Effects of dietary diversity on fatty acid and omega species composition of diverse fish species in the marshes of southern Iraq

By

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Abstract:

The present study was conducted to determine the effects of dietary diversity on the total composition of fatty acids and omega types of a variety of fish species from the wetlands of southern Iraq. Fish specimens were collected from the Al-Mashab marsh in East Al-Hamar, southern Iraq, and consisted of asp (Aspius vorax), abubola (Planiliza abu), blue tilapia (Oreochromis aureus) and carp (Cyprinus carpio). These fish feed on a variety of marine zooplankton and mixed plankton. Gas chromatography-mass spectrometry (GC-MS) was used to evaluate the content of saturated and unsaturated fatty acids, especially omega-3, in the oil extracted from all fish samples using the Dyer method. The study revealed the fatty acid composition of various species of fish, with varying average proportions of these acids and a notable proportion of specific acids. Significant differences in the presence of saturated fatty acids were observed in this study, and the variation was attributed to factors such as fish diet characteristics, fish species, and breeding season. Statistical analysis revealed significant variation ($p>0.05$) in the percentage of fatty acids under different conditions.

Keywords dietary diversity; overall fatty acid and omega composition; fish; marsh; Iraq**.**

Introduction

The southern Iraqi wetlands are recognized as a series of water bodies covering the lowlands of the southern Iraqi sedimentary plain, occupying a considerable area (Akbar et al., 2008). The southern region of these wetlands includes the cities of Basra, Maysan, and Dhi Qar (Habib, 2008) (2). These southern wetlands are classified into three main regions: the Haur al-Hawiza wetlands in the east, the Zaziri or Abu Alam wetlands in the center, and the Sharq al-Hama wetlands in the south.

With a total area of approximately 6852 km², this wetland consists of 65 species, including (Barbus sharbeyi, Luciobabus xanthopterus, Rastrineobola argentea, Aspius vorax, Barbus luteus, Bagre marinus It creates a suitable habitat for a diverse range of fish species. This favorable habitat is attributed to the abundance of water, light, and a variety of food sources including crustaceans, aquatic insects, plankton, and various types of algae.

Due to its high nutritional value, fish meat is an important dietary source for humans (Ochokwu et al., 2014) (4). The chemical composition of fish varies in terms of moisture, protein, lipids, and carbohydrates under the influence of diverse environmental factors. In addition, fish contain minerals and vitamins such as A, D, E, and B12. Assessing the quality of raw materials requires a basic understanding of the chemical composition of marine organisms (Gokoglu and Yerlikaya, 2015)(5).

Due to their remarkable impact on human health, fish contain high concentrations of unsaturated fatty acids, especially omega-3 fatty acids such as eicosapentaenoic acid (EPA) 20:5 and docosahexaenoic acid (DHA) 22:6 (Bera, 2010) (6). These acids and their metabolites reduce elevated cholesterol levels and

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decrease the risk of atherosclerosis and heart disease (Prato and Biandolino, 2015)(7). They also affect blood coagulation and platelet aggregation rates (Harris, 2004)(8). Furthermore, the storage and metabolism of triglycerides by omega-3 also confers a benefit of a 20% reduction in blood triglyceride levels (Woodman et al., 2002)(9).

The objective of this study was to evaluate the quality and total fatty acid composition of different species of fish within the study area. This assessment was made with respect to the dietary preference of each fish, addressing the lack of field studies in the southern Iraqi wetlands that specifically address the composition of total fatty acids, particularly omega-3 fatty acids, in fish from this region.

Materials and methods:

Fish samples were collected from the al-Mashab swamp in the Basrah region of southern Iraq. Samples were carefully weighed and placed in secure plastic containers with lids. After weighing, the samples were quickly transported under cooled conditions to the chemical analysis facility at the Marine Science Center's Marine Vertebrates Department. They were then stored at -18°C until the start of the study to ensure sample integrity for accurate analysis. To determine fatty acids, fish samples were cut into small pieces, leaving the skin and viscera, and pretreated. The prepared samples were placed in conical flasks of 250 ml volume and used for the subsequent oil extraction process. For the oil extraction process, samples were taken from each fish species under study, using the organic solvent extraction method outlined by AL-Hussainy (2007)(10) and introduced by Bligh and Dyer (1959)(11). Shimadzu GC-MS Qp

