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Effects of Natural Antioxidant (*Zataria multiflora* Boiss) on Fatty Acid Profiles in Cobia Fillets During Frozen Storage

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Abstract: Marine fishes are rich in n-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are extremely important for human health. In this study, the effect of thyme essential oil (*Zataria multiflora* Boiss) on the fatty acid profiles of fish fillets during a further frozen storage in -18° C was investigated. Cobia fillets were treated with thyme essential oil (250ppm and 500ppm) then stored at -18°C for up to 6 months and compared to control conditions. As a result of the frozen storage, 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. However, a preserving effect on such fatty acid parameters could be observed resulting from the thyme treatments. Assessment of the polyene index indicated an increased lipid oxidation development during the frozen storage time; this increase was partially inhibited by using the thyme essential oil. Results of our investigation revealed that thyme 250 and thyme 500 ppm retarded changes of fatty acids in fillets of Cobia fish during frozen storage although thyme 250 ppm was not as effective as thyme 500 ppm on stability of fatty acids.

Key words: Cobia · Fish · Fatty Acids · Frozen Storages · Thyme

INTRODUCTION

Cobia (*Rachycentron canadum*) is the only species in the family Rachycentridae. Cobia is a promising candidate for aquaculture because of rapid growth rates, reaching up to 4-6 kg in a year, hardiness, efficient feed conversion, excellent flesh quality and comparatively low production costs [1-3]. The different part of Cobia shows unique qualities. In particular, the content of n-3 polyunsaturated fatty acids (n-3 PUFA), mainly eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are high in the Cobia lipids. Nowadays, high content of PUFAs in most of the marine products leads to increase of their importance in human diet. So using aquatic products is become very important, especially when they contain high amounts of fat. Aquatic species are known as a valuable source to provide high content of important constituents for the human diet [4]. Lipid oxidation in seafoods contributes to the loss of freshness and n-3 PUFA, depending on the storage period and temperature, natural antioxidants, type of chemical or physical treatment and packaging. The application of synthetic and natural antioxidants to control lipid oxidation in seafoods is well established [5]. Freezing is one of the best methods for long-term fish preservation [6, 7]. However, it does not inhibit lipid oxidation [8]. One of the most employed preserving technologies has been the employment of antioxidant compounds. Since synthetic antioxidants have been reported to behave as carcinogen and mutating agents, most attention has been accorded to the employment of natural antioxidants [9]. So it is tried to replace natural antioxidants instead of artificial ones [6, 10]. Among natural antioxidants, thyme (*Zataria multiflora* Boiss) has been used successfully as an antioxidant in different kinds of fish species such as mullet fish (*Mugil capito*) [11] and tuna (*Thunnus thynnus*) [12]. Antioxidative effect of thyme is based essentially on phenolic compounds [12, 13]. Thymol and Carvacrol are the main constituent of thyme essence and

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have excellent antioxidant activities [14]. The aim of the present study was to investigate the effect of thyme essential oil (*Zataria multiflora* Boiss) on fatty acid profiles and extending shelf life of Cobia fish fillets (*Rachycentron canadum*) up 6 months.

MATERIALS AND METHODS

Sample Preparation: Fresh cobia (*Rachycentron canadum*; 20 individual fishes) were caught 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 individuals were placed in boxes, surrounded by 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, being the weight of each fillet of 200±5g. Then, fillets were divided into 3 groups. Samples of the first group were left untreated (control) and directly packaged in polyamide/polyethylene bags. Fillets belonging to the second group were immersed in a 250 ppm solution of thyme (250ppm-thyme treatment); after 5 minutes, fillets were removed and packaged in individual polyamide/polyethylene bags. Finally, samples of the third groups were immersed in a 500 ppm solution of thyme (500ppm-thyme treatment); after 5 minutes, fillets were removed and packaged in individual polyamide/polyethylene bags. All packaged samples were immediately frozen at -30°C. After 24 hours, all fish fillets were placed in -18° C. 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.

