect of the higher concentration of $CO_2$ (80%) on microbial growth. $CO_2$, because of its bacteriostatic effect, 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 5°C.**

| 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 changes in pH values of the fish fillets packed in modified atmospheres or in air and stored at 5°C are illustrated in Table 2. The initial pH value was 6.54.After 3 and 6 days of storage at 5°C, there was significant difference (P<0.05) between 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 (Devlieghere *et al.*, 1998; Sivertsvik *et al.*, 2004).

The effect of storage time X atmosphere interaction on *Enterobacteriaceae* count was significant (P<0.05), as shown in Table 3. The growth of *Enterobacteriaceae* on all samples packed in modified atmospheres was strongly inhibited. 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 5°C. 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 (2): Changes in pH of fish fillets packed in different modified atmospheres and stored at 5°C.**

| 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 odours (spoilage) on the next day.

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

| 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 effect of modified atmospheres packaging on the growth of lactic acid bacteria is shown in Table 4. The ANOVA indicates that storage time and treatment effects were significant (P< 0.05) in lactic acid bacteria counts. The initial number of lactic acid bacteria on the fish fillets was 1.94 $log_{10}$ CFU/g. 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 modified atmospheres (MAP) and samples packed in air (control). 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).

The inhibition of $H_2S$-producing bacteria on fish fillets packed in modified atmospheres and stored at 5 °C is illustrated in Table 5. The ANOVA indicates that storage time and treatment effects were significant ($P< 0.05$) in $H_2S$-producing bacteria counts. A strong inhibition was evidence in number of $H_2S$-producing bacteria for all samples packed in modified atmospheres. 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 modified atmospheres after 6 days of storage. A similar trained was obtained for the *Enterobacteriaceae* (Table 3).

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

| 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.

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

| 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%$N_2$ | $1.32^{Ea}$ | $1.95^{Db}$ | $2.85^{Cb}$ | $4.12^{Ba}$ | $5.18^{Aa}$ | 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 used was 4.82 mg/100g of fish (Table 6). The TVB-N continuously increased during storage for all the 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 5°C. However, the rates of increases for all samples packed in modified atmospheres packaging were significantly slower ($P<0.05$) than that of samples packed in air (control).

Fresh seafoods are generally considered to have little odour. As seafoods are stored, an odour develops and often characterized as being

"fishy". The product continues to deteriorate, ultimately having what is often described as intense and putrid odours. Sensory methods are frequently applied for estimating the quality of seafoods, and correlated to the microbiological data and chemical analyses (Kyrana and Lougovois, 2002). The results of sensory tests are presented in Table 7.The sensory evaluation of fish fillets revealed that colour 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 colour of samples packed in 80% $CO_2$ + 20% $N_2$ was evaluated as poor without off odour after 3 days of storage. However the, colour of samples packed in air 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 colour stability of the fish fillets. The changes in odour followed closely the changes in bacterial counts, which agreed with those of Kyrana and Lougovois, 2002. The odour of all fish fillets packed in modified atmospheres showed significantly improvement ($P< 0.05$) as compared with sample packed in air (control) after 3 and 6 days of storage at 5°C. Control samples were spoiled with persistent putrid odour after 6 days of storage.

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

| 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 ($P\geq 0.05$).
2. n.d. =not determined because of spoilage.
3. The total volatile bases nitrogen values stated refer to three samples.
4. *=Typical off odours (spoilage) on the next day.

**Table (7): Sensory evaluation of fish fillets held in different modified atmospheres and stored at 5°C. (A=Colour, B=Odour).**

**A: Colour.**

| 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%$N_2$ | $8.90^{Aa}$ | $4.68^{Bc}$ | $4.45^{Bd}$ | $3.86^{Cc}$ | $2.68^{Dc}$ | $2.35^{Db*}$ |

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 odours (spoilage) on the next day.

The level of total volatile bases nitrogen (TVB-N) 30 mg/100g of fish has been considered to the upper limit above which fish are considered unfit for human consumption (Vyncke *et al.*, 1987; EEC, 1995; Ashie *et al.*, 1996)

and critical spoilage level of $\log_{10}$ CFU/g 7-8 of total viable count followed by typical off odour 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 odour 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 5°C of 10, 12 and 12 days respectively. This signifies a prolongation of shelf life at 5°C of 4, 6 and 6 days respectively, as compared with samples packed in air (control). The best results were obtained by packaging fish fillets in 80% $CO_2$ + 10% $O_2$+ 10% $N_2$.This gas composition extended the shelf life of mussels for 6 days, as compared with control samples, retained higher sensory scores than all other packaged samples ($P< 0.05$) .