2010 Ultra system consisting of a gas chromatography unit coupled with a mass spectrometer was used. The analysis focused on assessing the percentage of fatty acids and omega-3 acids in the fish oil sample and used a Shimadzu GC-MS (2010) model equipped with a gas chromatography mass spectrometer featuring a split injection mechanism. a computer was used to process and generate the GC-MS solution, and a NSTA 08 curves and peaks were defined using a database linked to the library. The experimental setup included an air pump, a helium gas generator of 99.99% purity, and an Rtx-5MS capillary-type separation column (consisting of 5% diphenyl and 95% dimethylpolysiloxane). The gas chromatography-mass spectrometry system has an injection temperature of 250°C and a detection temperature of 200°C. The mass spectrometer, operating with electron impact ionization (EI), monitored the column temperature starting at 50°C. After stabilizing for 3 minutes, the temperature increased by 15°C per minute until it reached 250°C and held for 5 minutes before cooling. The sample injection volume was approximately 1 micromole. Statistical analysis of the data was performed using a special program for existing statistical systems (SPSS 20). The factors considered were tested with the least significant difference test (L.S.D.) at a probability level of 0.05. A completely randomized design (C.R.D.) was employed for experiments involving two, three, and four factors.

Results and discussion

1. sensory characteristics of fresh fish: As detailed in Table 1, sensory characteristics of fresh fish encompass important organoleptic properties. These include eye brightness, gill color

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and odor, texture, scale and skin integrity, and exterior examination. This sensory evaluation is important in assessing the freshness and overall condition of the fish.

According to Elshehawy (2016)(12), the evaluation of sensory qualities such as appearance, texture, smell, and color of fresh fish is considered an important indicator and a fundamental method for assessing its overall quality.

Table (1): Sensory characteristics of fresh fish inspected

Sensory characteristics	**Type of fish**			
	Planiliza abu	Aspius vorax	Oreochromis aureus	Cyprinus carpio
Odor	Herbal - slightly fishy	Herbal-pronounced fishiness	Herbal - slightly fishy	Herbal – fishy
Eyes sparkle	glamorous	Glamorous	glamorous	Glamorous
Color and smell of gills	Light red and clean fishy smell	Pinkish red and fishy smell	Dark red and fishy smell	Dark red and clean fishy smell
Texture	Inelastic knit	Inelastic knit	Inelastic knit	Inelastic knit
The consistency of the scales with the skin	The bond was strong and cohesive, and the scales are not easy to peel off	The bond was strong and cohesive, and the scales are not easy to peel off	The bond was strong and cohesive, and the scales are not easy to peel off	The bond was strong and cohesive, and the scales are not easy to peel off
Exterior test	Clean, free of blemishes and blemishes	Clean, free of blemishes and blemishes	Clean, free of blemishes and blemishes	Clean, free of blemishes and blemishes

Analysis of fatty acid composition, including saturated and unsaturated, of the target fish: The results of GC-MS analysis of raw fish oil are detailed in Tables (2) and (3). Total fatty acids (TFA) ranged from 70% to 97% and saturated fatty

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acids (SFA) from 0.1% to 28.25%. In addition, unsaturated fatty acids (UFA) were present in concentrations ranging from 0.22% to 39.15% Dvoretsky et al. (2023) (13) reported a similar composition of fatty acids in fish from the North Eastern Siberian River, with about 23 fatty acids accounting for 99.3% of the total, about 32.6% of saturated fatty acids (SFA) and %, while unsaturated fatty acids accounted for 15.1%. Because of the variety of foods and different types of fish studied, there were variations in the amounts of certain fatty acids, particularly C16:0, C18:0, C18:1, C18:3, C20:0, C20:4, C20:5, and C22:6. These variations were influenced by factors such as seasonal changes, fishing depth, and other variables, which is consistent with what was shown by both (Beca-Carretero et al., 2019; Jangaard et al., 1967) (14,15).. Although the conversion of acids to fatty acids, as well as the synthesis and storage of different fish species are consistent (Okland et al., 2005)(16), significant changes occur in their composition.