Fatty Acid Analysis: Total lipid was extracted according to AOAC standard method [15]. Lipid extracts were then saponified with 0.5 N methanolic NaOH and further transesterified with BF3 in methanol [1]. The fatty acid methyl esters (FAMEs) were analyzed on a Gas Chromatography (GC1000, DANI Instrument, Switzerland) equipped with a flame Ionization Detector (FID). The fatty acid esters were separated on a SGE column (30m × 0.25 mm i.d.). Helium was employed as 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). FAMEs were identified by comparison of the retention times with those of standard mixtures (Larodan, Qualmix Fish; Supelco, FAME Mix). Peak areas were electronically integrated and quantified; results are expressed as percentage of total FAME.). Each sample was repeated three times and its average was calculated.

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

The fatty acid profiles were identified for 18 classes by GC. Results obtained throughout a 6-month frozen storage corresponding to treated (thyme250 ppm and thyme500 ppm) and untreated (control) fish are shown in Tables 1-4. A great similarity in fatty acid profiles were observed by comparison of the three kinds of samples. The changes in fatty acids profiles during frozen storage were 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 and those treatments ($P<0.05$). Palmitic acid (C16:0) and stearic acid (C18:0) respectively were the major fatty acids among the SFA during storage (Table 1-3). The same results were obtained about Sardine [8], Mackerel and Shark [16] and Sturgeon [17].

The average amount of the saturated fatty acids of pentadecanoic acid (C15:0) was also in minimum value in all treatments (Table 1-3).

There were significant difference among MUFA (monounsaturated fatty acids) content during 6 months ($P<0.05$). Oleic acid (C18:1 n-9) in control, thyme250 ppm and thyme500 ppm with 25.76, 25.92 and 25,81 respectively was in the maximum value in the fish tissue as compared to other fatty acids in control and those treatments (Table 1-3). Similar results were reported by Sahari *et al.* [16] and Liu *et al.* [18].

There were significant difference among PUFA contents during 6 months ($P<0.05$). PUFA accounted for approximately 15.44%, 15.32% and 15.45% of TFA

Table 1: Changes in fatty acid composition of control Cobia fillets during frozen storage up to 6 months in -18°C,(Means ± SD (n = 3) ; P < 0.05; % of total lipids)

	Time of storage (months)			
Fatty Acids	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 at the same row with different letters indicate significant differences (P<0.05) as result of frozen storage time

Table 2: Changes in fatty acid composition of thyme (250ppm) treated Cobia fillets during frozen storage up to 6 months in -18°C (Means ± SD (n = 3); P < 0.05; % of total lipids)

	Time of storage (months)			
Fatty acids	0	1	3	6
C14:0 (Myristic acid)	4.21±0.05A	4.36±0.06A	4.91±0.05B	5.80±0.15C
C15:0 (n-Pentadecanoic)	0.64±0.01A	0.89±0.01B	1.17±0.01C	1.23±0.02D
C16:0 (Palmetic acid)	27.47±0.07A	27.73±0.1B	30.77±0.1C	31.90±0.1D
C17:0(Margaric acid)	1.12±0.02C	0.93±0.02B	0.40±0.02A	0.39±0.01A
C18:0 (Stearic acid)	12.66±0.03D	12.24±0.1C	10.16±0.02B	9.56±0.01A
C14:1 (Myristoleic acid)	0.65±0.02D	0.48±0.01C	0.30±0.02B	0.17±0.03A
C15:1 (Pentadec-10-enoic acid)	0.67±0.05D	0.56±0.02C	0.44±0.04B	0.27±0.02A
C16:1(Palmitileic acid)	4.90±0.10D	4.62±0.10C	3.86±0.10B	3.12±0.10A
C17:1 (Cis-10-heptadanoic acid)	0.92±0.02D	0.83±0.03C	0.70±0.05B	0.41±0.01A
C18:1n-7(Vaccenic acid)	0.05±0.005C	0.04±0.002B	0.05±0.005C	0.03±0.001A
C18:1n-9(Oleic acid)	25.92±0.10C	25.72±0.20C	25.36±0.10B	23.66±0.10A
C20:1n-9(Gadoleic acid)	0.74±0.03D	0.66±0.05C	0.55±0.03B	0.34±0.01A
C18:2n-6(Linoleic acid)	4.37±0.07D	4.08±0.05C	3.59±0.03B	3.16±0.04A
C18:3n-3(Linolenic acid)	0.59±0.03D	0.51±0.02C	0.40±0.02B	0.25±0.02A
C20:2n-6(Eicosadienoic acid)	0.31±0.01C	0.22±0.02B	0.17±0.02A	0.15±0.01A
C20:4n-6(Arachidonic acid)	2.48±0.08A	2.65±0.02B	2.88±0.08C	3.51±0.03D
C20:5n-3(Eicosapentaenoic acid)	1.75±0.04D	1.63±0.02C	1.18±0.02B	0.86±0.02A
C22:6n-3(Docosahexaenoic acid)	5.82±0.10D	5.46±0.02C	5.06±0.06B	4.40±0.10A

