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Influence of Vacuum Packaging and Long Term Storage on Some Quality Parameters of Cobia (*Rachycentron canadum*) Fillets During Frozen Storage

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Abstract: Cobia (*Rachycentron canadum*) has recently attracted a great interest as a farmed product. This study focuses on its commercialization as a frozen product. With this purpose, chemical and sensory evaluation of Cobia, with emphasis on the quality parameters in vacuum packaging (VP), was investigated. Quality assessment of Cobia stored in VP for up to 6 months at -18°C was done by the monitoring of sensory quality, free fatty acids (FFA), peroxide values (PV), thiobarbituric acid (TBA), pH and expressible moisture (EM). Results showed that free fatty acid, primary and secondary oxidation products, expressible moisture and pH value of vacuum packaging samples were significantly lower than those in control samples ($p<0.05$). Results indicated that VP was effective in reduce lipid oxidation and increased shelf life of Cobia frozen fillets. Thus the employment of VP alone or in combination with other protective strategies is recommended.

Key words: Cobia · Fish · Frozen storage · Lipid oxidation · Vacuum packaging

INTRODUCTION

Fish are considered an important part of human nutrition, because of their high content in polyunsaturated fatty acids (PUFAs), especially of the n-3 series. These unsaturated fatty acids are highly susceptible to oxidation [1]. Deterioration of fish begins immediately upon harvest and continues to varying degrees, depending on storage conditions [2]. Fish is one of the most highly perishable food products and the shelf life of such products is limited in the presence of normal air by the chemical effects of atmospheric oxygen and the growth of aerobic spoilage microorganisms [3].

Lipid oxidation is one of the major problems in the fish industry, due to the resultant flavor deterioration and loss of nutritional value [4]. In order to minimize such undesirable effects, different technological strategies have been applied such as low temperature storage, preserving packaging, glazing, including protecting chemicals and the incorporation of antioxidants [5, 6]. One of the appropriate methods to access this target is using vacuum packaging in order to control rancidity in oils and lipid containing foods [7]. Vacuum packaging is a way for delaying lipid oxidation (auto oxidation) because of limiting oxygen molecule. As reported by Anelich *et al.* [8], Fagan and Gormley [9] and Perez-Alonso *et al.* [10], packaging under vacuum has positive effect on extended shelf life of fish fillets.

Cobia (*Rachycentron canadum*) fish is the only species in the family Rachycentridae. Cobia is a promising candidate for aquaculture trade because of its rapid growth rate, reaching up to 4-6 kg in a year, hardiness, efficient feed conversion, excellent flesh quality and comparatively low production costs [11, 12]. Therefore, study has been recently carried out to assess the optimal commercial feeds to be employed [6, 13].

The present study focuses on the retention of the lipid nutritional value of this species when commercialized as a frozen product. The effect of vacuum packaging on the shelf life and lipid quality of Cobia fish fillets during frozen storage up to 6 months was investigated.

MATERIALS AND METHODS

Sample Preparation: Fresh Cobia (*Rachycentron canadum*) was caught (100 kg) in the Persian Gulf near Bandar Abbas (Hormozgan, South Iran). The average length and weight of the specimens employed were 92.23±1.04 cm and 5.32±1.02 kg, respectively. The fish individuals were placed in boxes, surrounded by ice and

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Table 1: Scale employed for evaluating the sensory quality of frozen Cobia fillets

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 Odour	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and incipient rancidity	Sharply sour and rancid
Flesh consistency	Presence or partial 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

transferred for processing to the Persian Gulf and Oman Sea Ecology Research Centre. Fish samples were beheaded, gutted and filleted by hand and washed with cold water carefully, being the weight of each fillet of 200±5g. Then, fillets were divided into 2 groups. Samples of the first group were left untreated (control) and directly packaged in polyamide/polyethylene bags. Fillets belonging to the second group were packaged under vacuum condition in individual polyamide/polyethylene bags.

All packaged samples were immediately frozen at-30°C. After 24 hours, all fish fillets were placed in a-18°C freezer. 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. For each kind of fillet, three different batches (n = 3) were considered and analysed separately in order to achieve the statistical analysis.

General Chemical Analyses: The moisture content in flesh of Cobia was determined by drying to constant weight at102-105°C for 20 to 24 h according to the AOAC standard method [14]. Crude ash was determined after heating the sample overnight at 550°C [14]. The crude protein content was determined as Kjeldal-protein using a factor 6.25 following the AOAC standard method [14]. Fillet fat content was determined by the AOAC [14] method, this including acid hydrolysis followed by petroleum ether extraction. In all cases, results are expressed as g/100 g muscle. 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) [15]. 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 [16]. Three replicates were used for each experiment.

