

Antioxidant effect of ascorbic acid on the quality of Cobia (*Rachycentron canadum*) fillets during frozen storage

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Abstract

The effect of aqueous solutions of ascorbic acid (AA) on the rancidity development in Cobia (*Rachycentron canadum*) fillets during frozen storage was studied. Cobia fillets were treated with ascorbic acid (AA 0.25% and AA 0.5%) then stored at -18°C up to 6 months. Rancidity development was measured by several biochemical indices including free fatty acids (FFA), peroxide value (PV), and thiobarbituric acid (TBA) and complemented by the sensory analysis (odor, consistency and appearance). In addition, pH and expressible moisture (EM) were measured during 6 months storage. Proximate composition was also determined in the first day. TBA, PV and FFA levels increased on all treatments due to lipid oxidation. Ascorbic acid showed antioxidative effect on Cobia fillets during frozen storage as indicated by TBA, PV and FFA levels. Results showed that free fatty acid, primary and secondary oxidation products, EM and pH value of AA- treated samples were significantly lower than those of the control samples ($P<0.05$). A gradual decrease ($P<0.05$) in sensory analysis were observed as the storage time increased. Results of our investigation revealed that ascorbic acid retarded oxidative changes in frozen Cobia fillets whereas AA 0.25% was not as effective as AA 0.5% on oxidative stability. Best oxidation inhibition results on fish fillets were obtained when employing a 0.5% AA solution.

Keywords: Cobia, Lipid oxidation, Ascorbic acid, Frozen storage

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Introduction

Lipid oxidation is one of the major forms of spoilage in foods, because it leads to the formation of off-flavors and potentially toxic compounds (Babovic et al., 2010). It affects the quality of the product due to the loss of a desirable color, odor, and flavor and reduces the shelf life (Wettasinghe and Shahidi, 1999; Babovic et al., 2010). Lipid oxidation is done by a series of chain reactions; this process which is based on Free-Radical formation contains three stages: Initiation, Propagation and Termination (Chaijan et al., 2006). Marine lipids have a high content of polyunsaturated fatty acids (PUFAs), so it is not surprising if oxidative rancidity is known as the most important problem in their output products technology. (Pazos et al., 2005; Khan et al., 2006). Different methods have been used for extending fish products shelf life such as low temperature storage, proper packaging and glazing with solution of protecting chemicals and antioxidants (Pourashouri et al., 2009; Lin and Lin, 2005; Rostamzad et al., 2011). Lipid oxidation is one of the most important factors responsible for quality deterioration of fish during both refrigerated and frozen storage (Serdaroglu and Felekoglu, 2005). The investigated studies show that freezing is one of the best methods for long-term fish maintenance (Verma and Sriker, 1994; Vidya Sagar Reddy and Sriker, 1996; Aubourg et al., 2004; Aubourg et al., 2005). Freezing prevents microbial spoilage and helps to reduce fat oxidation but cannot prevent it. One of the appropriate methods to access this target is using additives such as antioxidants. The

use of antioxidants is emerging as an effective methodology for controlling rancidity in oils and food (Pazos et al., 2005; Rostamzad et al., 2011). The application of synthetic and natural antioxidants to control lipid oxidation in sea foods is well established (Khan et al., 2006). Researchers are using antioxidants to prevent or reduce fat oxidation, now. Also they use synthetic antioxidants like Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA) and Tertiary butylhydroquinone (TBHQ) to solve oxidation problems. But nowadays synthetic antioxidants are known as carcinogen and mutating agents. So it is tried to replace natural antioxidants instead of artificial ones (Aubourg et al., 2004; Pourashouri et al., 2009). Natural antioxidants are used increasingly, to preserve meat and fishes (Sahoo et al., 2004; Lin and Lin, 2005). Recently the food industry focused on the use of natural antioxidants, such as tocopherols, various spices and herbs, vegetable extracts and ascorbic acid. The antioxidant properties of spices and herbs are attributed to their phenolic contents (Serdaroglu and Felekoglu, 2005). Many studies reported the effectiveness of these additives in retarding lipid oxidation (Aubourg et al., 2004; Serdaroglu and Felekoglu 2005; Pourashouri et al., 2009). The use of ascorbic acid as an antioxidant was reported by (Aubourg et al., 2004; Pourashouri et al., 2009; Rostamzad et al., 2011). Ascorbic acid can be used as an antioxidant agent to increase shelf life of processed food and conserves. This acid is a strong antioxidant and has a direct