**Table (7): Continues B: Odour.**

| Gas atmosphere | Days of storage at 5 °C | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 8 | 10 | 12 |
| 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-c) are not significantly different (P≥ 0.05).**
2. **n.d. =not determined because of spoilage.**
3. **9 =extremely good 5 =marginally acceptable 1 = extremely poor.**
4. ***=Typical off odours (spoilage) on the next day.**

**In 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 5°C.

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## تقيم ميكروبيولوجي وكيماوي وحسي لفيله سمك الهامور النجمي (الوقار) المخزن في جو غازي معدل

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تم تعبئة سمك الهامور النجمي (الوقار) ( *Epinephelus areolatus* ) ذو الأهمية الاقتصادية المرتفعة في توليفات مختلفة من الجو الغازي المعدل كالتالي: ٨٠% ثاني أكسيد الكربون + ٢٠% نيتروجين ، ٦٠% ثاني أكسيد الكربون + ٢٠% أكسجين + ٢٠% نيتروجين ، ٨٠% ثاني أكسيد الكربون + ١٠ أكسجين + ١٠% نيتروجين ثم العينة الكنترول في هواء جوي عادي .وذلك لتحديد صفات الجودة الميكروبية والكيماوية والحسية لتلك العينات المعبأة في توليفات مختلفة من الغازات والمخزنة في الثلاجة علي ٥م.

ولقد أظهرت النتائج أن تعبئة شرائح فليه السمك في الجو الغازي المعدل أدت إلي تثبيط كل من العدد البكتيري الكلي وبكتيريا الـ *Enterobacteriaceae* والبكتريا المنتجة لغاز كبريتيد الهيدروجين وبكتريا حامض اللاكتيك إذا ما قورنت بالعينة من نفس السمك المعبأة في الهواء العادي وكانت الفروق معنوية. وأشارت النتائج أن أعلي تثبيط كان في العينات المحتوية علي ٨٠% ثاني أكسيد الكربون حيث لم يكون هناك فرق معنوي بين العينات المعبأة في ٨٠% ثاني أكسيد الكربون + ٢٠% نيتروجين و ٨٠% ثاني أكسيد الكربون + ١٠ أكسجين + ١٠% نيتروجين من الناحية الميكروبية. أيضا أظهرت النتائج أن هناك فرق معنوي في رقم الـ pH بين العينات المعبأة في الجو الغازي المعدل والمعبأة في الهواء العادي وذلك يرجع إلي ذوبان ثاني أكسيد الكربون في الوسط المائي لشرائح السمك منتجا حامض الكربونيك.أيضا النيتروجين الكلي المتطاير انخفاض معنوي بعد ٣و٦ يوم من التخزين علي درجة حرارة ٥م في عينات السمك المعبأة في الجو الغازي المعدل إذا ما قورنت مع العينة المعبأة في الهواء. أوضحت أيضا نتائج التقييم الحسي تحسن لون شرائح السمك المعبأ في كل من ٨٠% ثاني أكسيد الكربون + ١٠ أكسجين + ١٠% نيتروجين وأيضا ٦٠% ثاني أكسيد الكربون + ٢٠% أكسجين + ٢٠% نيتروجين وعلي العكس من ذلك ظهر تغير غير مرغوب في لون العينات المعبأة في ٨٠% ثاني أكسيد الكربون + ٢٠% نيتروجين بعد ثلاثة أيام بينما كان لون العينة المعبأة في الهواء العادي مقبول لمدة ٦ أيام.أكدت النتائج فاعلية الجو الغازي المكون ٨٠% ثاني أكسيد الكربون + ١٠ أكسجين + ١٠% نيتروجين في أعطاء أعلي ثبات لون لشرائح السمك المعبأ. ولقد كانت التغيرات في الرائحة متوافقة مع التغيرات في العد البكتيري وهذا يوضح تطابق التغيرات الكيماوية والميكروبيولوجية في السمك مع نتائج الخواص الحسية. أوضحت النتائج أن مدة الصلاحية لشرائح السمك المعبأ في ٦٠% ثاني أكسيد الكربون + ٢٠% أكسجين + ٢٠% نيتروجين ، ٨٠% ثاني أكسيد الكربون + ١٠ أكسجين + ١٠% نيتروجين ، ٨٠% ثاني أكسيد الكربون + ٢٠% نيتروجين كان ١٠، ١٢، ١٢ يوم علي التوالي. وذلك يؤكد إطالة مدة الصلاحية ٤، ٦، ٦ يوم علي التوالي إذا ما قورنت بالسمك المعبأ في الجو العادي.

أتضح من الدراسة أن أحسن النتائج تم الحصول عليها بتعبئة السمك في ٨٠% ثاني أكسيد الكربون + ١٠ أكسجين + ١٠% نيتروجين والتي أدت إلي تحسين كل من الخواص الميكروبيولوجية والكيماوية والحسية مع أطالة مدة الصلاحية أثناء التخزين علي ٥م.