Besides, these results are consistent with a study by Ljubojevic (2013)(17) in which the importance of nutritional status in the fatty acid composition of seven freshwater fish species was demonstrated using GC-MS technology. This study supports this by revealing higher levels of omega-3 fatty acids in the fish species studied; studies by Zhang et al. (2020)(18) and Bayir et al. (2006)(19) on a variety of marine fish also contribute to our understanding of fatty acid type and composition. Figures (1), (2), (3), and (4) show fatty acid profiles of abu mullet (Planiliza abu), asp (Aspius vorax), blue tilapia (Oreochromis aureus), and carp (Cyprinus carpio).3. Saturated fatty acid (SFA)

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profiles observed in crude oils extracted from select studied fish species (table 2):

1- Caprylic acid (C8:0): statistical analysis showed that Asp, Blue tilapia, and Common carp were deficient with no significant difference ($p<0.05$) in the levels of caprylic saturated fatty acid found in their crude oils, as determined by GC-MS. In contrast, Abu mullet fish showed a content of 0.60%, which was lower than the findings reported by Ljubojevic et al. (2013) (17) in their research comparing the fatty acid composition of different fish species. Ljubojevic's study highlighted the presence of this acid in some fish and its absence in others. Unlike certain species (Barbus barbus, Cyprinus carpio, and Acipenser ruthenus), which lacked this acid, Aspius aspius, Abramis brama, and Esox lucius exhibited acid contents of (1.23%, 1.33%, and 3.64%), respectively.

2- Capric acid (C10:0): Based on the results of a study on fatty acids, it was observed that capric acid was present in lower concentrations in the crude oils of Abu mullet fish, Asp, and blue tilapia compared to the oil of common carp (3.28%, 1.64%, and 0.85% respectively). Statistical analysis of the studied species revealed significant differences ($p>0.05$), consistent with the findings of Alhaj Ali and Habbal (2017)(20); Visentainer et al. (2007)(21) conducted an analysis of fatty acid content and composition in coastal marine fish species and, notably, capric acid was not detected in the studied species. It is worth mentioning that the studied fish species are available in the market of Damascus City.

3- Myristic acid (C14:0): the percentage of myristic acid (C14:0) in the oil from all fish sources of Abu mullet, Asp, Blue tilapia

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and Common carp was 3.94%, 2.85%, 1.16% and 10.1%, respectively, and these values showed significant differences ($p > 0.05$). It is noteworthy that the percentage of myristic acid in crude fish oil falls within a range consistent with findings from other sources, as indicated by Zhang et al. (2020)(18) Fernandes et al. (2018)(22) also found a comparable range (2.1% ～to 7.47%) reported. Furthermore, studies on different organs of female Seriola rivoliana fish showed myristic acid content in muscle, liver, and gonads to be about 2.48%, 0.67%, 0.76%, and 1.79%, respectively. However, a recent study found that myristic acid levels were low by comparison.

4-Palmitic acid (C16:0): According to results obtained from GC-MS analysis, crude fish oils of abu mullet, asp, blue tilapia, and carp contained approximately 17.10%, 13.77%, 28.28%, and 13.75% palmitic acid, respectively. Interestingly, despite the significant differences between these fish species, statistical analysis ($p > 0.05$) showed no significant differences.Tenyang et al. (2014) (23) found that the chemical composition, fatty acid, amino acid profile, and mineral content of six fish species sold along the Cameroon River In a study conducted on the Palmitic acid emerged as one of the most abundant saturated fatty acids in the fish species studied. The observed percentages were consistent with the findings of other studies cited by Tenyang et al. 2014 (23). In addition, Alhaj Ali and Habbal (2010)(24) investigated the total fatty acid composition and omega-3 content of various types of fish available in the Damascus market. In this study, palmitic acid was significant in sardines, sultan ibrahim, and mullet, with percentages of 22.68%, 24.41%, and 26.18%, respectively.

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5- Margaric acid (C17:0): the margaric acid content of Abu mullet and Blue tilapia was about 1.39% and 1.98%, respectively, levels (0.62%) reported by Dvoretsky et al. (2023)(13) in their analysis of fatty acid composition in Esox lucius fish from northeast Siberia was markedly higher than that reported by (13). This difference was statistically significant ($p>0.05$), based on GC-MS results and subsequent statistical analysis. In contrast, diagnostic results did not indicate the presence of margaric acid in Asp fish. This discrepancy could be attributed to differences in environmental factors, fish species, and eating habits. Notably, Asp fish showed a diet concentrated on plant foods, which may explain the absence of margaric acid in this species. Interestingly, the percentage of margaric acid decreased by 0.16% in carp. This change may be influenced by several factors, including the fish's environment, eating habits, and inherent metabolic processes.

