



Determination of Fatty Acid Content in Six Common Marine Fishes in Bangladesh

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ABSTRACT

A comparative study on the fatty acid contents of six common marine fishes: *Poa (Poa poa)*, *Rupchanda (Chinese pomfret)*, *Hilsha (Tenualosa ilisha)*, *Loita (Harpadon nehereus)*, *Baila (Hemibagrus menoda)* and *Chapila (Gudusia chapra)* were carried out by Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). Saturated and unsaturated fatty acids, notably monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were shown to be more advantageous; these fatty acids are found in marine fishes. The polyunsaturated fatty acids Eicotennoic acid (C20:4), 12Z, and 15Z-heneicosadienoic acid (C21:2) are found in *Rupchanda* fish. *Hilsha* fish contained the monounsaturated fatty acids-Palmitoleic acid (C16:1) and Oleic acid (C18:1), which are respectively omega-7 and omega-9. *Chapila* fish contained the monounsaturated fatty acids such as cis-10 pentadecanoic acid (C15:1) and cis-15-tetracosenoic acid (C24:1). Only one mono unsaturated fatty acid, pentadecanoic acid (cis-10) (C15:1) is found in *Loita* fish species. However, the six selected marine fishes also showed several saturated fatty acids. Pentadecanoic acid (C15:0) was the most common saturated fatty acid identified in all studied marine fishes. However, this study suggests that, fatty acid profiles and concentration of the marine fishes will evaluate the fatty acids contents of that fishes to appraise the health benefits.

Keywords: Marine fishes, GC-MS/MS, Saturated fatty acids, Unsaturated fatty acids, Polyunsaturated fatty acids, Bangladesh.

INTRODUCTION

The Bay of Bengal, which has a 710 km of coastline and provides about 511 marine species including shrimp, crabs, sea foods, and other marine species, is located in the southern portion of Bangladesh [1]. Fish products' nutritional and medicinal advantages come from the proteins, lipids, minerals, and vitamins they contain. Because they are distinguished by the existence of necessary amino acids in proper amounts, fish proteins have great biological value [2-3]. Additionally, fish is the best supplier of PUFA omega-3[4]. Eicosapentaenoic acid (EPA or 20:5 ω 3) has been implicated in several investigations and docosahexaenoic acid (DHA or 22:6 ω 3) are found in important amounts in fish tissues [5-6]. It has been shown that these PUFAs are vital for survival nourishment [7]. They also have therapeutic and preventative benefits on a variety of human ailments, including cancer, rheumatoid arthritis, cardiovascular disease, and inflammatory processes [8-9].

When it comes to selecting fish for its nutritional value, nevertheless, not a lot of consideration has been given to the different species' levels of polyunsaturated fatty acids (PUFA) [10]. It is believed that the profile of fatty acids contained in fish, namely the proportion of long-chain polyunsaturated fatty acids (LC-PUFAs), is the key component responsible for the nutritional benefits of eating fish. These LC-PUFAs are crucial for the regulation of several biochemical and physiological processes. Recent research has shown that LC-PUFAs protect against the short- and long-term memory loss that results from chronic sleep loss[11]. Neurodegenerative and neurological symptoms may be relieved by increasing one's intake of n-3 PUFA, which also improves one's memory and mental acuity [12-13]. Studies have shown that n-3 PUFA-rich fish oil has positive effects on inflammation, autoimmune diseases, and cardiovascular disease [14-15]. As humans lack the ability to produce them, long-chain n-3 PUFAs must be consumed [16].

In most cases, the fatty acid profile and fatty acid makeup of fish must be taken into account when it is recommended for health purposes. There are a number of variables that may affect the fatty acid profile and fatty acid content of fishes, including species, sex, size, catch location, water temperature, diet, and time of year [17-18]. The nutritional and physiological consequences of fatty acids, particularly the connection between n-3 and n-6 fatty acids and cardiovascular heart disorders, have brought fatty acid analysis to the forefront in recent years [19].

The vast majority of free radicals and reactive oxygen species (ROS) are generated as a natural byproduct of the process of breathing. They were a significant contributor to the onset of a broad range of diseases, such as diabetes, Alzheimer's disease, hepatitis and cardiovascular disease [20-23]. Both Parkinson's disease (PD) and Alzheimer's disease (AD) were forms of neurodegenerative illnesses that affected the central nervous system and were associated with aging [24]. It's well known that fish is loaded with healthy polyunsaturated fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Fish is also a good source of protein [25]. Hepatoprotection, or the protective effect of fish on the liver, has been shown in a number of studies, leading to its use in the treatment of liver illness and, in particular, hepatic damage caused by medicines and other substances [26]. It is known that eating fish has a positive influence on human health, particularly with regard to the reduction of the risk of coronary artery disease, diabetes, hypertension, and cancer. Particularly significant in this regard are the roles played by DHA and EPA in the defense against illness [27-28]. A population-based, nested, case control study reported a strong inverse relationship between the

consumption of fish and the risk of sudden death (i.e., 5.6 g of n-3 fatty acids for every month, which is comparable to two fatty fish meals a week, was affiliated with a 50% of decreased risk of principal cardiac arrest) [29]. Gas chromatography (GC) is a typical type of chromatography used in analytical chemistry to separate and investigate volatile substances that may be evaporated without decomposition. Regular applications of GC include testing the purity of a certain material and isolating the various components of a mixture [30].