synergistic relationship with other antioxidants (Aubourg et al., 2004). Ascorbat acts as a metal chelator and oxygen scavenger agent and plays a reducing role (Boyd et al., 1993; Aubourg et al., 2004; Fernandez-Lopez et al., 2005). This acid is used in oil, fillet and fish mince and there is no limitation for ascorbic acid consumption (Aubourg et al., 2004). Cobia is a promising candidate for aquaculture because of rapid growth rates, reaching up to 4-6 kg in a year, hardness, efficient feed conversion, excellent flesh quality, and comparatively low production costs (Franks et al., 1999; Chou et al., 2001; Liao et al., 2004; Wang et al., 2005). At present, research on cobia mainly concentrates on breeding, culture, disease prevention, and feed. The study of cobia processing is, however, rarely reported. The aim of present study was to investigate the antioxidant activity of ascorbic acids on extending shelf life of Cobia fillets. Lipid hydrolysis and oxidation assessments were carried out during 6 months frozen storage.

Materials and methods

Sample preparation

Fresh Cobia (*Rachycentron canadum*) were caught from Persian Gulf in Bandar Abbas (Hormozgan province, Persian Gulf). The average length and weight of the specimens employed were 92.23 ± 1.04 cm and 5.32 ± 1.02 kg, respectively. The fish samples were put in ice box with ice and transferred to processing salon in Persian Gulf and Oman Sea Ecology Research Center in 30 minutes. Then, fish samples were deheaded, gutted and filleted

(240 fillet particles) by hand and washed by cold water carefully. The weight of each fillet was 200 ± 5 g. Then, fillets were divided into 3 groups. Samples of the first group were left untreated (blank control; BC treatment) directly packaged in polyamide/polyethylene (PA/PE) bags. The Second groups submerged in 0.25% of ascorbic acid aqueous solution (AA treatment 0.25%) and the third groups were submerged in 0.5% of ascorbic acid aqueous (AA treatment 0.5%) respectively. After 5 minutes, fillets were removed from all solutions and packaged in individual polyamide/polyethylene bags. Dipping time was chosen according to previous related research (Aubourg et al., 2004; Pourashouri et al., 2009). After packaging, first the fillets were frozen by air blast freezing method for 24 h at -30°C and then stored in -18°C for 6 months. For all kinds of fish fillets, analysis was carried out after the freezing process (0-month storage at -18°C), and after 1, 3 and 6 months of storage at -18°C . In all cases, thawing was carried out by refrigerated storage (4°C) over night. Also on the first day the amount of protein, fat, moisture and ash of fillets were measured. Each analysis was conducted in three replications.

Chemical analysis

The moisture content in flesh of cobia was determined by drying to constant weight (g) at 105°C for 20 to 24 h according to the AOAC standard method (AOAC, 2005). Crude ash was determined after heating the sample overnight at 550°C (AOAC, 2005). The crude protein content was determined as Kjeldal-protein using a

factor 6.25 following the AOAC standard method (AOAC, 2005). The fat content of Cobia was determined by a solvent extraction (Submersion) method for fat (Crude) in Meat and Meat Products (AOAC, 2005). For measurement of pH, five grams of fish mince was homogenized for 1 minute with 45 ml of distilled water. The pH value was measured using a standardized portable pH meter (TOA, Japan) (Suvanich et al., 2000). Expressible moisture (EM) content was determined by weight difference between the muscle (1-2g) of fish before and after being pressed under 0.5 and 1 kg load for 5 and 20 minutes respectively (Pourashouri et al., 2009). Three replicates were used for each experiment.

