Changes of fatty acid profiles in fillets of Cobia *(Rachycentron canadum)* during frozen storage

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Abstract

In this study changes in fatty acids profile during frozen storage at -18°C of Cobia *(Rachycentron canadum)*, caught from the Persian Gulf (Bandar Abbas) were studied. Changes in saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), EPA+DHA/C16, n-3 PUFA/n-6 PUFA (n-3/n-6) and polyunsaturated fatty acids /saturated fatty acids (PUFA/SFA) were investigated during a six-month storage at -18°C. Eighteen fatty acids were found in Cobia, with higher percentage of saturated fatty acids (46.07%), monounsaturated fatty acids (33.72%) and polyunsaturated fatty acids (15.44%). The MUFAs and PUFAs reduced from 33.72 to 26.26% and 15.44 to 10.78%, respectively. Palmitic acid (C16:0, 27.42% of total fatty acids) and stearic acid (C18:0, 12.62%) were the dominant saturated fatty acids. The major unsaturated fatty acids were determined as docosahexaenoic acid (C22:6n3, 5.76%), oleic acid (C18:1n9, 25.76%) and linoleic acid (C18:2n6, 4.38%). As a result of the frozen storage (up to 6 months), marked content decreases were found in fatty acid groups such as monounsaturated, polyunsaturated and n-3 polyunsaturated, as well as in the n-3/n-6 ratio and it means that the nutritional value of Cobia has decreased.

Keywords: Cobia, Fish, Fatty acids, Frozen storage

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Introduction

Cobia, with the scientific name of *Rachycentron Canadum* (Nelson, 2006) is a migratory pelagic species that is found worldwide in tropical, subtropical and warm-temperate waters except for the eastern Pacific Ocean (Mach, 2009). In marine aquaculture, Cobia is considered as a remarkable candidate species due to its fast growth, good fillet quality and high commercial prices. Cobia can reach the weight of 5 – 6 kg within one year and 8 – 10 kg in 16 months. The culture of cobia will presumably become an emerging aquaculture industry in the near future because of the fish's rapid growth and high quality flesh (Liu et al., 2004). Cobia has high nutritional and medicinal value due to its balanced composition of essential amino acids, its richness in polyunsaturated fatty acids, and its comprehensive supply of microelements (Liu et al., 2009). Marine lipids have a high content of polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic acids (DHA; 22:6n-3) (Pazos et al., 2005; Bayir et al., 2006; khan et al., 2006). There is strong evidence that consumption of fish is favorable to human health (Bayir et al., 2006). It is generally recognized that PUFA composition may vary among fish species. Degradation of PUFA by auto-oxidation during storage and the processing of fish oils and fatty fish easily lead to the formation of volatiles associated with rancidity (Pazos et al., 2005). For this reason, freezing and frozen storage have largely been employed to retain fish sensory and nutritional properties (Lugasi et al., 2007). Cobia contains an acceptable amount of fat (medium fat) and is among the most demanded marine species by market worldwide. At present, research on cobia mainly concentrates on breeding, culture, disease prevention and feed (Liu et al., 2009). There is no information on the fatty acids and proximate compositions of Cobia from Iran. Therefore, the aims of this study were to determine the values of lipid, protein, moisture, ash content of Cobias and monitoring the changes in fatty acids composition during frozen storage in -18°C for six months and to evaluate their nutritional value to provide scientific data for food processing and pharmaceuticals.

Materials and methods

Sample preparation

Cobia fish (*Rachycentron canadum;* 20 individual fishes) were caught from Bandar-e-Abbas (Hormozgan province, Iran). The average length of the specimens was 92.23±1.04 cm and their average weight was 5.32±1.02 kg. The fish samples were put in an ice box with ice and transferred to the processing salon in the Persian Gulf and Oman Sea Ecology Research Center in 30 minutes. Then, the fish samples were beheaded, gutted and filleted (240 fillet particles) by hand and washed by cold water carefully and dehydrated (sieved); each fillet weighed about 200±5g. Then each fillet was put in a separate polyamide/polyethylene bag and packed. After packaging, first the fillets were frozen by air blast freezing method for 24 h at - 30°C and then stored in -18°C

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for 6 months. Analysis of frozen fish fillets was carried out immediately after freezing (time 0), then after 1, 3 and 6 months of storage in -18°C. In each time a total of six samples were randomly drawn for analysis. The samples were thawed in a refrigerator (at a temperature of 4±1°C) overnight and a certain amount was taken from all six samples and homogenized by a mixer, then the final sample was taken from the homogenized mixture. The experiment was conducted with three replications and the mean was estimated. All analyses were carried out at the Laboratory of Fish Processing Technology, in the Persian Gulf and Oman Sea Ecology Research Center. All analyses were conducted in triplicates.