Lipid Damage Measurements: Free fatty acid (FFA) content was determined in the lipid extract by the Kirk & Sawyer [17] 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 [18]. Results are expressed as milli-equivalents peroxide per kg lipid (meq O_2/kg lipid). Thiobarbituric acid (TBA) was determined colorimetrically by the Porkony and Dieffenbacher method as described by Kirk & Sawyer [17]. 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 [19]. 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: flesh appearance, rancid odor and flesh consistency. At each sampling, the different fish fillets were thawed and then analyzed in the same session. The fish fillets were served to the panel members in individual polyamide/polyethylene bags in which they had been kept frozen and they were scored individually. Sensory analyses were carried out at 0, 1, 3 and 6 months after storage.

Statistical Analysis: Nonparametric statistics used to analyze the data. Repeated Measures analysis of variance 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 $P < 0.05$ used to compare sample means by using SPSS 16 software.

RESULTS AND DISCUSSION

Evolution of General Chemical Parameters: The proximate composition of the fish fillets in the first day was measured.The average (± standard deviation) Cobia fillet values obtained for moisture, protein, fat and ash were 75.27±0.04 %, 16.58± 0.25 %, 5.31± 0.85 % and 0.97±0.1 g/100 g muscle, respectively. Mach [20] and Daghoghi [21] reported that Cobia had high protein (16-21%) and medium fat (5.4%) content. Data confirm that cobia fillets can be considered among foods that provide a profitable protein content and show a lipid content that

Table 2: Changes of pH in Cobia fillets under different treatments during frozen storage (means ± SD (n = 3); P < 0.05) up to 6 months in-18°C

	Frozen storage time (months)			
Treatment	0	1	3	6
BC	5.90±0.05Aa	5.73±0.01Aa	5.45±0.23Ba	5.46±0.08Ba
VP	5.78±0.13Aa	5.67±0.02Ba	5.58±0.07Bb	5.34±0.03Cb

Mean values in column with different small letters indicate significant difference (p<0.05) between treatments and mean values in row with different capital letters indicate significant difference (p<0.05) as result of frozen storage time. SD: Standard Division. Treatment names: BC (blank control) and VP (vacuum packaging)

Table 3: Changes of Expressible moisture in Cobia fillets under different treatments during frozen storage (means ± SD (n = 3); P < 0.05) up to 6 months in-18°C

	Frozen storage time (months)			
Treatment	0	1	3	6
BC	26.38±1.96Aa	30.90±2.41Ba	35.15±1.37Ca	43.85±2.26Dc
VP	26.03±5.77Aa	30.53±0.19Aa	36.74±4.29Ba	38.83±1.51Ba

Mean values in column with different small letters indicate significant difference (p<0.05) between treatments and mean values in row with different capital letters indicate significant difference (p<0.05) as result of frozen storage time. SD: Standard Division. Treatment names are as expressed in Table2.

could be included among medium-fat wild fish species [22]. The lipid content in Cobia fillets in the present study was quite similar to Sea bass (5 - 6%) [23,24].

The pH and its alteration during storage time (0, 1, 3 and 6 months) in Cobia fillets in BC and vacuum packaging are shown in (Table 2). A decrease was observed in pH from 5.9 to 5.46 and 5.78 to5.34 in BC and VP respectively, during frozen storage in 6 months. There were significant differences in control and vacuum packaging during 6 months of frozen storage (P<0.05). In comparison between treatments, there were significant difference at zero time and first month (P> 0.05) but there was a significant difference between BC and VP at the 3rd and 6th month (P<0.05). Similar results were reported by Rostamzad *et al.* [19] on Persian sturgeon (*Acipenser persicus*) that pH was decreased after 6 months storage at -18°C. According to Grigorakis *et al.* [25] 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. The pH values between 6.8 - 7 were proposed as acceptance limit of fish and values above 7 were considered to be spoiled. Decrease or constant levels of pH might be attributed to increasing solubility of CO_2 at storage time, effecting on growth of aerobic microflora [26].

Changes in EM values of treatments stored at - 18°C are shown in (Table 3). Initial EM values of BC and VP were found to be 26.38 and 26.03 percent, respectively. All samples showed an increased EM value with storage (P <0.05) period. No difference was found in EM values of BC and VP at the zero (initial), 1st and 3rd month of storage time (P> 0.05), but there was significant difference between control and VP in the 6th month (P <0.05). The EM values of VP treatment were significantly lower than BC after 6 months of storage. During the frozen storage of fish, lipid oxidation has shown to enhance protein denaturation and detrimental texture changes [16]. 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. 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 [15]. This phenomenon leads to reduction of flavor agents and nutrition value [19].