Lipid oxidation measurements

As frozen fish quality has shown a great dependence on lipid hydrolysis and oxidation, the following lipid measurements were chosen as representative of lipid hydrolysis free fatty acids (FFA), primary peroxide value (PV) and secondary thiobarbituric acid (TBA) lipid oxidation. Free fatty acid (FFA)

content was determined in the lipid extract by the Kirk & Sawyer (1991) method. Results are expressed as grams of oleic fatty acid per kilogram lipids. Peroxide value (PV) was determined in the lipid extract according to the method described by AOAC (2000). Results are expressed as milli-equivalents oxygen per kg lipid (meq O₂/kg lipid). Thiobarbituric acid (TBA) was determined colorimetrically by the Porkony and Dieffenbacher method as described by Kirk and Sawyer (1991). Results are expressed as mg malondialdehyde /kg (mg ma/kg) fish muscle.

Sensory analysis

Sensory analyses were conducted by a taste panel consisting of five experienced judges, according to the guidelines presented in Table 1 (Stodolnik et al., 2005; Pourashouri et al., 2009; Rostamzad et al., 2011). Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and poor quality (C). Sensory assessment of the fish fillet included the following parameters: appearance, odor and consistency.

Table1: Sensory evaluation of frozen Cobia fillets stored at -18°C

| Attribute | E (Highest quality) | A (Good quality) | B (Fair quality) | C (poor quality) |
|-------------------|--|---|--|---|
| Flesh appearance | Strongly hydrated and pink; myotomes totally adhered | Still hydrated and pink; myotomes adhered | Slightly dry and pale; myotomes adhered in groups | Yellowish and dry; myotomes totally separated |
| Rancid Odor | Sharp seaweed and shellfish | Weak seaweed and shellfish | Slightly sour and incipient rancidity | Sharply sour and rancid |
| Flesh consistency | Presence or partial disappearance of rigor mortis symptoms | Firm and elastic; pressure signs disappear immediately and completely | Presence of mechanical signs; elasticity notably reduced | Important shape changes due to mechanical factors |

Statistical analysis

Nonparametric statistics used to analyze the data. Repeated Measures analysis of variance was used to compare between the groups and times. Repeated Measures was made with the General Linear Models (GLM) with a significant level of $P < 0.05$. The Duncan's multiple range tests with significant difference at $P < 0.05$ was used to compare sample means by using SPSS 16 software.

Results

Evolution of general chemical parameters

Proximate composition of fish involves the determination of moisture, lipid, protein and ash content. In this study moisture, crude protein, crude fat and crud ash contents of fresh Cobia were determined as 75.27 ± 0.04 %, 16.58 ± 0.25 %, 5.31 ± 0.85 % and 0.97 ± 0.1 %, respectively.

The pH value and its changes during storage (0, 1, 3 and 6 months) in Cobia fillets in BC and both treatments (AA 0.25% and AA 0.5%) are shown in (Table 2). The decreased were observed in pH from 5.90 to 5.46, 5.73 to 5.29 and 5.68 to 5.20 in BC, AA 0.25% and AA 0.5% respectively, during frozen storage in 6 months. There were Significant differences in control and both treatments during frozen storage in 6 months ($P < 0.05$). In a comparison between all treatments there were no Significant differences at zero and 3 month ($P > 0.05$) but there were Significant difference between control and both treatments in 1 to 6 month ($P < 0.05$). No significant difference was observed between treatment groups (AA 0.25% and AA 0.05%) in pH at 6 months ($P > 0.05$).