Chemical composition

Determination proximate compositions

The moisture content of flesh was determined by drying to constant weight at 102-105°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). Crude protein content was determined by the Kjeldahl method (AOAC, 2005), employing the 6.25 conversion factor. The fat content of Cobia was determined by a solvent extraction (Submersion) method for fat (crude) in meat and meat products (AOAC, 2005).

Fatty acid analysis

Total lipid was extracted according to the AOAC standard method (AOAC, 2000). Lipid extracts were then saponified with 0.5 N methanolic NaOH and further transesterified with BF3 in methanol (AOAC, 2000). The fatty acid methyl esters (FAMEs) were analyzed on a Gas Chromatography (GC1000, DANI Instrument, Switzerland) equipped with a flame ionization detector (FID). The esters were separated on a SGE column (30m × 0.25 mm i.d.). The carrier gas was helium. The temperature profiles were as follows: initial temperature, 175˚C; heating rate, 1˚C/min; final temperature, 220˚C (final time, 20 min); injector temperature, 250˚C and detector temperature, 270˚C. The fatty acids were identified by comparison of the retention times with those of standard (C19:0, Sigma) purified fatty acids (Shirai et al., 2006). Each sample was repeated three times and its average was calculated.

Statistical analysis

Data from the different quality parameters were subjected to one-way ANOVA and differences among the means were determined for significance at $p < 0.05$ using Duncan's multiple range test by using SPSS 16 software.

Results

Moisture, Protein, Fat and Ash content

Proximate compositions of fillets were measured just in the first day. 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±0.10 g/100 g muscle, respectively.

Fatty acid composition

Different individual fatty acids were identified and quantified in Cobia muscle; results obtained throughout a 6-month frozen storage time (0, 1, 3 and 6 months) in Cobia fillets are shown in Table 1. The changes in fatty acid profiles during storage in frozen conditions were statistically significant ($P<0.05$). Except

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for Zero time and the first month, significant differences were observed among the SFA (saturated fatty acids) during frozen storage ($p<0.05$). Palmitic acid (C16:0) and stearic acid (C18:0) were the major fatty acids among the SFAs during storage and pentadecanoic acid (C15:0) with 0.65% was also in minimum value (Table 1). There were significant differences among MUFA contents during 6 months ($p<0.05$). Oleic acid (C18:1 n-9) and palmitoleic acid (C16:1) with 25.76 % and 4.91 % respectively were in the maximum value of fish tissue as compared to other fatty acids. There were significant differences among PUFA contents during 6 months ($p<0.05$). PUFA accounted for approximately 15.44% of TFA (Total Fatty Acids). It is noticeable that both linoleic (C18:2n-6) and arachidonic acids (C20:4n-6) were predominant in the total n-6 polyunsaturated fatty acids in fillets of Cobia, and Eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) were the major total n-3 polyunsaturated fatty acids in fillets of Cobia.

The PUFA accounted for 15.44% of the total fatty acids. Distribution of fatty acid in Cobia was as SFA> MUFA > PUFA but unsaturated fatty acids were more than saturated fatty acids (SFA<PUFA+MUFA). An increase was observed in the percentage of SFA from 46.07% to 51.32% which was statistically significant between 1, 3 and 6 months of storage time ($p<0.05$). Also a significant reduction was observed in the percentage of MUFA and PUFA from 33.73% to 26.26% and 15.44% to 10.78% respectively after 6 months storage under frozen conditions ($p<0.05$). Results showed that the PUFA/SFA (P/S) ratio was less than 1 (Table 2) and the decrease of PUFAs, in contrast to SFA, led to a significant decrease in this ratio ($p<0.05$) during frozen storage.

Table 2 shows the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated (PUFA), n-6 PUFA, n-3PUFA, PUFA/SFA, EPA+DHA/C16 and the n-3/n-6 ratios. The SFAs were the most abundant fatty acids in the tissues of cobia, accounting for 46.07% of the total fatty acids. The second-most abundant fatty acids were the MUFA, which accounted for 33.73% of the total fatty acids.

Correlation

The correlation coefficient of storage time and fatty acid indices were tested. The storage time (Table 3) showed the closest correlation with such parameters as SFAs, MUFAs, PUFAs, PUFA/SFA, n-3, n-6, n-3/n-6 and

EPA+DHA/C16 (polyene index) in Cobia fish.