Means at the same row with different letters indicate significant differences (P<0.05) as result of frozen storage time

Table 3: Changes in fatty acid composition of thyme (500ppm) treated Cobia fillets during frozen storage up to 6 months in -18°C (Means ± SD (n = 3); P < 0.05; % of total lipids)

	Time of storage(months)			
Fatty acids	0	1	3	6
C14:0 (Myristic acid)	4.26±0.04A	4.38±0.06B	4.94±0.04C	5.41±0.04D
C15:0 (n-Pentadecanoic)	0.64±0.01A	0.84±0.01B	0.94±0.02C	1.14±0.01D
C16:0 (Palmetic acid)	27.44±0.1A	27.63±0.03A	29.73±0.2B	30.85±0.1C
C17:0(Margaric acid)	1.12±0.12C	1.08±0.01C	0.45±0.01A	0.59±0.01B
C18:0 (Stearic acid)	12.74±0.04A	12.37±0.1B	10.93±0.1C	10.31±0.05C
C14:1(Myristoleic acid)	0.72±0.02D	0.52±0.02C	0.38±0.01B	0.25±0.02A
C15:1(Pentadec-10-enoic acid)	0.69±0.01D	0.53±0.02C	0.46±0.02B	0.34±0.01A
C16:1(Palmitileic acid)	4.88±0.10C	4.73±0.10C	4.36±0.06B	3.26±0.10A
C17:1 (Cis-10- heptadanoic acid)	0.89±0.01D	0.79±0.02C	0.63±0.05B	0.51±0.01A
C18:1n-7(Vaccenic acid)	0.05±0.005B	0.08±0.01C	0.04±0.005B	0.03±0.005A
C18:1n-9(Oleic acid)	25.81±0.10C	25.74±0.10C	25.44±0.20B	24.01±0.20A
C20:1n-9(Gadoleic acid)	0.65±0.02D	0.56±0.04C	0.42±0.02B	0.28±0.02A
C18:2n-6(Linoleic acid)	4.36±0.06C	4.27±0.07C	3.75±0.04B	3.38±0.08A
C18:3n-3(Linolenic acid)	0.60±0.05C	0.55±0.03C	0.49±0.02B	0.31±0.01A
C20:2n-6(Eicosadienoic acid)	0.35±0.02C	0.27±0.02B	0.24±0.02B	0.21±0.02A
C20:4n-6(Arachidonic acid)	2.58±0.08A	2.77±0.04B	2.88±0.07B	3.31±0.05C
C20:5n-3(Eicosapentaenoic acid)	1.79±0.09D	1.61±0.01C	1.25±0.02B	0.89±0.02A
C22:6n-3(Docosahexaenoic acid)	5.77±0.02D	5.53±0.03C	5.21±0.02B	4.89±0.09A

Means at the same row with different letters indicate significant differences (P<0.05) as result of frozen storage time

Table 4: Changes in fatty acid series of control and thyme- treated Cobia fillets during frozen storage up to 6 months in -18°C, (Means ± SD (n = 3); P < 0.05; % of total lipids)

