

Seasonal changes in fatty acid profiles of economically important fish species from Arabian Gulf and Shatt Al-Arab River

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Abstract

The present investigation aimed to comparative fatty acid levels in three species of fish muscle from Shatt Al-Arab river (*Cyprinus carpio*, *Oreochromis aureus*, *Planiliza abu*) with three species from Arabian Gulf (*Pampus argenteus*, *Epinephelus coioides*, *Otolithes ruber*). Fat content`s extracted by organic solvent extraction, and fatty acids were estimated using Gas Chromatography/Mass Spectrometry (GC-MS) (Calculated from the total amount of fatty acids) For the period from August 2018 to May 2019. The proportion of saturated fatty acids (SFA) ranged from 56.967% in *P. argenteus* during winter to 23.783% in *C. carpio* during spring. The percentage of monounsaturated fatty acids (MUFA) ranged from 50.771% in *C. carpio* during winter to 12.908% In *O. aureus* during winter. The ratio of polyunsaturated fatty acids (PUFA) ranged from 31.777% in *O. aureus* during spring to 2.608% in *C. carpio* fish during autumn. Also, omega-3, EPA, and DHA were identified for fish species and were found to be higher in marine species compared to freshwater fish.

Keywords: Fatty acids, GC-MS, Marine fishes, Freshwater fishes, Omega-3, Northwest Arabian Gulf, Shatt Al-Arab River .

Introduction

Fish`s fats are mainly composed of triglycerides containing saturated and unsaturated fatty acids, which makes them easy to digest. Fat in fish ranges between 0.2-25% of wet weight (Kulkarni *et al.*, 2020). The interest continues in many fatty acids and associated compounds such as Eicosapentaenoic (EPA) and Docosahexaenoic (DHA) Omega-3 fatty acids, which are found mainly in marine fish where they originate from phytoplankton and seaweed, which are an important part of the marine food chain. They also have important impact on the health and immune competence of various species including human beings, and they are essential for the modulation of immune responses in health



and disease (Al-Khalaifah, 2020). In recent years there has been a great interest in the role of fatty acids in various biological activities, especially in the field of health. Omega-3 fatty acids possess beneficial properties for cardiovascular (CV) health and elevated O₃FA levels are associated with lower incident risk for CV disease (Sherratt *et al.*, 2023).

The American Heart Association recommended patients with heart disease and vascular problems to eat a variety of fish, especially fatty ones, at least twice a week or to consume the equivalent of a gram of fatty acid EPA + DHA that is characterized by (Kris-Etherton *et al.*, 2002), which are useful in arthritis, rheumatism and other diseases, besides the US-EPA suggest that they are concentrated early in the brain as they have a major role in the development Cognitive property and visual acuity in children (Birch *et al.*, 2000). Fish are also used in many of industries using their uniforms in the field of food, using the right methods in fishing, conservation and transport where it is possible to produce oils With no fishes' smell. this study aimed to compare the fatty acid composition of three freshwater and three economically important marine fish species in Basra, highlighting seasonal differences and their nutritional importance.

Materials And Methods

The current study was conducted for the period from August 2018 to May 2019 on three species of marine fish: *Pampus argenteus*, *Epinephelus coioides* and *Otolithes ruber* collected from the Northwest Arabian Gulf and three types of freshwater fish (*Cyprinus carpio*, *Oreochromis aureus* and *Palaniza abu*) collected from Shatt Al-Arab river (Fig. 1).



Figure 1: Location map for Northern Arabian Gulf Shows the stations aria of samples: (1) National Iraqi waters, and (2) Shatt Al-Arab River.

The samples were taken to the laboratory and transported by using cork boxes and refrigeration and the muscles were extracted after removal of the head, skin, gills and internal intestines of the studied fish and The muscle tissues of each species were homogenized separately using an electric grinder to obtain uniform samples. The samples

were then dried by the Freeze dryer using a vacuum dryer (Model Modulyo; Edwards, UK), and the procedure was carried out under a vacuum pressure ranging from 0.01 to 0.20 mbar for a continuous period of 24 hours to ensure complete dehydration and sample stability. The samples were then well grinded and stored in sealed glass containers at a temperature of -15°C until chemical analysis was carried out.

