Inhibition of fatty acids profile changes of Cobia (*Rachycentron canadum*) fillets during frozen storage by packaging under vacuum system

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Abstract

This study was aimed to investigate the effect of vacuum packing (VP) on the fatty acids profiles in cobia (*Rachycentron canadum*) fillets during an extended frozen storage period. Cobia fillets were treated under vacuum system then stored at -18°C for up to 6 months and compared to control conditions. As a result of a frozen storage period of 6 months, a marked content decrease was found in the fatty acid groups such as MUFA, PUFA and ω -3 PUFA, as well as in the ω -3/ ω-6 ratio. However, a preserving effect on such fatty acid parameters could be observed due to the VP treatment. Assessment of the polyene index (PI) indicated an increased lipid oxidation development as a result of the frozen storage time; however, this increase was partially inhibited by the vacuum packaging. Results indicate that vacuum packaging was a proper way to reduce lipid oxidation in Cobia fillets and extend their shelf life by omitting available oxygen. Thus the employment of VP alone or in combination with other protective strategies is recommended.

Keywords: Fatty acids, Vacuum packaging, Frozen storage, Cobia Fish

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Introduction

Cobia is an important fish species with high economic value as a result of its growing demand in international markets. 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 (Franks *et al*., 1999; Liao *et al*., 2004). Cobia has high nutritional and medicinal values 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). In particular, the content of ω-3 polyunsaturated fatty acids (ω-3 PUFA), mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), is high in the cobia lipids. 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). Lipid oxidation is one of the major problems in the fish industry, due to the resultant flavor deterioration and loss of nutritional value (Pettersen *et al*., 2004). 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 (Medina *et al*., 2009; Taheri *et al*., 2012). 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 vacuum packaging in order to control rancidity in oils and lipid containing foods (Rostamzad *et al*., 2010). Vacuum packaging is a way for delaying lipid oxidation (auto oxidation) by limiting oxygen molecules. As reported by Anelich *et al*. (2001), Fagan and Gormley (2004) and Perez-Alonso *et al*. (2004), packaging under vacuum has a positive effect of extending shelf life of fish fillets. Vacuum packaging, along with refrigeration, have become increasingly popular preservation techniques, which have brought major changes in storage, distribution, and marketing of raw and processed products to meet consumer demands (Ozogol *et al*., 2004). 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 packing on the fatty acids profiles during an extended frozen storage period was investigated.

Materials and methods

Sample preparation

Fresh cobia (*R. canadum*) were caught (100 kg) in the Persian Gulf near Bandar Abbas (Hormozgan Province, 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 were placed in

boxes with ice and 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. The weight of each fillet was 200±5g. The fillets were then divided into 2 groups. Samples of the first group were left untreated (blank control; BC treatment) 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 -30ºC. After 24 hours, all fish fillets were placed in a -18º C freezer. Analyses of the fish fillet in both groups were carried out after the freezing process (0-month storage at –18º C), and after 1, 3 and 6 months of storage at -18 ºC. In all cases, thawing was carried out by refrigerated storage (4ºC) over night. For each kind of fillet, three different batches (n=3) were considered and analysed separately in order to achieve the statistical analysis.

Fatty acid analysis

Total lipids were extracted by a chloroform-methanol mixture, according to the Bligh and Dyer (1959) method. Lipid extracts were then saponified with 0.5 N methanolic NaOH and further trances terified with BF3 in methanol (AOAC, 2000). The resulting fatty acid methyl esters (FAME) were analyzed on a Gas Chromatograph (DANI 1000) equipped with a Flame Ionization Detector (FID). The fatty acid esters were separated on a SGE column (30m 3 0.25mm i.d.). Helium was employed as the carrier gas. The temperature and other chromatographic conditions employed were as follows: initial temperature (175°C), heating rate (1°C/ min), final temperature (220°C), end time (20 min), injector temperature (250°C) and detector temperature (270°C). FAME were identified by comparison of the retention times with those of standard (C19:0, Sigma) purified fatty acids (Shirai *et al*., 2006). Peak areas were electronically integrated and quantified; results are expressed as percentage of total FAME. The polyene index (PI) was calculated as the following fatty acid concentration ratio: (20:5n-3 + 22:6n-3)/16:0.). Each sample was repeated three times and its average was calculated.