		Frozen storage time (months)			
Fatty Acid series	Treatment	0	1	3	6
ΣSFA	Control	46.07±1.65Aa	46.52±0.54Aa	48.91±0.27Bc	51.32±0.23Cc
	Thyme 250ppm	46.10±0.18Aa	46.15±0.29Aa	47.41±0.20Bb	48.88±0.29Cb
	Thyme 500ppm	46.20±0.31Aa	46.30±0.21Aa	46.99±0.37Bb	48.30±0.21Ca
ΣMUFA	Control	33.72±0.57Ca	32.80±0.19Ca	30.38±0.1Ba	26.26±1.07Aa
	Thyme 250ppm	33.83±0.31Da	32.91±0.41Ca	31.26±0.34Bb	28.00±0.27Ab
	Thyme 500ppm	33.69±0.27Da	32.95±0.31Ca	31.73±0.35Bb	28.68±0.25Ab
ΣPUFA	Control	15.44±0.28Da	14.51±0.18Ca	12.57±0.23Ba	10.78±0.27Aa
	Thyme 250ppm	15.32±0.33Da	14.55±0.11Ca	13.28±0.23Bb	12.33±0.21Ab
	Thyme 500ppm	15.45±0.32Ca	15.00±0.20Cc	13.82±0.19Bc	12.99±0.27Ac
PUFA/SFA	Control	0.335±0.004Da	0.312±0.003Ca	0.257±0.002Ba	0.210±0.002Aa
	Thyme 250ppm	0.332±0.004Da	0.315±0.003Ca	0.280±0.002Bb	0.252±0.002Aa
	Thyme 500ppm	0.334±0.004Da	0.324±0.003Ca	0.294±0.002Bc	0.269±0.002Ac
Σn-3PUFA	Control	8.19±0.08Da	7.32±0.06Ca	5.69±0.06Ba	4.33±0.05Aa
	Thyme 250ppm	8.16±0.08Da	7.60±0.06Cb	6.64±0.06Bb	5.51±0.05Ab
	Thyme 500ppm	8.16±0.08Da	7.69±0.06Cb	6.95±0.06Bc	6.09±0.05Ad
Σn-6PUFA	Control	7.25±0.09Ca	7.19±0.09Cb	6.88±0.08Bb	6.45±0.05Aa
	Thyme 250ppm	7.16±0.11Ca	6.95±0.12Ba	6.64±0.09Aa	6.82±0.12Bb
	Thyme 500ppm	7.29±0.10Ba	7.31±0.10Bb	6.87±0.07Ab	6.90±0.15Ab
n-3/n-6	Control	1.129±0.01Da	1.010±0.03Ca	0.827±0.02Ba	0.671±0.01Aa
	Thyme 250ppm	1.139±0.01Ca	1.093±0.02Ca	1.000±0.06Bb	0.807±0.01Ab
	Thyme 500ppm	1.119±0.10Ba	1.050±0.05Ba	1.010±0.06Bb	0.880±0.04Ab
PA+DHA /C16	Control	0.276±0.007Da	0.238±0.004Ca	0.169±0.001Ba	0.123±0.003Aa
	Thyme 250ppm	0.276±0.005Da	0.256±0.001Cb	0.203±0.002Bb	0.165±0.003Ab
	Thyme 500ppm	0.276±0.004Da	0.258±0.002Cb	0.217±0.001Bd	0.187±0.003Ad

Means in column with different small letters indicate significant differences (P<0.05) between treatments and means in row with different capital letters indicate significant differences (P<0.05) as result of frozen storage time

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, 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; EPA+DHA/C16: Ecosapentaenoic acid + docosahexaenoic acid/palmitic acid.

(Total Fatty Acids) at the zero time in control sample, thyme250 ppm and thyme 500 ppm 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 third omega-3 fatty acids followed Linolenic acid (C18:3n-3), Eicosapentaenoic acid (C20:5n-3) and Docosahexaenoic acid (C22:6n-3) were identified in our study. A comparison between control, thyme 250 ppm and thyme 500 ppm showed the samples with thyme 500 ppm had higher amount of omega-3 fatty acids. Similar results were reported by Marichamy *et al.* [19].

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 same results were found for Mackerel (*Scomberomorus Commerson*) and Shark (*Carcharhinus Dussumieri*) by Sahari *et al.* [16].

Present results agree to those obtained by Liu *et al.* [18] for cobia that was farmed in marine cages located offshore of Hainan province (China). In such a research, different tissue locations were analysed so that all of them 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.