6- Stearic Acid (C18:0): In this study, the stearic acid percentage in carp fish oil samples was low, approximately 14.63%. Furthermore, fish oils from abu mullet, asp, and blue tilapia showed stearic acid values of about 2.96%, 2.86%, and 6.82%, respectively. Xu et al. (2022)(25) observed that the percentage of stearic acid in Larimichthys crocea fish oil treatments was affected by substituting oil from various sources for fish meal. Specifically, the stearic acid contribution of LO (linseed oil), linseed oil, and soybean oil was 4.26%, 3.95%, and 3.76%, respectively. These values were within the range of 3.63% to 9.96% reported by Zhang et al. (2020)(18) in their study of fatty acid composition of various commercial fish species collected from Chinese rivers. Zhang attributed the variation in fatty acid

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percentages among fish species to differences in diet, and suggested that fatty acids in fish diets may contribute to fish oil composition of fish oil.

Statistical analyses in both the current study and Zhang's study support the notion that dietary factors contribute to differences in fatty acid percentages among the species studied. It is noteworthy that the fish from Khishni and Shilluk did not differ significantly from each other, while the surveyed species did. This underscores the influence of diet on the fatty acid composition of fish oil.

7- Arachidic acid (C20:0): statistical analysis showed significant differences ($p>0.05$) between carp and tilapia fish species. Furthermore, a clear difference was observed when comparing the analysis of total fatty acids in the oil samples of blue tilapia, which eat mainly vegetables, and carp, which eat a mixed diet. The percentages of these acids were approximately 0.54% and 0.12%, respectively. These percentages are lower than those reported by Dvoretsky et al. (2023)(13). In contrast, no arachidic acid was detected in the oil of abalone, which is known for its planktonic and animal diet habits; in the study by Dvoretsky et al. (2023)(13), the percentage of arachidic acid was 7.29%, highlighting the possibility of differences in fatty acid composition in fish oil depending on dietary preferences and habits The study also found that the percentage of arachidic acid in fish oil was 7.29%. These findings highlight the influence of diet on specific fatty acid profiles observed in different fish species.

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Table (2): content of saturated fatty acids (SFA) in crude oil extracted from samples obtained from various fish species, expressed in grams per 100 grams.

N	Saturated fatty acids	fish types				overall average %
		Planiliza abu	Aspius vorax	Oreochrom is aureus	Cyprinus carpio	
1	C8:0	0.60	-	-	-	٠.١٥
2	C10:0	3.28	1.64	0.85	-	١.٤٤
3	C14:0	3.94	2.85	1.16	1`.1	2.26
4	C16:0	17.10	13.77	28.28	13.75	١٨.٢٣
5	C17:0	1.39	-	1.98	0.16	٠.٨٨
6	C18:0	2.96	2.86	6.82	14.63	٦.٨٢
7	C20:0	-	-	0.54	0.12	0.17

4. unsaturated fatty acid content in crude oil from selected fish species as measured by total monounsaturated fatty acids (TMUSF).

1- Oleic acid (C18:1): Oleic acid (C18:1): Crude oil from carp whole fish had the highest concentration of omega-9 type oleic acid, reaching 39.20%. In contrast, the crude oil of asp fish had the lowest percentage of this fatty acid, at 5.93%. These values were within the range observed by Belghit et al. (2019) (26) in their study on dietary replacement of Atlantic salmon fish meal, with percentages for the different treatments (IM0, IM33, IM66, and IM100) ranging from 40.0% to 30.0%. For vaccenic acid type omega-7, the percentages were (5.27%, 3.77%, and 9.15%) for rough, asp, and blue tilapia fish species in the current study, respectively, compared to (2.5%, 2.3%, 2.2%, and 2.0%) in Belghit's study. Statistical analysis of the test fish species showed significant differences at the significance level ($p>0.05$), with no omega-7 fatty acids present in the common carp fish.

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These variations were attributed to differences in fish species, eating habits, reproductive cycle, fishing season, and living environment, as noted by Chukwuemeka et al. (2009)(27).