Oil extraction

The oil was extracted from the muscles of fish described by A.O.A.C (2004) method, After grinding and drying taking the weight of the samples from the dried and grated fish and placed in the extraction apparatus with the use of the n-Hexane solvent for 24 hrs., separating the extract from the solvent by evaporation and collecting in sealed glass bottles, dry and the preservation of oil by refrigeration .

Methyl degradation of fatty acids

using A.O.A.C method (2004) was adopted in a triglyceride solution by reacting with a methyl potassium hydroxide solution by dissolving 11.2 g of potassium hydroxide in a small amount of pure methanol, then completing the volume to 100 mL by adding methanol using a volumetric flask. Quantity of fish oil extracted from the samples was placed in a 15 mL test tube, and 5 mL of potassium methyl hydroxide solution was added. The tube was vigorously shaken using a vortex mixer for 5 minutes, and then 5 mL of purified hexane was added. The mixture was left to stand until the upper layer of fatty acid methyl ester (FAMES) dissolved in the hexane, while the lower layer contained the reaction products. A microliter of the upper layer containing hexane and fatty acid (FAMES) was isolated using gas chromatography-mass spectrometry (GC-MS). The samples were analyzed for fatty acid content at the Basra Oil Company's quality control laboratories in Nahran Omar and expressed in a mg/100mg unit using a Agilent 7693 Agronomist GC-MS gas chromatograph with a vertical column as a static phase type (HP-5MS 5 % phenyl methyl siloxane 30m x 250 μm x 0.25 mm) with the use of high purity helium gas as a carrier gas. Injection of 1 μl under the separation conditions in both GC and MS are as follows:

Gas Chromatography 7890 B	Mass Spectrometer Agilent 5977 A
Column Oven Temp.: initial 40°C hold 5 mint. Rate 1 10c/min Final Temp 310°C to end run Injection Temp.: 260°C Injection Mode: Split, Split ratio 75:1 Flow Control Mode: Constant Flow Pressure: 7.0699 Psi Total Flow: 79 ml/min. Colum Flow: 1 ml/min. Purge Flow:3 ml/min.	Ion Source Temp.: 250°C Quad Temp.: 150°C Interface Temp.: (MSD Transfer Line) 2830°C Solvent Cut Time: 4.00 min. Start Time: 4.00 min. End Time: 35.00-40 min. ACQ Mode: Scan Scan Speed: 1562 (N2) Start m/z: 30 End m/z: 600

Data were analyzed using one-way ANOVA followed by LSD test in SPSS version 20, with significance set at $P \leq 0.05$.

Results and Discussion

By using the gas chromatography technique related to mass spectrometry, the type of fatty acids found in the fish were identified during the seasons and their quantity. The analysis revealed qualitative and quantitative differences in fatty acid composition among species and seasons, with some fatty acids consistently present across all samples, while others were species- or season-specific. Also, the percentage of fatty acids between one type and the other, and the percentage of saturated and unsaturated fatty acids. Tables (1, 2, 3 and 4) and Figures (2, 3, 4 and 5) shows the content of saturated and unsaturated fatty acids for fish studied during four seasons. The results showed that the total percentage of saturated fatty acids (SFA) ranged from 23.783 to 56.967%. The total monounsaturated fatty acids (MUFA) ranged between 12.908-50.771%. The total percentage of polyunsaturated fatty acids (PUFA) varied between 2.608-31.777%.