Table 4 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 frozen storage led in all kinds of cobia fillets to a progressive content increase in SFA, while MUFA, PUFA, n-6 PUFA and n-3 PUFA presence showed to decrease by increasing the frozen storage time. Additionally, a progressive decrease with frozen time could be observed for the n-3/n-6, PUFA/SFA ratio and the PI.

The SFAs were the most abundant fatty acids in the tissues of cobia in all treatments. In comparison between treatments, there was no significant difference between zero and first time ($P>0.05$) but there were significant differences between control and those treatments in 3rd and 6th months ($P<0.05$) whereas significant differences were recorded between treatment groups (thyme 250 and 500 ppm) in the 6th month ($P>0.05$). The percent of SFA increased from 46.07 to 51.32, 46.1 to 48.88 and 46.2 to 48.3 in control samples, thyme 250 ppm and thyme 500 ppm respectively. The highest incremental rate was found in the control sample (Table 4). Similar to our results, Serdaroglu and Felekoglu [8] reported that SFA percent increased in Sardine mince after 5 months storage at -20°C.

The second most abundant fatty acids were the MUFA, which in control samples, thyme 250 ppm and thyme 500 ppm, the MUFA percent decreased from 33.7 to 26.26, 33.83 to 28 and 33.69 to 28.68 respectively of the total fatty acids. There were significant differences in control and those treatments during 6 months frozen storage ($P<0.05$). In a comparison between all treatments there was not significant differences at zero and first time ($P>0.05$) but there were significant differences between control and those treatments in 3rd and 6th months ($P<0.05$) whereas no significant differences were recorded between treatment groups (thyme 250 and 500 ppm) in MUFA in the 3rd and 6th months ($P > 0.05$). MUFA content (Table 4) was found lower in control samples than in both - thyme-treated fish fillets when considering at frozen storage for 1, 3 and 6 months. The same results were found for Sardine (*Sardina pilchadus*) by Serdaroglu and Felekoglu [8] and Sardine (*Sardinella gibbosa*) by Chaijan *et al.* [20].

The PUFA accounted 15.44%, 15.32% and 15.45% of the total fatty acids in control, thyme 250 and thyme 500 ppm respectively. Distribution of fatty acid in Cobia was as SFA> MUFA > PUFA but unsaturated fatty acids were more than saturated fatty acids (SFA<PUFA+MUFA) in all treatments. A significant reduction were observed in control samples, thyme 250 and thyme 500 ppm PUFA percent from 15.44 to 10.78%, 15.32 to 12.12.33% and 15.45 to 12.99% respectively after 6 months storage under frozen condition ($P<0.05$). Similar to our results, Serdaroglu and Felekoglu [8] reported that PUFA percent decreased from 43.81 to 32.78 in Sardine mince and mentioned that the decrement in PUFA percent reflect enzymatic hydrolysis of sardine lipids. A significant differences were observed among the PUFAs in control samples and both treatments during frozen storage ($P<0.05$). The decrease of PUFAs percentage may indicate the oxidation of these unsaturated fatty acids during storage time. The similar results were reported by Selmi and Sadok [12], Sahari *et al.* [16], Hedayatifard and Moeini [17], Pirestani *et al.*[21] and Nazemroay *et al.*[22]. The total n-3 fatty acids in control samples and both treatments were found to be higher than of n-6 fatty acids in fillets of cobia fish (Table 4). Significantly lower level of n-3 and n-6 fatty acids were found in control samples ($P<0.05$). The n-3 PUFA was present as 8.19, 8.16 and 8.16% in control samples, thyme250 and thyme500 ppm respectively of the total fatty acids, most abundant of which was DHA (C22:6n-3) in all treatments (Table 1-3) .

The n-6 PUFA were present as 7.25, 7.16 and 7.29% in control samples, thyme 250 and thyme 500 ppm of the total fatty acids and were mainly linoleic acid (C18:2n-6) and arachidonic acid (C20:4n-6). The significant decrease were 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 [23]. Liu *et al.* [18] 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 [23]. Tawfik [24] reported that the total percentage n-3 polyunsaturated fatty acids, it is higher than n-6 in the Spanish mackerel, (*Scomberomorus maculates*), Grouper (*Epinephelus coioides*) and Yellow-spotted trevally (*Carangoides fulvoguttatus*).