Statistical analysis

Data (n = 3) obtained from the different FAME analyses were subjected to the Repeated Measures analysis (p<0.05) to compare the treatment effect and frozen storage time effect (SPSS 16 software). Comparison of the means was performed using the Duncan's multiple range test (p< 0.05).

Results

Fatty acid composition

Different individual fatty acids were identified and quantified in cobia muscle; the results obtained throughout a 6-month frozen storage period corresponding to treated (VP) and untreated (BC) fish are shown in Tables (1 and 2). The changes in fatty acid

profiles during storage in frozen conditions were statistically significant (p<0.05). Except for zero time and first month, significant differences were observed among the SFA (saturated fatty acids) during frozen storage in control samples (p<0.05) but no significant differences were detected in samples treated with VP after 0,1 and 3 months storage (p>0.05). All samples showed an increased SFA value with increased storage (p<0.05). Palmitic acid (C16:0) and stearic acid (C18:0) were the major fatty acids among the SFAs during storage. Also minimum values of 0.65 % and 0.63% of pentadecanoic acid (C15:0) were found in control samples and vacuum packaging, respectively (Tables 1, 2).

There were significant differences among MUFA (monounsaturated fatty acids) contents during 6 months storage (p<0.05). A decrease was observed in MUA value with increased storage period in both treatments (p<0.05). Oleic acid content (C18:1 n-9) in control (25.76) and vacuum packaged samples (25.90) was higher than that of other fatty acids (Tables 1, 2).

There were significant differences among PUFA contents during 6 months (p<0.05). All samples showed a decreased PUF value with increased storage (p<0.05). PUFA accounted for approximately 15.44% and 15.43% of TFA (Total Fatty Acids) at the zero time in control samples and VP, respectively. 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 fish in all treatments. Totally three omega-3 fatty acids including Linolenic acid (C18:3n-3), Eicosapentaenoic acid (C20:5n-3) and Docosahexaenoic acid (C22:6n-3) were identified in our study. A comparison between BC and VP samples showed that the samples with VP had higher amounts of omega-3 fatty acids.

Distribution of fatty acid in BC and VP samples were in the order of SFA> MUFA > PUFA, but unsaturated fatty acids were more than saturated fatty acids (SFA<PUFA+MUFA) in both treatments. An increase was observed in the percentage of SFA in BC and VP from 46.07% to 51.32% and 46.01% to 48.01, respectively. A significant reduction was observed in the percentage of MUFA and PUFA from 33.73% to 26.26%, 33.94% to 28.85% and 15.44% to 10.78%, 15.43% to 13.10 in BC and VP, respectively after 6 months storage under frozen conditions (p<0.05). Table 3 shows the total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated (PUFA), ω -6 PUFA, ω -3PUFA, PUFA/SFA, EPA+DHA/C16 and the ω -3/ ω -6 ratios. As a result of the frozen storage period (up to 6 months), marked decreases were found in the contents of fatty acid groups such as ω -6 PUFA, ω -3PUFA, PUFA/SFA, EPA+DHA/C16, as well as in the ω -3/ ω -6 ratio in both treatments.

Table 1: Changes in fatty acids profiles of control cobia fillets during frozen storage up to 6 months in -18˚C(Means ± SD (n = 3); $p<0.05$).

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(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
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	0.335±0.004 A	0.312±0.003 B	0.257±0.002C	0.210±0.002D
Σ ω3PUFA	8.19±0.08A	7.32±0.06B	5.69±0.06C	4.33±0.05D
Σ ω6PUFA	7.25±0.09A	7.19±0.09A	6.88±0.08B	6.45±0.05C
ω 3/ ω 6	1.129±0.01A	1.010±0.03B	0.827±0.02C	0.671±0.01D
EPA+DHA/C16	0.276±0.007 A	0.238±0.004 B	0.169±0.001C	0.123±0.003D

Means in a row with different letters indicate significant differences ($p<0.05$) as result of frozen storage time.ND, Non-detected

ND, Non-detected

Table 2: Changes in fatty acids profiles of vacuum treated cobia fillets during frozen storage up to 6 months in -18˚C(Means ± SD (n=3); p<0.05).