It was noted that *P. argenteus* fish contained the highest percentage of SFA 56.967% during winter and the lowest percentage 23.783% in the *C. carpio* during the spring. The *C. carpio* fish recorded the highest percentage of MUFA which reached 50.771% during winter and the lowest percentage 12.908% in *O. aureus* during winter, the highest percentage of PUFA was 31.777% in *O. aureus* during spring and the lowest percentage 2.608% in *C. carpio* during autumn. The results of the statistical analysis showed no significant differences between the samples ($P > 0.05$) and no significant differences between the seasons ($P > 0.05$) and significant differences between the fatty acids compounds ($P \leq 0.05$). Differences in the content of saturated and unsaturated fatty acids between species during the seasons are due to variation in fish genetics as well as to their culture areas and the environment in which they differ in the presence of food and its types. This causes a difference in the number of fatty acids diagnosed in fish oil, to the method of fishing and the studied season in the type and difference in the age and sex of fish (Othman, 2015). Within the group of saturated fatty acids, Lauric acid (C12:0) was found in only three samples during the seasons and in a few percentages, this agreed with Othman (2015). Palmitic acid (C16:0) is the most abundant in this group, as this fatty acid is completely stable during the life cycle of marine animals and is a major future in the synthesis of other fatty acids (Bandarra *et al.*, 2009). Among the monounsaturated fatty acids, Oleic acid (C18:1) was predominant in most samples because this acid has an external and recurrent source that reflects the type of diet in fish (Jabeen and Chaudhry, 2011), and there was a significant difference between acids C16:1 and C18:1, this variation is due to differences in food status and temperature, in addition to the maturity of the species. Freshwater fish and marine water fish are no differences in storage and transformation, but there are differences in structure, and these results were agreed with (Al-Husseini and Al-Tai, 2013). The percentage of fatty acid (C20:1) decreased during the winter is due to the lack of presence of crustaceans rich in wax esters in the region. It is worth mentioning that the lack of light affects the growth of the phytoplankton during the

winter, leading to the lack of presence of crustaceans that can store fat in the form of wax esters in addition to triglycerides. The results of this study showed that the omega-3 ratio was found in marine species higher than omega-6, since the origin of omega-3 PUFA is naturally occurring from marine phytoplankton and other microorganisms (Jasim and Al-Shatty, 2014; Nogueira *et al.*, 2013), and in the spring, omega-3 was found to be very high in river fish, this may be due to the blooming of vegetation and phytoplankton during spring, which is a stable food for river species. All fish samples showed higher DHA than EPA, DHA in these samples is due to the high content of phospholipids, which usually contain high amounts of Polyunsaturated fatty acids (PUFA), and these results agreed with (Bandarra *et al.*, 2009; Tenyang *et al.*, 2014; Nogueira *et al.*, 2013). The differences in DHA and EPA ratios for the same species by several studies may be due to several factors such as food available to fish. Also, the reproduction status, size, and fishing seasons are factors that affect the fatty acid compositions in fish (Khonji *et al.*, 2023).

Table (1): Percentage of saturated and unsaturated fatty acids in fish studied during summer.