Table2: Changes of pH during frozen storage of Cobia fillets that were pretreated under different conditions, (means \pm SD (n = 3); $P < 0.05$) up to 6 months at -18°C

| Treatment | Frozen storage (months) | | | |
|-----------|--------------------------|--------------------------|--------------------------|--------------------------|
| | 0 | 1 | 3 | 6 |
| BC | $5.90 \pm 0.05\text{Aa}$ | $5.73 \pm 0.01\text{Aa}$ | $5.45 \pm 0.23\text{Ab}$ | $5.46 \pm 0.08\text{Ab}$ |
| AA 0.25 % | $5.73 \pm 0.01\text{Aa}$ | $5.70 \pm 0.02\text{Ba}$ | $5.42 \pm 0.08\text{Ab}$ | $5.29 \pm 0.13\text{Bb}$ |
| AA 0.5 % | $5.68 \pm 0.12\text{Aa}$ | $5.59 \pm 0.09\text{Ba}$ | $5.28 \pm 0.07\text{Ab}$ | $5.20 \pm 0.05\text{Bb}$ |

Means in column with different capital letters indicate significant differences ($p < 0.05$) among treatment and means in row with different small letters indicate significant differences ($p < 0.05$) as result of frozen storage.

SD, Standard Division, Treatment names, BC (blank control), AA (ascorbic acid 0.25% and 0.5%)

Changes in EM values of treatment groups stored at -18°C are given in (Table 3). Initial EM values of BC, AA 0.25% and AA 0.5% treatments were found to be 26.38, 29.8 and 32.83%, respectively. All

samples showed an increased EM value with storage ($P < 0.05$) period. No difference was found in EM values between BC and both treatments at the zero (initial) and 3 months of storage ($P >$

0.05) but there were Significant differences between control and both treatments in 1 and 6 months ($P < 0.05$) whereas significant difference was recorded between treatment groups (AA

0.25% and AA 0.05%) in 1 and 6 months ($P < 0.05$). The EM values of AA 0.25 and AA 0.5% treatments were significantly lower than BC after 6 months of storage.

Table3: Changes of Expressible moisture (%) during frozen storage of Cobia fillets that were pretreated under different conditions, (means \pm SD (n = 3); $P < 0.05$) up to 6 months at -18°C

| Treatment | Frozen storage (months) | | | |
|-----------|-------------------------|--------------------|--------------------|--------------------|
| | 0 | 1 | 3 | 6 |
| BC | 26.38 \pm 1.96Aa | 30.90 \pm 2.41Ab | 35.15 \pm 1.37Ac | 43.85 \pm 2.26Ad |
| AA 0.25 % | 29.80 \pm 1.57Aa | 34.55 \pm 1.26Bb | 35.86 \pm 1.98Ab | 37.26 \pm 1.63Bb |
| AA 0.5 % | 32.83 \pm 1.82Aa | 36.41 \pm 0.45Cb | 37.43 \pm 0.35Ab | 41.45 \pm 0.55Cc |

Means in column with different capital letters indicate significant differences ($p < 0.05$) among treatment and means in row with different small letters indicate significant differences ($p < 0.05$) as result of frozen storage.

SD, Standard Division, Treatment names as expressed in Table 1.

Lipid hydrolysis

The FFA values and its changes during storage (0, 1, 3 and 6 months) in Cobia fillets in BC and both treatments (AA 0.25% and AA 0.5%) are shown in (Table 4). All samples showed an increased FFA value with increased storage ($P < 0.05$). The increase was observed in FFA from 0.28 to 5.39, 0.16 to 2.36 and 0.19 to 1.28 (g of oleic acid kg^{-1} lipids) in BC, AA 0.25% and AA 0.5% respectively, during frozen storage in 6 months. At zero time there was no significant difference between treatment groups (Table 4); however, FFA value had a sharp increase in control samples after 1 month of storage. Significant difference was observed between BC and both treatments in 1, 3 and 6 months of storage ($P < 0.05$). No difference was observed in FFA levels between AA 0.25% and AA 0.5% in 1

month ($P > 0.05$) but there were Significant difference between both treatments in 3 and 6 months of storage ($P < 0.05$).