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Table 1: Changes in the fatty acid profile of stored Cobia (*Rachycentron canadum*) up to 6 months in -18°C (means ± SD (*n* = 3); P < 0.05; % of total lipids)

Fatty acids, grams per 100 g of total fatty acids	Time of storage (months)			
	0	1	3	6
C14:0 (Myristic acid)	4.23±0.1a	4.53±0.3a	5.45±0.05b	6.83±0.1c
C15:0(n-Pentadecanoic)	0.65±0.02a	0.90±0.05b	1.40±0.01c	1.74±0.01d
C16:0 (Palmetic acid)	27.42±1.0a	28.07±0.07a	31.69±0.1b	33.87±0.1c
C17:0 (Margaric acid)	1.15±0.03a	0.92±0.02b	0.26±0.01c	0.15±0.005d
C18:0 (Stearic acid)	12.62±0.5a	12.1±0.1b	10.11±0.1c	8.73±0.03d
C14:1(Myristoleic acid)	0.71±0.01a	0.69±0.01b	0.58±0.01c	0.31±0.01d
C15:1 (Pentadec-10-enoic acid)	0.67±0.04a	0.57±0.03b	0.55±0.01b	0.36±0.01c
C16:1 (Palmitileic acid)	4.91±0.10a	4.73±0.10b	3.91±0.04c	2.82±0.02d
C17:1 (Cis-10- heptadanoic acid)	0.93±0.03a	0.81±0.01b	0.69±0.01c	0.39±0.02d
C18:1n-7 (Vaccenic acid)	0.04±0.005	ND	ND	ND
C18:1n-9 (Oleic acid)	25.76±0.30a	25.38±0.08a	24.09±0.10b	22.02±0.99c
C20:1n-9 (Gadoleic acid)	0.71±0.08a	0.62±0.02b	0.56±0.01b	0.36±0.01c
C18:2n-6 (Linoleic acid)	4.38±0.10a	4.08±0.08b	3.53±0.10c	2.55±0.02d
C18:3n-3 (Linolenic acid)	0.63±0.03a	0.56±0.01b	0.32±0.01c	0.15±0.005d
C20:2n-6 (Eicosadienoic acid)	0.31±0.02a	0.25±0.01b	0.15±0.005c	0.10±0.01d
C20:4n-6 (Arachidonic acid)	2.56±0.06a	2.86±0.01b	3.20±0.10c	3.80±0.10d
C20:5n-3(Eicosapentaenoic acid)	1.80±0.05a	1.50±0.01b	1.19±0.01c	0.78±0.03d
C22:6n-3(Docosahexaenoic acid)	5.76±0.02a	5.26±0.06b	4.18±0.01c	3.40±0.10d

Means in a row with different letters indicate a significant difference (p<0.05)
ND, Non-detected

Table 2: Changes in fatty acid series of stored Cobia (*Rachycentron canadum*) up to 6 months in -18°C (means ± SD (n = 3); P < 0.05; % of total lipids)

Fatty acid series	Time of storage (months)			
	0	1	3	6
ΣSFA	46.07±1.65a	46.52±0.54a	48.91±0.27b	51.32±0.23c
ΣMUFA	33.73±0.57a	32.80±0.19a	30.38±0.1b	26.26±1.07c
ΣPUFA	15.44±0.28a	14.51±0.18b	12.57±0.23c	10.78±0.27d
PUFA/SFA	0.33±0.00a	0.31±0.00b	0.25±0.00c	0.21±0.00d
Σn-3PUFA	8.19±0.08a	7.32±0.06b	5.69±0.06c	4.33±0.05d
Σn-6PUFA	7.25±0.09a	7.19±0.09b	6.88±0.08b	6.45±0.05a
n-3/n-6	1.12±0.01a	1.01±0.03b	0.82±0.02c	0.67±0.01d
EPA+DHA/C16	0.27±0.00a	0.23±0.00b	0.16±0.00c	0.12±0.00d

Means in a row with different letters indicate a significant difference (p<0.05)

ΣSFA: sum of saturated fatty acids.
ΣMUFA: sum of monounsaturated fatty acids
ΣPUFA: sum of polyunsaturated fatty acids
PUA/SFA: polyunsaturated/saturated fatty acids ratio
Σn-3: sum of n-3 fatty acids (linolenic + EPA + DHA)
Σn-6: sum of n-6 fatty acids (linoleic + Eicosadienoic + Arachidonic)
n-3/n-6: n-3/n-6 fatty acid ratio

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Table 3: Correlation coefficient for different parameters (storage time and fatty acids content) measurement during frozen storage in fillets of Cobia fish (*Rachycentron canadum*)