Common Name	Systematic Name	Carbons Number	<i>P. argenteus</i>	<i>E. coioides</i>	<i>O. ruber</i>	<i>C. carpio</i>	<i>O. aureus</i>	<i>P. abu</i>
Saturated Fatty Acids (SFA) %								
Myristic acid	Tetradecanoic acid	C14:0	5.795	2.742	5.257	2.698	3.137	4.654
Pentadecylic acid	Pentadecanoic acid	C15:0	1.756	ND	0.987	ND	2.753	3.399
Palmitic acid	Hexadecanoic acid	C16:0	19.407	21.971	22.086	25.066	29.743	24.451
Margaric acid	Heptadecanoic acid	C17:0	2.238	1.255	1.433	0.793	ND	0.797
Stearic acid	Octadecanoic acid	C:18:0	9.659	10.746	9.915	9.560	11.150	9.120
Total			38.855	36.714	39.678	38.117	46.783	42.421
Monounsaturated Fatty Acids (MUFA) %								
Palmitoleic acid	Hexadecenoic acid	C16:1 n-7	5.427	10.746	10.719	13.985	4.900	15.13
10-Heptadecenoic acid	cis-10-Heptadecenoic acid	C17:1	0.567	ND	ND	ND	1.631	2.143
Oleic Acid	Octadecenoic acid	C18:1 n-9	16.329	23.299	16.279	25.127	12.595	23.087
Gondoic acid	cis-11-Eicosenoic acid	C20:1 n-9	4.270	4.723	2.159	10.091	ND	8.892
Total			26.593	38.768	29.157	49.203	19.126	49.252
Polyunsaturated Fatty Acids (PUFA) %								
Linoleic acid	Octadecadienoic acid	C18:2 n-6	0.781	2.076	ND	2.595	1.521	2.013
Arachidonic acid	Eicosatetraenoic acid	C20:4 n-6	3.170	2.439	2.824	1.494	3.197	1.411
Timnodonic acid	Eicosapentaenoic acid (EPA)	C20:5 n-3	2.031	2.592	4.787	ND	2.082	ND
Docosahexaenoic acid	Docosahexaenoic acid (DHA)	C22:6 n-3	10.545	9.307	11.991	0.795	8.814	ND
Total			16.527	16.414	19.602	4.884	15.614	3.424
Others			18.024	8.829	11.563	7.796	18.477	4.905

ND: not detected

Table (2): Percentage of saturated and unsaturated fatty acids in fish studied during Autumn.

Common Name	Systematic Name	Carbons Number	<i>P. argenteus</i>	<i>E. coioides</i>	<i>O. ruber</i>	<i>C. carpio</i>	<i>O. aureus</i>	<i>P. abu</i>
Saturated Fatty Acids (SFA) %								
Lauric acid	Dodecanoic acid	C12:0	ND	ND	ND	1.962	ND	ND
Myristic acid	Tetradecanoic acid	C14:0	3.865	2.794	2.511	2.574	6.260	4.368
Pentadecylic acid	Pentadecanoic acid	C15:0	1.053	ND	ND	ND	1.716	3.116
Palmitic acid	Hexadecanoic acid	C16:0	22.238	21.679	32.766	30.92	25.439	24.078
Margaric acid	Heptadecanoic acid	C17:0	1.977	1.356	ND	ND	1.291	0.726
Stearic acid	Octadecanoic acid	C:18:0	10.566	10.789	12.137	6.447	10.644	9.364
Total			39.699	36.618	47.414	41.903	45.35	41.652
Monounsaturated Fatty Acids (MUFA) %								
Palmitoleic acid	Hexadecenoic acid	C16:1 n-7	6.889	10.469	5.390	11.122	10.123	14.720
10-Heptadecenoic acid	cis-10-Heptadecenoic acid	C17:1	ND	0.694	ND	ND	0.899	1.938
Oleic Acid	Octadecenoic acid	C18:1 n-9	21.344	18.674	15.243	27.694	11.571	22.738
gondoic acid	cis-11-Eicosenoic acid	C20:1 n-9	3.275	4.781	ND	7.424	0.826	8.411
Total			31.508	34.618	20.633	46.24	23.419	47.807
Polyunsaturated Fatty Acids (PUFA) %								
Linoleic acid	Octadecadienoic acid	C18:2 n-6	1.204	3.075	ND	ND	1.930	2.375
Arachidonic acid	Eicosatetraenoic acid	C20:4 n-6	4.058	2.792	1.386	1.486	2.322	1.345
Timnodonic acid	Eicosapentaenoic acid (EPA)	C20:5 n-3	1.407	2.739	1.386	ND	2.291	ND
Docosahexaenoic acid	Docosahexaenoic acid (DHA)	C22:6 n-3	6.043	10.832	11.674	1.122	6.473	ND
Total			12.712	19.438	14.446	2.608	13.016	3.72
Others			16.080	9.327	17.508	9.249	19.404	6.821

ND: not detected

Table (3): Percentage of saturated and unsaturated fatty acids in fish studied during Winter.