Lipid oxidation

Lipid oxidation development was measured according to the PV formation (primary oxidation compounds) and TBA (secondary oxidation compounds). Changes in PV values of BC and both treatments (AA 0.25% and AA 0.5%) during frozen storage at -18°C for 6 months are shown in (Table 5). Initial PV values of BC, AA 0.25% and AA 0.5% treatments were found 3.45, 2.97 and 2.86 meq O_2/kg and increased to 18.65, 8.11 and 7.34, respectively. All samples showed an increased PV value in Cobia fillets when the frozen storage increased ($P < 0.05$). At zero time of storage there was no significant difference in peroxide

values of the treatment groups ($P > 0.05$) but in the 1st, 3rd and 6th month significant difference was observed between BC and both treatments ($P < 0.05$).

No difference ($P > 0.05$) was detected between AA 0.25% and AA 0.5% treated samples during storage.

Table4: Changes of Free fatty acid (grams of oleic fatty acid kg^{-1} lipids) during frozen storage of Cobia fillets that were pretreated under different conditions, (means \pm SD (n = 3); $P < 0.05$) at -18°C

| Treatment | Frozen storage (months) | | | |
|-----------|-------------------------|-------------------|-------------------|-------------------|
| | 0 | 1 | 3 | 6 |
| BC | 0.28 \pm 0.12Aa | 0.66 \pm 0.10Aa | 2.35 \pm 0.42Ab | 5.39 \pm 0.97Ac |
| AA 0.25 % | 0.16 \pm 0.09Aa | 0.21 \pm 0.07Ba | 1.21 \pm 0.15Bb | 2.36 \pm 0.51Bc |
| AA 0.5 % | 0.19 \pm 0.09Aa | 0.27 \pm 0.07Ba | 0.67 \pm 0.27Cb | 1.28 \pm 0.26Cc |

Means in column with different capital letters indicate significant differences ($p < 0.05$) among treatment and means in row with different small letters indicate significant differences ($p < 0.05$) as result of frozen storage. SD, Standard Division, Treatment names as expressed in Table 1.

Table5: Changes of Peroxide value (meq oxygen kg^{-1} lipids) during frozen storage of Cobia fillets that were pretreated under different conditions, (means \pm SD (n = 3); $P < 0.05$) at -18°C

| Treatment | Frozen storage (months) | | | |
|-----------|-------------------------|-------------------|--------------------|--------------------|
| | 0 | 1 | 3 | 6 |
| BC | 3.45 \pm 0.23Aa | 7.91 \pm 1.71Ab | 10.11 \pm 1.83Ab | 18.65 \pm 0.58Ac |
| AA 0.25 % | 2.97 \pm 0.29Aa | 4.71 \pm 0.68Bb | 6.26 \pm 1.44Bb | 8.11 \pm 0.91Bc |
| AA 0.5 % | 2.86 \pm 0.86Aa | 3.70 \pm 0.99Ba | 6.00 \pm 1.22Bb | 7.34 \pm 1.81Bb |

Means in column with different capital letters indicate significant differences ($p < 0.05$) among treatment and means in row with different small letters indicate significant differences ($p < 0.05$) as result of frozen storage. SD, Standard Division, Treatment names as expressed in Table 1.

Changes in TBA values of BC and both treatments (AA 0.25% and AA 0.5%) during frozen storage at -18°C for 6 months are shown in (Table 6). Initial TBA values of BC, AA 0.25% and AA 0.5% treatments were found to be 0.102, 0.096 and 0.08 mg ma/kg and increased to 2.34, 0.877 and 0.347, respectively. All samples showed an increased TBA value in Cobia fillets when the frozen storage

increased ($P < 0.05$). No difference was found in TBA values between BC and both treatments at the zero (initial) time and first month ($P > 0.05$). After 3 months of storage significant difference was observed in TBA value between the BC and both AA treatments ($P < 0.05$) but no difference was found in TBA value between AA 0.25% and AA 0.5% ($P > 0.05$).