	ST	SFA	MUFA	PUFA	PUFA/SFA	n-3	n-6	n-3/n-6	EPA+DH /C16
ST	1	0.94**	-0.983**	-0.986**	-0.984**	-0.986**	-0.976**	-0.981**	-0.976**
SFA	**	1	-0.893**	-0.905**	-0.935**	-.0912**	-0.87**	-0.909**	-0.941**
MUFA	**	**	1	0.978**	0.965**	0.967**	0.984**	0.961**	0.947**
PUFA	**	**	**	1	0.991**	0.998**	0.979**	0.996**	0.986**
PUFA/SFA	**	**	**	**	1	0.991**	0.968**	0.994**	0.989**
n-3	**	**	**	**	**	1	0.967**	0.998**	0.992**
n-6	**	**	**	**	**	**	1	0.963**	0.938**
n3/n-6	**	**	**	**	**	**	**	1	0.992**
EPA+DHA/C16	**	**	**	**	**	**	**	**	1

ST,storage time
**Correlation is significant at the 0.01 level (2- tailed)

Discussion

The results presented in this work show that Cobia fish are rich in protein and medium in lipid content. According to Mach (2009) and Daghoghi (2008), Cobia has high protein (16 – 21%) and medium fat (5.4%) content. Data confirm that Cobia fillets can be considered among those foods providing a profitable protein content and showing 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%) (Testi et al., 2006; Yanar et al., 2007; Yildizet al., 2008). The results obtained in this study were also within the levels reported by Daghoghi (2008) and Mach (2009). Eighteen fatty acids were found in Cobia, with a higher percentage of saturated fatty acids (46.07%), monounsaturated fatty acids (33.72%) and polyunsaturated fatty acids (15.44%). The MUFAs and PUFAs reduced from 33.72 to 26.26% and 15.44 to 10.78%, respectively. The results showed that unsaturated fatty acid contents were greater than saturated fatty acids in Cobia fillets. The content of unsaturated and saturated fatty acids of the Cobia fillets was 49.17 and 46.07 respectively. Similar results were reported by Hedayatifard and Moeini, 2007; Sahari et al., 2009 and Pirestani et al., 2010. Palmitic acid (C16:0) and stearic acid (C18:0) with 27.42 % and 12.62 %, respectively were the major fatty acids among the SFAs during storage. The same results were obtained about Mackerel and Shark (Sahari et al., 2009), farmed Cobia (Liu et al., 2009) and Sturgeon (Hedayatifard and Moeini, 2007). The average amount of the saturated fatty acids of pentadecanoic acid (C15:0) with 0.65% was also in minimum value (Table 1). The similar result was reported by Liu et al. (2009. Oleic acid (C18:1 n-9) and palmitoleic acid (C16:1) with 25.76 % and 4.91 % respectively were in the maximum value of fish tissue as compared to other fatty acids. Similar results were reported by Liu et al., (2009) (farm Cobia) and Sahari et al., (2009) (Mackerel and Shark). Eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) were the major total n-3 polyunsaturated fatty acids in fillets of Cobia. Eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) also played a major role in the total n-3 polyunsaturated fatty acids. The

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same results were found (Sahari et al., 2009) for Mackerel (*Scomberomorus Commerson*) and Shark (*Carcharhinus Dussumieri*).

The n-3 PUFA was present as 8.19% of the total fatty acids, most abundant of which was DHA above 5.76%. The n-6 PUFA were present as 7.25 % of the total fatty acids and were mainly linoleic acid (4.38%) and arachidonic acid (2.56%). Significant decrease was observed in the percentage of n-3 PUFA and n-6 PUFA during 6 months ($p<0.05$). Marine fish are rich in n-3 fatty acids, especially DHA and EPA (Celik et al., 2005). Liu et al., (2009) determined that farmed Cobia (*Rachycentron canadum*) from china had higher total n-3 quantity than in the results of this study. It has been reported that the types and amount of fatty acids in fish tissues vary with the geographic location, size, age, what the fish eat, reproductive status and season (Celik et al., 2005). In our study, the amount of n-3 was more than the n-6 compounds. A significant decrease in this ratio from 1.129 to 0.671 in Cobia showed that the nutritional value of this fish had declined during frozen storage.