Common Name	Systematic Name	Carbons Number	<i>P. argenteus</i>	<i>E. coioides</i>	<i>O. ruber</i>	<i>C. carpio</i>	<i>O. aureus</i>	<i>P. abu</i>
Saturated Fatty Acids (SFA) %								
Lauric acid	Dodecanoic acid	C12:0	ND	1.745	ND	ND	ND	ND
Myristic acid	Tetradecanoic acid	C14:0	5.851	7.646	4.651	ND	4.228	6.931
Pentadecylic acid	Pentadecanoic acid	C15:0	ND	1.506	ND	ND	ND	2.890
Palmitic acid	Hexadecanoic acid	C16:0	34.803	22.418	27.641	21.065	36.599	23.57
Margaric acid	Heptadecanoic acid	C17:0	1.239	2.240	1.063	1.274	ND	2.444
Stearic acid	Octadecanoic acid	C:18:0	15.074	11.974	11.062	10.298	14.055	6.153
Total			56.967	47.529	44.417	32.637	54.882	41.988
Monounsaturated Fatty Acids (MUFA) %								
Palmitoleic acid	Hexadecenoic acid	C16:1 n-7	1.524	12.046	10.587	6.293	4.812	16.448
10-Heptadecenoic acid	cis-10-Heptadecenoic acid	C17:1	ND	0.729	ND	ND	ND	1.469
Oleic Acid	Octadecenoic acid	C18:1 n-9	16.932	20.739	18.243	38.528	8.096	10.844
gondoic acid	cis-11-Eicosenoic acid	C20:1 n-9	3.597	ND	1.335	5.950	ND	ND
Total			22.053	33.514	30.165	50.771	12.908	28.761
Polyunsaturated Fatty Acids (PUFA) %								
Linoleic acid	Octadecadienoic acid	C18:2 n-6	ND	1.790	ND	2.200	1.333	3.516
Arachidonic acid	Eicosatetraenoic acid	C20:4 n-6	ND	1.932	1.425	3.296	1.152	2.181
Timnodonic acid	Eicosapentaenoic acid (EPA)	C20:5 n-3	3.033	1.736	2.335	1.158	1.609	6.165
Docosahexaenoic acid	Docosahexaenoic acid (DHA)	C22:6 n-3	9.609	3.083	10.539	4.053	4.089	3.011
Total			12.642	8.541	14.299	10.707	8.183	14.873
Others			8.341	11.144	11.119	5.885	24.027	14.378

ND: not detected

Table (4): Percentage of saturated and unsaturated fatty acids in fish studied during Spring.

Common Name	Systematic Name	Carbons Number	<i>P. argenteus</i>	<i>E. coioides</i>	<i>O. ruber</i>	<i>C. carpio</i>	<i>O. aureus</i>	<i>P. abu</i>
Saturated Fatty Acids (SFA) %								
Lauric acid	Dodecanoic acid	C12:0	ND	ND	ND	0.401	ND	ND
Myristic acid	Tetradecanoic acid	C14:0	5.445	3.402	4.510	3.367	6.402	6.147
Pentadecylic acid	Pentadecanoic acid	C15:0	1.401	0.885	0.976	1.033	0.789	3.329
Palmitic acid	Hexadecanoic acid	C16:0	16.693	18.232	17.677	11.698	21.727	19.387
Margaric acid	Heptadecanoic acid	C17:0	2.111	1.649	1.543	1.013	1.545	1.649
Stearic acid	Octadecanoic acid	C:18:0	9.284	10.169	8.702	6.271	11.656	5.045
Total			34.934	34.337	33.408	23.783	42.119	35.557
Monounsaturated Fatty Acids (MUFA) %								
Palmitoleic acid	Hexadecenoic acid	C16:1 n-7	4.896	9.317	10.730	12.032	10.570	13.235
10-Heptadecenoic acid	cis-10-Heptadecenoic acid	C17:1	ND	0.766	0.657	1.065	ND	2.579
Oleic Acid	Octadecenoic acid	C18:1 n-9	11.408	12.421	11.772	16.079	ND	8.222
gondoic acid	cis-11-Eicosenoic acid	C20:1 n-9	6.179	2.527	2.290	2.678	3.562	1.543
Total			22.483	25.031	25.449	31.854	14.132	25.579
Polyunsaturated Fatty Acids (PUFA) %								
Linoleic acid	Octadecadienoic acid	C18:2 n-6	0.617	1.524	0.868	2.981	8.841	4.094
Arachidonic acid	Eicosatetraenoic acid	C20:4 n-6	2.237	4.333	4.014	4.029	2.956	3.827
Timnodonic acid	Eicosapentaenoic acid (EPA)	C20:5 n-3	7.555	4.879	4.594	10.021	7.695	8.843
Docosahexaenoic acid	Docosahexaenoic acid (DHA)	C22:6 n-3	11.377	15.410	16.723	10.246	12.285	4.433
Total			21.786	26.146	26.199	27.277	31.777	21.197
Others			20.799	14.488	14.944	17.086	11.973	17.666