Table6: Changes of Thiobarbituric acid (mg malondialdehyde kg⁻¹ fish muscle) during frozen storage of Cobia fillets that were pretreated under different conditions, (means \pm SD (n = 3); P < 0.05) at -18°C

| Treatment | Frozen storage (months) | | | |
|-----------|-------------------------|---------------------|---------------------|---------------------|
| | 0 | 1 | 3 | 6 |
| BC | 0.102 \pm 0.004Aa | 0.131 \pm 0.009Aa | 0.768 \pm 0.089Ab | 2.340 \pm 0.280Ac |
| AA 0.25 % | 0.096 \pm 0.014Aa | 0.144 \pm 0.023Aa | 0.293 \pm 0.026Bb | 0.877 \pm 0.091Bc |
| AA 0.5 % | 0.080 \pm 0.006Aa | 0.152 \pm 0.020Aa | 0.222 \pm 0.023Bb | 0.347 \pm 0.047Cb |

Means in column with different capital letters indicate significant differences (p<0.05) among treatment and means in row with different small letters indicate significant differences (p<0.05) as result of frozen storage.

SD, Standard Division, Treatment names as expressed in Table 1.

Sensory analysis

Sensory scores obtained by the frozen Cobia fillets are shown in (Table 7). At the first time of the storage odor, consistency, color and appearance of fillets were fresh. As expected, a progressive quality loss was observed as a result of increasing the frozen storage. Comparison among treatments showed no difference when considering the flesh consistency, odor, color and appearance at the zero and first month but significant difference was observed in the 3rd and 6th months storage time (P<0.05). However, no difference was recorded between AA

0.25% and AA 0.5% during frozen storage. Attribute indices (odor, consistency and appearance of fillets) decreased at the 3rd month of storage. Flesh appearance assessment showed a lower score at the 6th month for the control samples than AA treatments. Also flesh consistency assessment showed a better score at the 3rd month for AA treatments than control samples. Odor analysis led to a better quality score (P<0.05) at the 3rd month for AA treatments than control samples.

Table7: Changes of sensory parameters during frozen storage of Cobia fillets those were pretreated under different conditions at -18°C

| Frozen storage (months) | Flesh appearance | | | Rancid odor | | | Flesh consistency | | |
|-------------------------|------------------|---------|--------|-------------|---------|--------|-------------------|---------|--------|
| | BC | AA0.25% | AA0.5% | BC | AA0.25% | AA0.5% | BC | AA0.25% | AA0.5% |
| 1 | E | E | E | E | E | E | E | E | E |
| 3 | A | A | A | B | A | A | B | A | A |
| 6 | B | A | A | C | A | A | B | A | A |

Freshness categories: E (excellent), A (good), B (fair) and C (poor).

All fish were category E for all attributes initially.

Treatment names are as expressed in Table 1.

Discussion

According to Mach (2009) and Daghoghi (2008) Cobia had high protein (16 – 21%) and medium fat (5.4%) content. Also according to Ackman (1990) classified lipid content of fish; Cobia has medium fat (5.31%) content. Data confirm that Cobia fillets can be considered among foods that provide a profitable protein content and show a lipid content that could be included among medium-fat wild fish species (Álvarez et al., 2009). The lipid content in Cobia fillets in the present study was quite similar to Sea bass (5 – 6%) (Mach, 2009; Testi et al., 2006; Yanar et al., 2007; Yildiz et al., 2008). The results obtained in this study also were within levels reported by Daghoghi, 2008; Mach, 2009. Results of pH measurements showed that pH of antioxidant treated samples was lower than control samples in frozen fish fillets during six months storage. According to Grigorakis et al. (2003) post mortem pH can vary from 5.4–7.2, depending on fish species. Several authors have reported different results about decrease or increase of pH in various fish species (Mahmoudzadeh et al., 2010). Similar to our results were reported by Pourashouri et al. (2009) on wells catfish and Rostamzad et al. (2011) on Persian sturgeon that the amount of pH decreased after 6 months storage at -18°C. The pH values between 6.8 -7.0 were proposed as acceptance limit of fish, and values above 7 were considered to be spoiled (Kose et al., 2006; Mahmoudzadeh et al., 2010). However, pH value is not a suitable index and it can be useful as a guideline for quality control of fish (Selami and Sadoki, 2008).