Fatty acids	Time of storage (months)			
	0	1	3	6
C14:0 (Myristic acid)	4.19±0.04A	4.36±0.03B	4.95±0.05C	5.28±0.06D
C15:0 (n-Pentadecanoic)	0.63±0.02A	0.89±0.01B	0.95±0.05C	1.14±0.01D
C16:0 (Palmetic acid)	27.52±0.1A	27.56±0.1A	29.24±0.1B	31.10±0.6C
C17:0(Margaric acid)	1.07±0.07C	1.01±0.01B	0.49±0.01A	0.48±0.01A
C18:0 (Stearic acid)	12.60±0.05C	12.28±0.05C	11.2±0.05B	10.01±0.48A
C14:1(Myristoleic acid)	0.77±0.02D	0.62±0.02C	0.53±0.02B	0.42±0.02A
C15:1 (Pentadec-10-enoic acid)	0.70±0.05C	0.60±0.01B	0.60±0.02B	0.36±0.01A
C16:1(Palmitileic acid)	4.89±0.10D	4.66±0.10C	4.39±0.10B	3.43±0.03A
C17:1(Heptadanoic acid)	0.88±0.04C	0.87±0.01C	0.65±0.02B	0.41±0.02A
C18:1n-7(Vaccenic acid)	0.0ʸ±0.01	0.02±0.005	ND	ND
C18:1n-9(Oleic acid)	25.90±0.10C	25.77±0.10C	25.22±0.10B	23.84±0.10A
C20:1n-9(Gadoleic acid)	0.72±0.01C	0.68±0.01C	0.47±0.04B	0.39±0.02A
C18:2n-6(Linoleic acid)	4.35±0.03D	4.26±0.06C	3.91±0.06B	3.53±0.03A
C18:3n-3(Linolenic acid)	0.66±0.06D	0.59±0.02C	0.52±0.02B	0.31±0.02A
C20:2n-6(Eicosadienoic acid)	0.38±0.03C	0.26±0.01B	0.16±0.01A	0.15±0.01A
C20:4n-6(Arachidonic acid)	2.51±0.10A	2.63±0.02A	2.98±0.08B	3.22±0.02C
C20:5n-3(Eicosapentaenoic acid)	1.73±0.03C	1.67±0.02C	1.56±0.06B	1.21±0.03A
C22:6n-3(Docosahexaenoic acid)	5.80±0.05D	5.58±0.02C	5.08±0.06B	4.68±0.08A
ΣSFA	46.01±0.22A	46.10±0.20A	46.83±0.26A	48.01±1.16B
ΣMUFA	33.93±0.33D	33.22±0.25C	31.86±0.30B	28.85±0.20A
ΣPUFA	15.43±0.30C	14.99±0.15C	14.21±0.29B	13.10±0.19A
PUFA/SFA	0.335±0.004D	0.325±0.003C	0.303±0.002B	0.273±0.002A
Σ ω3PUFA	8.19±0.08D	7.84±0.06C	7.16±0.06B	6.20±0.05A
Σ ω6PUFA	7.24±0.06B	7.15±0.10B	7.05±0.12A	6.90±0.15A
ω 3/ ω6	1.131±0.01B	1.090±0.09B	1.010±0.11A	0.890±0.12A
EPA+DHA/C16	0.274±0.002D	0.263±0.001C	0.227±0.003B	0.189±0.001A

Means in a row with different letters indicate significant differences (p<0.05) as result of frozen storage time.
ND, Non-detected

Table 3: Changes in fatty acid series of control and vacuum-treated cobia fillets during frozen storage up to 6 months in -18˚C, (Means ± SD (n = 3); $p<0.05$).