Turan et al., (2007) and Pirestani et al., (2010) suggested that the n-3/n-6 ratio is a better index in comparing relative nutritional value of fish oils of different species. The n-3/n-6 ratio of 1:1 is considered to be optimal for nutritional purposes (Turan et al., 2007). As shown in Table 2, the n-3/n-6 ratio of Cobia was 1.129. The n-3/n-6 ratio in mackerel and shark were reported by Sahari et al. (2009), 4.16 and 2.02, respectively and gilthead sea bream was found between 1.6 to 3.6 in different months (Senso et al., 2007). Decrease in unsaturated fatty acid content, particularly PUFA, and lower n-3/n-6 ratios were also obtained by Pirestani et al. (2010) in different kinds of fresh water fishes (Caspian kutum, golden grey mullet, common carp, pike perch and common kilka) belonging to the South Caspian sea during frozen (-24º C) storage.The PUFA/ SFA (P/S) ratio reveals that marine fish are a good source of PUFA regarding saturate fatty acids. According to the result, this ratio in Cobia (0.335) was less than the minimum value (0.45) of PUFA/SFA ratio recommended (HMSO, 1994). The same results were reported by Pirestani et al. (2010) in Golden grey mullet (0.35), farmed Cobia (0.332) by Liu et al., (2009) and Nile tilapia (0.35) by de Castro et al. (2007).

The EPA+DHA/C16:0 ratio (polyene index) is a good index to determine lipid oxidation (Sahari et al., 2009; Nazemroay et al., 2009 and Pirestani et al., 2010). In this study, this ratio was decreased (from 0.276 to 0.123) and also significant decrease was observed in the percentage of EPA+DHA/C16:0 during 6 months ($p<0.05$). The same results were found in mackerel and shark (Sahari et al., 2009). The negative relationship between this ratio and storage time showed that oxidation mechanisms are active during frozen storage.

The correlation coefficients of storage time with all fatty acids were higher than 0.9. The storage time (Table 3) showed to be best correlated with PUFAs ($r_=$ 0.986) and n-3 ($r_=$ 0.986) in Cobia fish. Among the fatty acid indices (Table 3), the most satisfactory results yielded after

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comparing PUFA content with the n-3 and n-3 with the n-3/n-6 ratio ($r_= 0.998$) .

Effect of frozen storage

The frozen storage led to important changes in the fatty acid profile in Cobia fillets (Table 1). Results showed an increase with time in most saturated fatty acids (C14:0, C15:0 and C16:0) and in C20:4n-6 (arachidonic acid). On the contrary, most of the remaining fatty acid showed a content loss throughout the whole storage period. Considering fatty acid series (Table 2), the frozen storage led to a progressive content increase in SFA in Cobia fillets, while MUFA, PUFA and n-3 PUFA showed a decrease by increasing the frozen storage time. Additionally, a progressive decrease with frozen time could be observed for the n-3/n-6 ratio and the polyene index (PI). Freezing storage is known to be associated with fish lipid oxidation processes that could be explained as a result of the presence of pro-oxidant enzymes in the fish muscle such as lipoxygenases, peroxidases and chemical pro-oxidant molecules named hemoproteins and metal ions (Sikorski and Kolakowski, 2000). Previous research related to fish frozen storage has already shown that unsaturated lipids are likely to be oxidized. Thus, Serdaroglu and Felekoglu (2005) reported that SFA and PUFA presence increased and decreased, respectively, in minced sardine (*Sardina pilchadus*) muscle when stored at -20º C up to 5 months. A similar behaviour was found for both fatty acid group presence in frozen (-30º C) Spanish mackerel (*Scomberomorus commersoni*) and white cheek shark (*Carcharhinus dussumieri*) fillets (Nazemroaya et al., 2009). Regarding the polyene index (PI) evolution during the frozen storage, present results agreed to previous research where a decrease in such a quality index was found for mackerel (*Scomberomorus commerson*), shark , *Carcharhinus dussumieri* (Nazemroaya et al., 2009) and coho salmon, *Oncorhynchus kisutch* (Ortiz et al., 2009).

It is concluded that Cobia contains large amounts of PUFA, particularly n-3 fatty acids and also considerable amounts of protein. The effects of storage time on the fatty acids profile were examined in the study. As a result of frozen storage during 6 months, a marked content decrease was found in fatty acid groups such as MUFA, PUFA and n-3 PUFA, as well as in the n-3/n-6 ratio. Increasing SFA and decreasing PUFA concentrations indicate that oxidation is in progress, and this has an important effect on quality. Assessment of the polyene index (PI) indicated an increased lipid oxidation development as a result of the frozen storage time. In addition, the decrease in unsaturated fatty acids, especially polyunsaturated fatty acids and EPA+DHA/C16 (PI), n-3/n-6 and PUFA/SFA ratios, showed that the nutritional value of Cobia fish has decreased.

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