ND: not detected

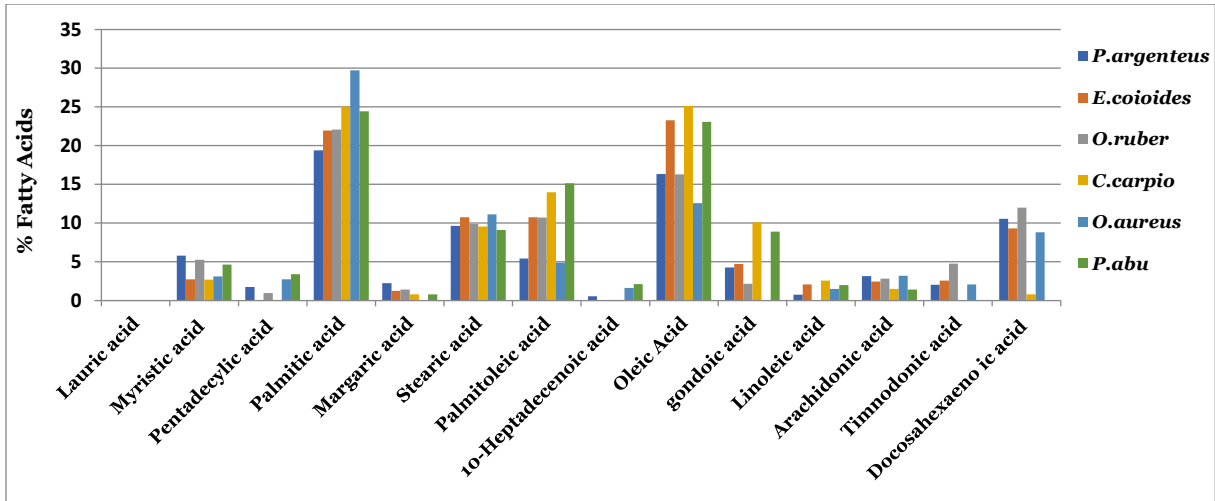


Figure (2): Percentage of fatty acids in studied fish during summer.