		Frozen storage time (months)			
Fatty Acid series	**Treatment**	**0**	**1**	**3**	**6**
ΣSFA	BC	46.07±1.65a	46.52±0.54a	48.91±0.27a	51.32±0.23a
	VP	46.01±0.22a	46.10±0.20a	46.83±0.26b	48.01±1.16b
ΣMUFA	BC	33.72±0.57a	32.80±0.19a	30.38±0.1a	26.26±1.07a
	VP	33.94±0.33a	33.22±0.25a	31.86±0.30b	28.85±0.20b
ΣPUFA	BC	15.44±0.28a	14.51±0.18a	12.57±0.23a	10.78±0.27a
	VP	15.43±0.30a	14.99±0.15b	14.21±0.29b	13.10±0.19b
PUFA/SFA	BC	0.335±0.004a	0.312±0.003a	0.257±0.002a	0.210±0.002a
	VP	0.335±0.004a	0.325±0.003a	0.303±0.002b	0.273±0.002b
Σω3 PUFA	BC	8.19±0.08a	7.32±0.06a	5.69±0.06a	4.33±0.05a
	VP	8.19±0.08a	7.84±0.06b	7.16±0.06b	6.20±0.05b
Σω6 PUFA	BC	7.25±0.09a	7.19±0.09a	6.88±0.08a	6.45±0.05a
	VP	7.24±0.06a	7.15±0.10a	7.05±0.12b	6.90±0.15b
ω 3/ ω6	BC	1.129±0.01a	1.010±0.03a	0.827±0.02a	0.671±0.01a
	VP	1.131±0.01a	1.090±0.09a	1.010±0.11b	0.890±0.12b
EPA+DHA /C16	BC	0.276±0.007a	0.238±0.004a	0.169±0.001a	0.123±0.003a
	VP	0.274±0.002a	0.263±0.001b	0.227±0.003b	0.189±0.001b

Means in column with different small letters indicate significant differences ($p<0.05$) between treatments SD: standard division, ΣSFA: sum of saturated fatty. acids. ΣMUFA: sum of monounsaturated fatty acids, ΣPUFA: sum of polyunsaturated fatty acids, PUFA/SFA: polyunsaturated/saturated fatty acids ratio, Σ ω-3: sum of ω-3 fatty acids (linolenic+EPA+DHA), Σ ω-6: sum of ω -6 fatty acids (linoleic + Eicosadienoic + Arachidonic), ω -3/ ω -6: ω -3/ ω -6 fatty acid ratio; EPA+DHA/C16: Ecosapentaenoic acid + docosahexaenoic acid/palmitic acid.

Discussion

The fatty acid profiles were identified for 18 classes by GC. A great similarity in fatty acid profiles could be observed on comparison of the two kinds of samples. In all cases, the most abundant fatty acids were C16:0 (palmitic acid) and C18:1n-9 (oleic acid), followed by C18:0 (stearic acid). With regards to PUFA, C22:6n-3 (docosahexaenoic acid) and C18:2n-6 (linoleic acid) were found to be the most abundant. The present results agree with those obtained by Liu *et al*. (2009) for cobia that was farmed in marine cages located offshore of the Hainan province (China). In this research, different tissue locations were analyzed all of which showed C18:1n-9 and C16:0 as the most abundant fatty acids; among PUFA, C22:6n-3 and C20:5n-3 (eicosapentaenoic acid) were predominant. With regard to previous research on cobia fish belonging to the wild, the results of the present study can be considered quite different. Kotb *et al*. (1991) found similar values for the four most abundant fatty acids (C22:6n-3, C16:0, C18:1n-9 and C18:0) in fish captured in the Qatari waters of the Persian Gulf. Similarly, Khristoferzen (1969) found a higher PUFA content in wild fish from the Indian Ocean when compared to the current study. It may be concluded that previous research on cobia fish agrees with previous research carried out on different kinds of fish species where lower contents in SFA and MUFA, but higher values in PUFA have been obtained for wild fish in comparison with the farmed samples (Alasalvar *et al*., 2002; Aubourg *et al*., 2007; Álvarez *et al*., 2009). When

compared to most wild fish species, the present results on the fatty acid profile of cobia fillets can be considered as having higher contents of SFA and MUFA, but lower contents of PUFA.