2- Linoleic acid (C18:2)(The linoleic acid (C18:2) content of fresh carp whole oil was 11.08%, exceeding the finding of Dvoretsky et al. (2023)(13) for this omega-6 fatty acid in Esox lucius fish oil (about 3.08%). Furthermore, the percentages of linoleic acid in Asp and Blue tilapia fish oils were 2.63% and 2.75%, respectively. In contrast, the present study revealed no value for this acid in fish oil from Abubola, a result that is different from the findings of Sushchik et al. (2017), who reported the amount of this acid in perch, pike, roach, and snapper fish oils as (1.8%, 2.2%, 3.1%, and 2.9%), respectively (28), which differs from the findings of Sushchik et al. In addition, Zhang et al. (2020) (18) reported that Sihama sole, Odontamblyopus rubicundus, Harpadon numerou, Lateolabrax japonicus, Leptolepis selaroidea, Cynoglossus lida, Branchiostegus lbus, Nemipterus virgatus, Arius sinensis, and Larimichthys polyactis were observed to have linoleic acid content in various fish oils ranging from 1.21% to 0.77%. These percentages were relatively lower than those obtained in the present study. No significant differences were observed between abu mullet and asp fish with respect to the presence of linoleic acid, but considerable differences were observed between asp fish, blue tilapia, and carp.

3- Arachidonic acid (C20:4): Blue tilapia fish had a significantly lower content of omega-3 arachidonic fatty acids in their oil, only 0.55%. Carp, on the other hand, contained between 0.87% and 0.60% omega-6. Interestingly, statistical analysis showed no

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significant difference between the two ($p<0.05$). These percentages are lower than those reported in certain studies. For instance, Huyben et al. (2020)(29) found higher levels of arachidonic acid in fish fillets of Sparus aurata, reaching 1.01%, 0.78%, and 0.73% when oils from various sources were added to the given diet. Similarly, Xu et al. (2022) (25)discovered a 1.3% arachidonic acid level in Larimichthys crocea fish oil in their investigation. The observed variations may be attributed to factors such as species-specific differences, diet, and environmental conditions, emphasizing the influence of these factors on the fatty acid composition of fish oils.

4- Eicosapentaenoic acid (EPA): (C20:5): The ratio of eicosapentaenoic acid (EPA) type omega-3 in the extracted crude oil differed significantly between blue tilapia and common carp ($p>0.05$). In blue tilapia and carp, the EPA content was about 0.60% and 0.57%, respectively. This variation in EPA content highlights the differences in fatty acid profiles of these two fish species Al-Asheeri et al. (2020)(30) compared the EPA (C20:5 n-3) and DHA (C22:6 n-3) content of various species of farmed fish (local and imported) and several species commonly consumed in the Bahraini market, and the results fell within their predicted range. This suggests that the EPA content in the fish species studied was as expected and may vary depending on factors such as fish diet, habitat, and other environmental conditions.

5- Docosapentaenoic acid (DPA): (C22:5(: Blue tilapia and abalone consider the fatty acid docosapentaenoic acid (DPA) as an omega-3 fatty acid because of its significant contribution to fish metabolism and health DPA is present in fish tissues and

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cells and has been shown to enhance activity in various diseases and tissue integrity, and plays an important role in defense against infection. In addition, it is essential for fish development and reproduction. However, the crude oil obtained from the abalone and asp did not contain any detectable total fatty acids when analyzed by GC-MS. Among the fish species studied, carp contained the lowest percentage of DPA, but blue tilapia samples had the highest percentage at 1.28%, in contrast to about 0.21% for carp. Statistical analysis revealed significant differences between these percentages ($p>0.05$). In a study by Saini and Keum (2018)(31) that investigated the role of omega-3 and omega-6 polyunsaturated fatty acids in metabolism and their presence in various foods, the proportions of DPA found in the oils of sabal, sardines, salmon, cod liver and herring were (4.92%, 2 .99%, 1.97%, 0.94%, and 0.62%, respectively. This emphasizes that different fish oils have different levels of DPA and highlights the importance of considering specific fish species when aiming to include omega-3 fatty acids in the diet.