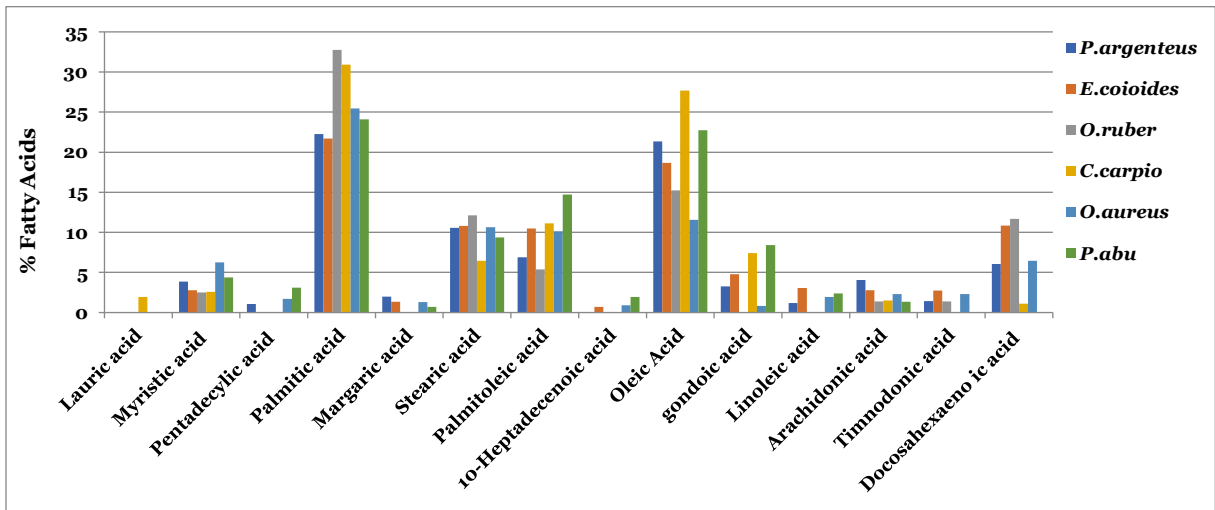


Figure (3): Percentage of fatty acids in studied fish during Autumn.

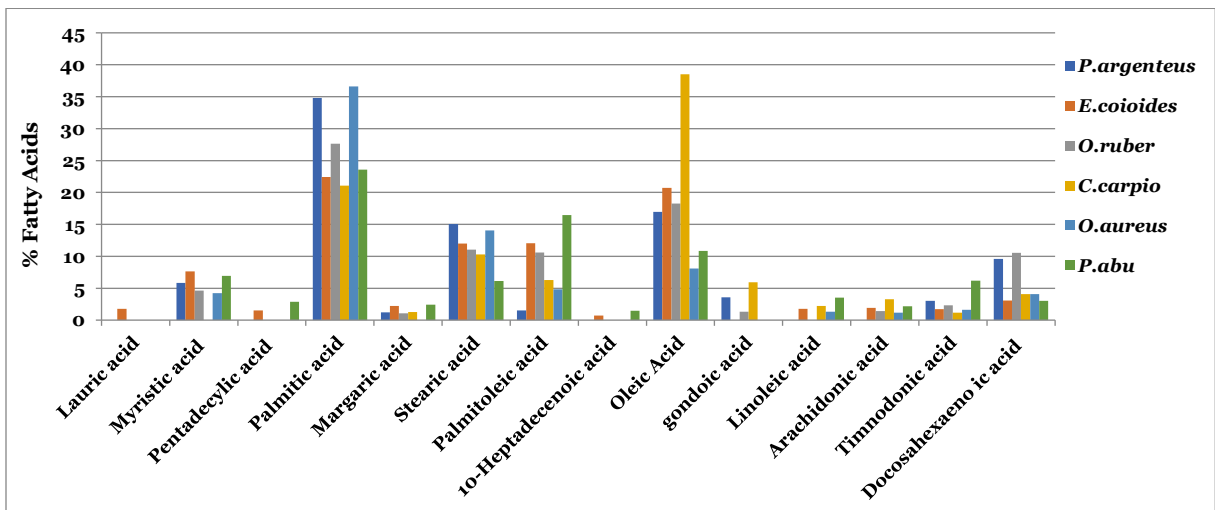


Figure (4): Percentage of fatty acids in studied fish during Winter.