When the fatty acid groups of all kinds of cobia fillets are considered (Table 3), frozen storage led to a progressive increase in the contents of SFA, while the presence of MUFA, PUFA and ω -3 PUFA showed a decrease with the frozen storage time. Additionally, a progressive decrease with frozen storage time could be observed for the ω -3/ ω -6 ratios and the polyene index (PI). Frozen 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 (lipoxygenases, peroxidases, and so on) and chemical pro-oxidant molecules (namely, hemoproteins and metal ions) in the fish muscle (Sikorski and Kolakowski, 2000). Oxidative damage during frozen storage has been reported to be especially important in the case of unsaturated fatty acids, as a result of an increased susceptibility of the carbon-carbon double bond to being attacked during the oxidation mechanism (Harris and Tall, 1994; Decker, 1998).

Previous research related to the frozen storage of fish has already shown that unsaturated lipids are likely to be oxidized. Thus, Serdaroglu and Felekoglu (2005) reported that SFA and PUFA content increased and decreased, respectively, in minced sardine (*Sardina pilchadus*) muscle when stored at –20°C for up to 5 months. A similar behavior was found for both fatty acid groups present in frozen (–30°C) Spanish mackerel (*Scomberomorus commersoni*) and white cheek shark (*Carcharhinus dussumieri*) fillets (Nazemroaya *et al*., 2009). A decrease in unsaturated fatty acid content, particularly PUFA, and lower ω -3/ ω -6 ratios were also found by Pirestani *et al*. (2010) in different kinds of fresh water fish species (Caspian kutum, golden grey mullet, common carp, pike perch and common kilka) belonging to the South Caspian Sea during frozen (–24°C) storage.

After 6 months of storage, a significant decrease ($p<0.05$) in the total amounts of the polyunsaturated fatty acids (PUFA) were observed with losses of 30% for BC and 15% for VP. The decrease in the PUFA concentrations is commonly attributable to oxidation and it is normally accepted that the oxidation rate increases dramatically with the degree of unsaturation (Saldanha and Bragagnolo, 2008).

The ω-3/ ω -6 ratio is a better index in comparing relative nutritional value of fish oils of different species (Turan *et al*., 2007; Pirestani *et al.*, 2010). The ω-3/ω-6 ratio of 1:1 is considered to be optimal for nutritional purposes (Turan *et al*., 2007). As shown in Table 3, the ω -3/ ω -6 ratio of cobia were 1.129 and 1.131 in BC and VP, respectively. The ω -3/ω -6 ratio in mackerel and shark were reported by Sahari *et al*. (2009) as 4.16 and 2.02 respectively and that in gilthead sea bream was found to be between 1.6 to 3.6 in different months (Senso *et al*., 2007). A significant decrease in this ratio from 1.129 to 0.671

in control samples, from 1.131 to 0.89 in vacuum packaging in cobia showed that the nutritional value of this fish had declined during frozen storage.

The PUFA/SFA (P/S) ratio reveals that marine fish are a good source of PUFA related to saturated fatty acids. In cobia, the PUFA/SFA (P/S) ratio was less than 1 (Table 3) and the decrease of PUFAs, in contrast to SFA, led to a significant decrease in this ratio (p<0.05) during frozen storage. This ratio in the control and VP were 0.335 and 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) Liu *et al.* (2009) and Nile tilapia (0.35) by (De Castro *et al*., 2007). In a comparison between control and vacuum packaging there were significant differences between both treatments during storage time (Table 3). Lin *et al*. (2008) reported that the PUFA/SFA ratio of the seahorses ranged from 0.40 to 0.93, which is higher than the general nutritional guidelines for humans recommended by the Department of Health of the UK, where high levels of SFA are not recommended.

The total ω-3 fatty acids in control samples and vacuum treatment were found to be higher than of ω -6 fatty acids in fillets of cobia fish (Table 3). Significantly lower level of ω -3 and ω -6 fatty acids were found in control samples (p<0.05). The ω-3 PUFA was present as 8.19 in control samples and vacuum packaging of the total fatty acids, most abundant of which was DHA (C22:6n-3) in all treatments (Tables 1,2).