During the frozen storage of fish, lipid oxidation has been shown to enhance protein denaturation and detrimental texture changes (Saeed and Howell, 2002; Pourashouri et al., 2009). In such storage conditions, one consequence of protein denaturation has been reported to be the reduction of water holding capacity (WHC) of the fish muscle (Suvanich et al., 2000; Pourashouri et al., 2009). Water holding capacity in meat tissue is strongly related to myofibril proteins. Increase of expressible moisture is a sign of reduction of water holding capacity due to denaturising of proteins (Suvanich et al., 2000). This phenomenon leads to reduction of flavor agents and nutrition value (Rostamzad et al., 2011). In this study expressible moisture content showed a progressive increase in all samples during frozen storage. Similar results were reported by Pourashouri et al., 2009 (on Wels catfish) and Rostamzad et al., 2011 (on Persian sturgeon).

FFA formation as a result of lipid hydrolysis (triglyceride and phospholipids classes) has provided a suitable means for evaluation of fish damage during frozen storage (Serdaroglu and Felekoglu, 2005). At the end of storage time the lowest FFA level was found in samples treated with AA 0.5%. A very good agreement of FFA increase with storage time was observed for BC, AA 0.25% and AA 0.5% ($r = 0.97$, $r = 0.96$ and $r = 0.93$, respectively). As a result of the hydrolysis of phospholipids and triglycerides through the action of lipases and phospholipases, an increase in FFA is observed (Serdaroglu and Felekoglu, 2005). Although the formation

of FFA does not directly result in nutritional losses, examining the extent of lipid hydrolysis need to be studied. Since FFA are known to undergo oxidation, low molecular weight compounds are produced that are responsible for the rancid off-flavour and taste of fish and fish products (Aubourg et al., 2004; Losada et al., 2007; Pourashouri et al., 2009; Sahari et al., 2009; Rostamzad et al., 2011;). FFA has great influence on protein denaturation and texture deterioration by interaction with proteins (Losada et al., 2004; Pourashouri et al., 2009; Rostamzad et al., 2011). The increase of FFA in Cobia fillets could be related to the lipase and phospholipase activity in Cobia fillets during frozen storage. Similar results were reported by other researchers (Aubourg et al., 2004; Losada et al., 2004; Serdaroglu and Felekoglu, 2005; Chaijan et al., 2006; Losada et al., 2007; Lugasi et al., 2007; Pourashouri et al., 2009; Sahari et al., 2009).

The peroxide value of a sample indicates the concentrations of peroxides and hydro peroxides that are produced during the early stages of lipid oxidation. The peroxide values are monitored for a sample and when it sharply increases, it indicates the end of the shelf life for that sample. Peroxide values are measured in terms of meq O₂/kg of sample. The main use of a peroxide value is to determine the quality of oil sample (Kaya et al., 1993). Increase of PV in control samples in contrast with both treatments showed development of off-flavor is one of the major effects of lipid oxidation (Fagan et al., 2003; Sahari et al., 2009) and at the further stage of lipid peroxidation; changes

in color and nutritional value are observed (Sahari et al., 2009). Moreover, oxidized products of lipids formed during storage of fishery products are known to influence the soluble proteins (sarcoplasmic and myofibrillar proteins) (Sahari et al., 2009). In control samples PV < 20 meq O₂/kg were obtained at the end of the period. However, both treatments (AA 0.25% and AA 0.5%) showed a progressive but slow increase ($P < 0.05$) with frozen time, so that values above 10 were not attained even at the end of the storage. A very good agreement of PV content increase with storage time was observed for BC, AA 0.25% and AA 0.5% ($r = 0.96$, $r = 0.90$ and $r = 0.84$, respectively). The lowest PV was observed for Cobia fillets treated with AA 0.5%. According to the results, it is concluded that AA treatments had significant effect on delaying lipid oxidation. Similar results were reported by others (Pourashouri et al., 2009; Rostamzad et al., 2011).