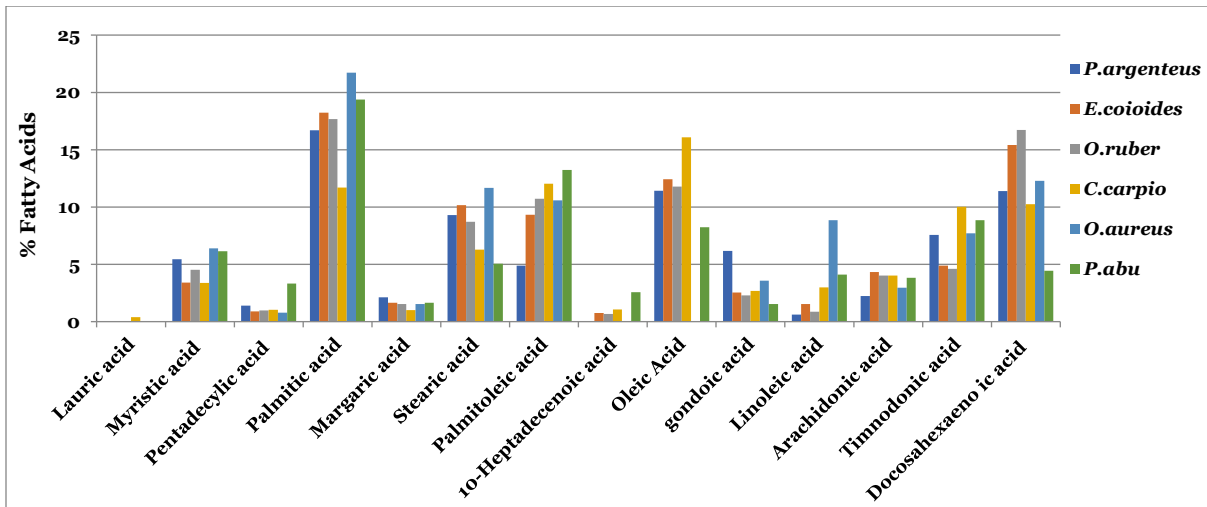


Figure (5): Percentage of fatty acids in studied fish during Spring.

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التغيرات الموسمية في خصائص الأحماض الدهنية لأنواع الأسماك ذات الأهمية الاقتصادية من الخليج العربي ونهر شط العرب

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المستخلص

هدفت هذه الدراسة إلى مقارنة مستويات الأحماض الدهنية في عضلات ثلاثة أنواع من اسماك نهر شط العرب *Cyprinus carpio* الكارب الاعتيادي و *Oreochromis aureus* البلطي و *Planiliza abu* الخشني وثلاثة أنواع من اسماك الخليج العربي *Pampus argenteus* الزبيدي و *Epinephelus coioides* الهامور و *Otolithes ruber* النوبيي. تم استخلاص محتوى الدهون عن طريق الاستخلاص بالمذيبات العضوية، وتم تقدير الأحماض الدهنية باستخدام كروماتوغرافيا الغاز / مطياف الكتلة (GC-MS) (محسوبة من إجمالي كمية الأحماض الدهنية) للفترة من أغسطس 2018 إلى مايو 2019، تراوحت نسبة الاحماض الدهنية المشبعة SFA بين 56.967% في اسماك الزبيدي خلال فصل الشتاء الى 23.783% في اسماك الكارب الاعتيادي خلال فصل الربيع، وتراوحت نسبة الاحماض الدهنية غير المشبعة أحادية الاصرة المزدوجة MUFA بين 50.771% لأسماك الكارب الاعتيادي خلال فصل الشتاء الى 12.908% في اسماك البلطي خلال فصل الشتاء، اما نسبة الاحماض الدهنية غير المشبعة المتعددة الاصرة المزدوجة PUFA فتراوحت بين 31.777% في اسماك البلطي خلال فصل الربيع الى 2.608% في اسماك الكارب الاعتيادي خلال فصل الخريف. وأيضاً تم تحديد الأوميكا-3 بنوعيه EPA و DHA للأنواع السمكية ووجد نسبها كانت اعلى في الأنواع البحرية مقارنة باسمك المياه العذبة.

الكلمات المفتاحية: الأحماض الدهنية، جهاز كروماتوغرافيا الغاز - مطياف الكتلة GC-MS، أسماك بحرية ونهرية، أوميكا 3، شمال غرب الخليج العربي، شط العرب .