Lipid Hydrolysis Development: A significant (P < 0.05) hydrolysis development was observed for the BC and VP treatments during the frozen storage (Fig.1). All samples showed an increased FFA value with increased storage time (P <0.05). The increase was observed in FFA from 0.28 to 5.39and 0.13 to 1.93 (g of oleic acid kg^{-1} lipids) in BC and VP respectively, during frozen storage in 6 months. At zero time, there was no significant difference among treatment groups (Fig. 1); however, FFA value had a sharp increase in control samples after 1 month of storage. Significant difference was observed between BC and VP in the 1st, 3rd and 6th months of storage (P<0.05). A very good agreement of FFA increase with storage time was observed for BCand VP (r = 0.97and r = 0.95, respectively). The increase is due to the hydrolysis of phospholipids and triglycerides by the action of lipases and phospholipases [27]. The increase of FFA in Cobia fillets could be attributable to the lipase and phospholipase activity in Cobia fillets during frozen storage. Similar results were reported by other researchers Rostamzad *et al.* [7], Rodríguez *et al.* [28] and Rostamzad *et al.* [29].

Lipid Oxidation Development: Lipid oxidation development was measured according to the PV formation (primary oxidation compounds) and TBA (secondary oxidation compounds). The peroxide value a sample indicates the concentrations of peroxides and hydroperoxides that are produced during the early stages

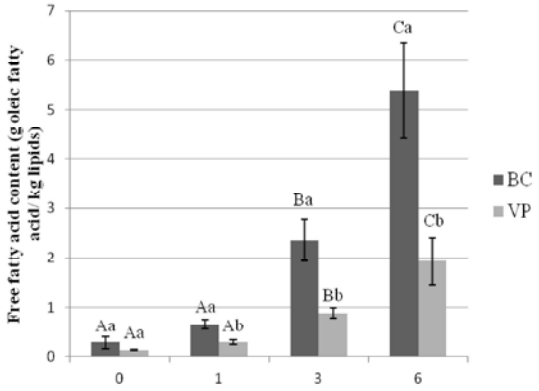

Fig. 1: Changes of Free fatty acid in Cobia fillets under different treatments during frozen storage up to 6 months in -18°C

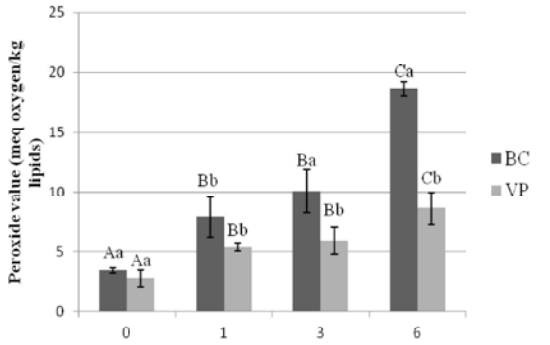

Fig. 2: Changes of Peroxide value in Cobia fillets under different treatments during frozen storage up to 6 months in-18°C

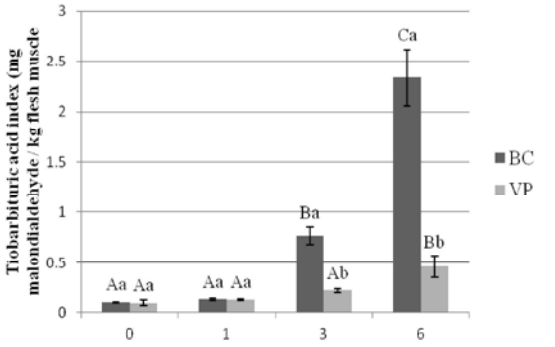

Fig. 3: Changes of Tiobarbituric acid in Cobia fillets under different treatments during frozen storage up to 6 months in -18°C

Mean values with different small letters indicate significant difference ($p<0.05$) between treatments and mean values with different capital letters indicate significant differences ($p<0.05$) as result of frozen storage time. Standard deviations are indicated by bars. Treatment names are as expressed in Table2.