Secondary lipid oxidation was studied by the TBA. The presence of TBA in a sample of meat indicates that lipid peroxidation has taken place. The level of TBA shows the amount of peroxidation that has already occurred (Lukaszewicz et al., 2004). The main thiobarbituric acid that is measured is the compound malonaldehyde (MA), which is a secondary product formed as a result of lipid peroxidation (Ulu, 2004). TBA values of the control samples increased sharply during the 3rd to 6th months of storage. This was probably due to the destruction of hydroperoxides into secondary oxidation products, especially aldehydes in the later stages of lipid oxidation (Chaijan et al., 2006). Samples

treated with AA showed a gradual increase in TBA values during frozen storage. However, significant differences were observed in TBA value between AA 0.25% and AA 0.5% treatments in 6th month ($P < 0.05$). A very good agreement of TBA increase with storage time was observed for BC, AA 0.25% and AA 0.5% ($r = 0.96$, $r = 0.95$ and $r = 0.96$, respectively). At the end of the storage, the lowest TBA value was recorded as 0.347 mg ma/kg for the AA 0.5% treatment. The TBA values indicated that control samples and samples submerged in aqueous AA 0.25% were more rancid than samples treated with AA 0.5% throughout the storage at -18°C . Comparison among treatments revealed the order of TBA increase ($P < 0.05$) at the end of the storage as: AA 0.5% < AA 0.25% < BC. Totally, the results showed that usage of ascorbic acid had positive influence on delaying lipid oxidation and increasing shelf-life of fillets ($P < 0.05$). Soaking the fillets with an antioxidant solution has been recommended (Aubourg et al., 2004). According to Goulas and Kontominas (2007), TBA value of 1–2 mg ma/kg of fish flesh is usually regarded as the limit beyond which fish will normally develop an objectionable odor/taste. TBA values tend to have a good correlation with sensory testing when being used to detect rancidity of foods (Rhee and Myers, 2003; Campo et al., 2006). The TBA values of the present Cobia fillets exceeded the value of 1 mg ma/kg only for control samples (2.34 mg ma/kg) and in AA 0.25% and AA 0.5% were recorded (0.877 and 0.347 mg ma/kg) respectively in the end of storage. This indicated that both treatments finally had an antioxidative

activity on the lipids of stored fillets. Lower TBA value in samples which were treated by antioxidants in compare to control samples were reported by Rostamzad et al., (2011); Pourashouri et al., (2009); Benjakul et al., (2005); Khan and Shahidi, (2001); Khan et al., (2006) and Stodolnik et al., (2005).

Rancid odor in control samples at the 6th month of storage was considered as a limiting factor. Among different kinds of molecules produced as a result of lipid oxidation, secondary ones are considered the chief compounds responsible for oxidized flavors (Pourashouri et al., 2009; Rostamzad et al., 2011). Results of sensory evaluation tests indicated that usage of ascorbic acid can slow down improper changes during frozen storage. A close relationship between the rancid odor development and the PV assessment has been obtained for BC, AA 0.25% and AA 0.5% ($r = 0.76$, $r = 0.74$ and $r = 0.74$, respectively). Similar to our results were reported by other researchers (Stodolnik et al., 2005; Losada et al., 2007; Pourashouri et al., 2009; Rostamzad et al., 2011;) who found that antioxidant treatment increased shelf-life and preserved sensory attributes during storage.

The effect of ascorbic acid in order to delay lipid oxidation was studied in Cobia fillets. In order to check the lipid damage development, FFA, PV, TBA and odor assessment were found to be accurate methods in the present experiment. In the present study FFA, PV, TBA and EM increased and also pH and sensory analysis decreased significantly ($P < 0.05$) during frozen storage. Results showed that the samples which were soaked in solutions of

ascorbic acid (0.25% and 0.5%) had significant differences in biochemical parameters which were studied in compare with control during frozen storage. Results of our investigation revealed that ascorbic acid retarded oxidative changes in frozen Cobia fillets whereas AA 0.25% was not as effective as AA 0.5% on oxidative stability. The efficiency of antioxidant inhibiting lipid oxidation throughout frozen storage was in the following order: ascorbic acid 0.5% > ascorbic acid 0.25% > control ($P < 0.05$).

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