6- Docosahexaenoic acid (DHA) (C22:6): an omega-3 unsaturated fatty acid, DHA is considered essential for maintaining overall health, especially since it cannot be produced in vivo and must be obtained from the diet (Pereira et al. Freshwater fish such as asp, blue tilapia, ab rami are part of a fish food chain structure in which marine fish are known to be rich in omega-3 fatty acids, including DHA (Zhang et al., 2020) (18). The integrity of muscle cell membranes depends on their resistance to oxidative changes, and fatty acid formation and saturation are key elements in this process (Zajic, 2013) (32). The percentage of DHA acid in the analyzed crude oil was

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0.59% for blue tilapia and 0.24% for carp, showing a significant difference between the two (p>0.05). These results are consistent with those of Arslan et al. (2012) (33), who investigated the effects of substituting fats and oils from different sources in the diets of salmonids. in the Arslan et al. study, the percentage of DHA acid ranged from 14.1% to 0.4% depending on the type of oil added to the diet (1985). However, abalone and asp did not contain DHA in the crude oil extracted. This may be due to the superiority of the fish oil treatment compared to other treatments using plant-derived oils such as hazelnut oil, soybean oil, and linseed oil.

Table (3): Monounsaturated Fatty Acids (MUFA) Content (g/100g) in Crude Oils Extracted from Various Fish Species

N	Saturated fatty acids	fish types				overall average %
		Planiliza abu	Aspius vorax	Oreochromis aureus	Cyprinus carpio	
1	C18:1 n-7	5.27	3.77	9.15	-	٤.٥٥
2	C18:1 n-9	10.18	5.93	18.15	39.20	17.61
3	C18:2 n-6	-	2.63	2.75	11.08	٤.١٢
4	C18:3 n-3	-	-	0.87	0.27	٠.٢٩
5	C18:3 n-6	0.59	2.18	0.53	0.39	٠.٩٢
6	C20:3 n-9	-	-	0.56	0.53	٠.٢٧
7	C20:4 n-3	-	-	0.55	0	0.14
8	C20:4 n-6	-	-	0.87	0.60	0.37
9	C20:5 n-3	-	-	0.60	0.57	0.29
10	C22:5 n-3	-	-	1.28	0.21	0.37
11	C22:6 n-3	-	-	0.59	0.24	0.21

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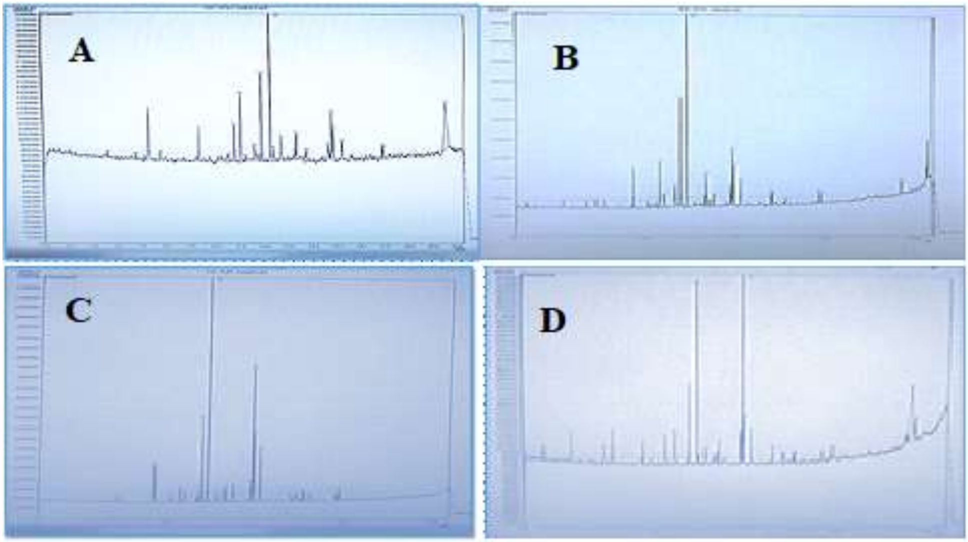

Figure (1): Fatty Acid Identification Using GC-MS Technology in (A)Planiliza abu

(B) Aspius vorax Fish, (C)Oreochromis aureus , (D) Cyprinus carpio Fish

Conclusions This study demonstrated that raw fish oil contains a spectrum of saturated and unsaturated fatty acids, especially those belonging to types -3, -6, -7, and -9, which provide diverse nutritional and physiological benefits. The GC-MS analysis performed in this study revealed significant differences in the composition of saturated and unsaturated fatty acids depending on the origin of the fresh fish oil. This study highlights that factors such as the variety of fish species studied, the breeding season, the specific nature of fish feeding and the environment comprehensively affect the amount of fatty acids detected.

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