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. The main use of a peroxide value is to determine the quality of oil samples [30]. Changes in PV values of BC and VP during frozen storage at -18°C for 6 months are shown in (Fig. 2). Initial PV values of BC and VP treatments were found to be 3.45 and 2.78 meq O_2/kg and increased to 18.65and 8.65, respectively. All samples showed an increased PV value in Cobia fillets when the frozen storage time increased ($P< 0.05$). At zero and 1st month of storage there was no significant difference in peroxide values of the treatment groups ($P>0.05$), but in the 3rd and 6th month of storage time, significant difference was observed between BC and VP treatment ($P<0.05$). In contrast to samples treated with VP, PV in the control sample increase. Such increase in PV could lead to colour changes and loss of nutritional value [31]. Moreover, oxidized products of lipids formed during storage of fishery products are known to influence the soluble proteins (sarcoplasmic and myofibrillar proteins). In control samples PV < 20 meq O_2/kg were obtained at the end of the period. However, VP treatment showed a progressive but slow increase ($P < 0.05$) with frozen time, so that values above 9 were not attained even at the end of the storage time. A very good agreement of PV content increase with storage time was observed for BC andVP ($r = 0.96$and $r = 0.88$, respectively). According to the results, it is concluded that VP treatment had significant effect on delaying lipid oxidation. Similar results were reported by others [8,9]. According to Rostamzad *et al.* [29], the Persian sturgeon (*Acipenser persicus*) fillets that treated by vacuum packaging showed the lowest rate of peroxide formation and the highest values were found in control sample during any time of storage.

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 [32]. The main thiobarbituric acid that is measured is the compound malonaldehyde (MA), which is a secondary product formed as a result of lipid peroxidation [33]. Changes in TBA values of BC and VP treatment during frozen storage at - 18°C up to 6 months are shown in (Fig.3). Initial TBA values of BC and VP treatment were found to be 0.102 and 0.097 mg ma/kg and increased to 2.34and 0.459 mg ma/kg respectively. All samples showed an increased TBA value in Cobia fillets when the frozen storage time increased ($P< 0.05$). No difference was found in TBA values between BC and VP treatment at the zero (initial) time and first month ($P>0.05$).

Table 4: Evolution of sensory parameters during frozen storage of Cobia fillets those were pretreated under different conditions in-18°C

Frozen storage time (months)	Flesh appearance		Rancid odour		Flesh consistency	
	BC	VP	BC	VP	BC	VP
1	E	E	E	E	E	E
3	A	A	B	A	B	A
6	B	A`	C`	A	B	A

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

All fish were category E for all attributes initially.

Treatment name is as expressed in Table 2.

After 3 month of storage significant difference was observed in TBA value between the BC and VP treatment ($P<0.05$). 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 [34]. A very good agreement of TBA increase with storage time was observed for BC and VP ($r = 0.96$and $r = 0.93$, respectively). Results showed that usage of VP had positive influence on delaying lipid oxidation and increasing shelf-life of fillets ($P<0.05$). According to Goulas and Kontominas [35], 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 and taste. TBA values tend to have a good correlation with sensory testing when being used to detect rancidity of foods [36]. The TBA values of the present Cobia fillets exceeded the value of 2 mg ma/kg only for control samples (2.34 mg ma/kg) and in VP was recorded (0.459 mg ma/kg) at the end of storage time. Rostamzad *et al.* [19] and Anelich *et al.* [8] reported lower TBA values in samples which were treated by vacuum packaging compare to control samples. The increase in TBA indicated formation of secondary lipid oxidation products such as aldehydes and other volatiles compounds.

Sensory Analysis: Sensory scores obtained by the frozen Cobia fillets are shown in (Table 4). 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 time. 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 ($P<0.05$). Attribute indices (odor, consistency and appearance of fillets) decreased at the 3rd month of storage. Flesh appearance assessment showed a lower score at 6th month for the control samples than VP treated sample. Also flesh consistency assessment showed a better score at 3rd month for VP treatment than control samples. Odor analysis led to a better quality score ($P<0.05$) at the 3rd month for VP treatments than control samples. Flesh odor in control samples at 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 [16,19]. A close relationship between the rancid odor development and the PV assessment has been obtained in the present study ($r = 0.76$). Sensory analyses of attributes considered indicate that vacuum packaging can slow down the quality loss during frozen storage. Similar to our results were reported by other researchers Perez-Alonso *et al.* [10] and Rostamzad *et al.* [19] who found that vacuum packaging treatment increased shelf-life and preserved sensory attributes during storage.

CONCLUSION

The effect of vacuum packaging in order to delay lipid oxidation was studied in Cobia fillets. As a result of a frozen storage period up to 6 months, a marked content increase was found in the FFA, PV and TBA, as well as in the EM value. A decrease was observed in pH and sensory analysis ($P <0.05$) during frozen storage. According to the present results, packaging samples under vacuum conditions, was a proper way for reducing lipid oxidation of Cobia (*Rachycentron canadum*) fillets and extended their shelf life by omitting available oxygen.

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