The n-3/n-6 ratio is a better index in comparing relative nutritional value of fish oils of different species [21, 25]. The n-3/n-6 ratio of 1:1 is considered to be optimal for nutritional purposes [25]. As shown in Table 4, 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.* [16] 4.16 and 2.02 respectively and gilthead sea bream was found between 1.6 to 3.6 in different months [26]. 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 0.671in control samples, from 1.139 to 0.807 in thyme 250 ppm and from 1.19 to 0.88 in thyme 500 ppm 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 saturate fatty acids. In Cobia, the PUFA/SFA (P/S) ratio was less than 1 (Table 4) 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 control, thyme 250 and thyme 500 ppm were 0.335, 0.332 and 0.334 respectively and less than the minimum value (0.45) of PUFA/SFA ratio recommended [27]. The same results were reported by Pirestani *et al.* [21] in Golden grey mullet (0.35), farmed Cobia (0.332) [18] and Nile tilapia (0.35) by De Castro *et al.* [28]. In a comparison between control, thyme 250 and thyme 500 ppm there were significant differences between control and both treatments during storage time (Table 4) whereas significant differences were recorded between treatment groups (thyme 250 and thyme 500 ppm) in PUFA/SFA ratio in 3rd and 6th months ($P<0.05$). Lin *et al.* [29] 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 polyene index (EPA+DHA/C16:0) ratio is a good index to determine lipid oxidation [16, 21, 22]. In this study this ratio was decreased from 0.276 to 0.123, 0.276 to 0.165 and from 0.276 to 0.187 in control, thyme 250 and thyme 500 ppm respectively and also significant decrease was observed in the percentage of PI (polyene index) during 6 months ($P<0.05$). In a comparison between control, thyme 250 and thyme 500 ppm there were significant differences between control and both treatments in 1st, 3rd and 6th months (Table 4) whereas significant differences were recorded between treatment groups (thyme 250 and thyme 500 ppm) at 3 and 6 months in EPA+DHA/C16:0 ratio ($P<0.05$). It has been reported that Tuna (*Thunnus thynnus*) decreased the polyene index (EPA+DHA/C16:0) from 1.7 to 1.52 following 15 days of storage [12]. Related to the PI evolution during the frozen storage, present results agree to previous research where a decrease in such a quality index was found for mackerel (*Scomberomorus commerson*), shark (*Carcharhinus dussumieri*) Nazemroaya *et al.* [22] and coho salmon (*Oncorhynchus kisutch*) Ortiz *et al.* [30]. The negative relationship between this ratio and storage time showed that oxidation mechanisms are active during frozen storage. Finally, treated fish showed a higher n-3/n-6, PUFA/SFA ratio and polyene index when compared to its counterpart untreated fish.

Present research agrees to previous research where a dipping treatment in an antioxidant solution was found useful in order to increase the quality retention and shelf life of frozen fish. Thus, thyme and other natural antioxidants were successfully employed in different kinds of fish species such as Mullet fish (*Mugil capito*) Nessrien Yasin and Abou-Taleb [11], Tilapia (*Oreochromis niloticus* L.) Ibrahim and EL-Sherif, 2008 [31], Carp (*Cyprinus carpio*) Mahmoud *et al.* [32] and Tuna fish (*Thunnus thynnus*) Selami and Sadoki [12]. Similarly, different kinds of vegetable extracts were employed as previous soaking treatment such as rosemary extract and onion juice with sardine (*Sardina pilchardus*) mince Serdaroglu and Felekoglu [8] and flaxseed extract on whole mackerel (*Scomber scombrus*) Stodolnik *et al.* [33].

CONCLUSION

As a result of a frozen storage during 6 months, a marked content decrease was found in fatty acid groups such as MUFA, PUFA, n-3 PUFA and n-6 PUFA as well as in the n-3/n-6 ratio. However, a preserving effect on such fatty acid parameters could be observed provoked by the thyme treatment, being this effect greater when applying the thyme 500 ppm as soaking pre-treatment.

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