The ω -6 PUFA were present as 7.25 and 7.24 in control samples and vacuum packaging of the total fatty acids and were mainly linoleic acid (C18:2n-6) and arachidonic acid (C20:4n-6). Significant decreases were observed in the percentage of ω-3 PUFA and ω-6 PUFA during 6 months (p<0.05). Marine fish are rich in ω -3 fatty acids, especially DHA and EPA (Celic *et al*., 2005). Liu *et al*. (2009) determined that farmed cobia (*R. canadum*) from china had higher total n-3 content than those reported in this study. It has been reported that the types and amounts of fatty acids in fish tissues vary with the geographic location, size, age, what the fish eat, reproductive status and season (Celic *et al*., 2005). Tawfik, (2009) reported that the total percentage ω-3 polyunsaturated fatty acids is higher than that of ω -6 in the Spanish mackerel, (*S. maculates*), Grouper (*Epinephelus coioides*) and Yellow-spotted trevally (*Carangoides fulvoguttatus*).

It is known that EPA and DHA have an essential role in the human diet to prevent diseases. Since these compounds are typical of seafood and cobia fillets contain higher amounts of these fatty acids, the losses observed in the EPA and DHA contents are especially important. A significant decrease was observed from 1.8 to 0.78 and 1.73 to 1.21 for EPA and 5.76 to 3.4 and 5.8 to 4.68 for DHA, in BC and VP respectively. The losses of these

products were probably related to autoxidation of the lipids. These results are in agreement with other research (Saldanha and Bragagnolo, 2008; Selami and Sadoki, 2008), which reported a decrease in the EPA and DHA levels during frozen storage in fish samples.

The polyene index (EPA+DHA/C16:0) ratio is a good index to determine lipid oxidation (Nazemroaya *et al*., 2009: Sahari *et al*., 2009; Pirestani *et al*., 2010). Regarding the PI evaluation during frozen storage, the present results agree with those of previous research where a decrease in this quality index was found for mackerel (*S. commerson*), shark (*Carcharhinus dussumieri*) (Nazemroaya *et al*., 2009) and coho salmon (*Oncorhynchus kisutch*) (Ortiz *et al*., 2009).

The results obtained which are related to the effect of vacuum packaging on the fatty acids profiles of cobia fillets throughout the frozen storage period can be seen in Table 3. Almost no effect could be observed during the 0-1-month storage period. Thus, after the freezing process (0-month storage), no differences could be observed for any of the fatty acid groups and ratio values as a result of the vacuum treatment. After 1 month of frozen storage, a preserving effect of such treatment could be depicted for the ω -3 PUFA content. However, when fish fillets corresponding to three months of frozen storage are considered, a greater effect of the VP treatment is observed. Thus, a preserving effect is observed for MUFA, PUFA and ω -3 PUFA, which is stronger in the case of vacuum packaging. Finally, the treated fish showed a higher ω -3/ ω -6 ratio and PI when compared to the untreated fish. Results related to a 6-month storage period are practically the same as those mentioned for 3 months. Totally, the results showed that usage of vacuum packaging had a positive influence on delaying lipid oxidation and increasing shelf-life of fillets ($p<0.05$).

Industrial requirements are always related to the search for valuable and practical treatments that may lead to enhancing commercial possibilities. In this sense, a vacuum packaging can be considered as most suitable, especially if such a method includes properties like easy availability, and low commercial cost. The commercial frozen storage with a low-oxygen permeability packaging was more effective in preventing lipid oxidation than high-oxygen permeability packaging (Saldanha and Bragagnolo, 2008). The present research agrees with previous research where a packing treatment in a vacuum system was found useful in order to increase the quality retention and shelf life of frozen fish. Thus, vacuum packaging has been successfully employed in different kinds of fish species such as sardines (*S. pilchardus*) Ozogol *et al*. (2004) and Persian sturgeon (*Acipenser persicus*) (Rostamzad *et al*., 2010).

As a result of a frozen storage period of 6 months, a marked content decrease was found in the fatty acid groups such as MUFA, PUFA and ω -3 PUFA, as

well as in the ω -3/ ω -6 ratio. However, a preserving effect on such fatty acid parameters could be observed due to the VP treatment. Assessment of the PI indicated an increased lipid oxidation development as a result of the frozen storage time; however, this increase was partially inhibited by the vacuum packaging. According to the present results, packaging samples under vacuum conditions was a proper way to reduce lipid oxidation of cobia (*R. canadum*) fillets and extend their shelf life by limiting available oxygen.

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