The analytical approach known as GC-MS combines the benefits of tandem mass spectrometry to determine the presence of certain chemicals in a given sample. In this study, six popular species of marine fishes such as Poa (*Poa poa*), Rupchanda (*Chinese pomfret*), Hilsha (*Tenualosa ilisha*), Loita (*Harpadon nehereus*), Baila (*Hemibagrus menoda*) and Chapila fish (*Gudusia chapra*) were selected and aimed to assess the amount of fatty acids from local market of Bangladesh and to detect the saturated and unsaturated fatty acids.

MATERIALS AND METHODS

Materials

Rotary evaporator (RE -100D, PHOENIX Instrument, Spain), sampling vial, microfilter, GC-MS machine (TRACE -1310 series, Thermo –scientific, USA), MS detector. Chloroform (ACS, 99.8% pure, Merck, Germany), Methanol (HPLC Grade, 99.8+% pure, Merck, Germany), $MgCl_2$ (Merck, Germany). Supelco 37 Component FAME Mix; Sigma Aldrich RTC, USA was used as a reference standard for fatty acid.

Methods

The Poa (*Poa poa*), Hilsha (*Tenualosa ilisha*), Rupchanda (*Chinese pomfret*), Loitta (*Harpadon nehereus*), Baila (*Hemibagrus menoda*) and Chapila fish (*Gudusia chapra*) were procured. The biological traits of entire marine fishes were identified and quickly dissected. As quickly as feasible, fish samples were brought to the lab and kept there at 4°C until analysis.

Each fish sample had its 25g of dorsal muscle tissue removed after it had partly thawed. A solvent: tissue ratio of 20:1, a chloroform/methanol solvent combination (2:1 v/v) was applied to a sample of fish muscle tissue. The samples were homogenized three times at 3000 rpm to 4000 rpm for a total of 10 minutes. Following each homogenization process, the material was cooled for an hour at 4°C. For every 1 g of tissue, 4 mL of 0.034% $MgCl_2$ were added to the extracts. The organic (which included the extracts of total lipids) and aqueous layers could be entirely separated when the chloroform/methanol extracts were incubated for a whole night at 4°C. Lower (organic) layer was washed with chloroform: methanol (2:1) solution and put in glass tubes after upper (aqueous) layer was removed. The complete lipid fraction was produced by the bottom phase's evaporation. In a rotary evaporator operating at 40°C under vacuum, the solvent was eliminated. These extracts were weighted and dissolved once again in a tiny amount (1-2 mL) of chloroform/methanol, reflecting the total lipids (2:1). For subsequent investigation, the complete lipid extract that was produced was kept at 4°C. The tissue's total lipid content was given as g/100 g. In this study, a GC-MS was used to assess the fatty acid contents of lipid extracts. Lipid extracts were transesterified to produce fatty acid methyl esters. The Thermo Fisher TRACE 1310 Gas chromatograph, both of which were outfitted with TR-FAME columns (30m X 0.25 mm X 25 μ m) were used to carry out the gas chromatograph analysis of methylated fatty acids. Fused silica with a non-polar bounded phase made up the column. TR-FAME column received an injection of one microliter of sample. Helium was

employed as the carrier gas, with a flow rate of 1 ml/min and a column head pressure of 43.0 kPa. Temperature programming was used to create the gas chromatograph, starting with a temperature of 100°C. The temperature was raised to 250°C at a rate of 15°C/min. Temperatures of 270°C were chosen for the injection and detector. After obtaining the mass spectrum from the chromatogram, it was checked against the NIST and NBS Mass Spectra Library.

Statistical Analysis

Results were analysed using Graphpad Prism version 9.4.1 in terms of fatty acid concentration and the varieties of fatty acids contain the different type of fishes.

RESULTS

Determination of Fatty Acids in Marine Fish Samples

From six varieties of marine fishes, the following 18 fatty acids have been identified: C4:0, C6:0, C8:0, C12:0, C13:0, C14:0, C15:0, C15:1, C17:0, C18:0, C18:1, C20:0, C20:4, C21:0, C21:2, C22:2, C24:0, C24:1. Figures 1.1, 1.2, 1.3, 1.4, 1.5 and 1.6 represent the fatty acid content of 6 selected marine fish samples. Finally, the data were compared to the fatty acid standard curve. The standard curve presents 37 specified fatty acids with their retention times. By comparing to the standard curve fatty acid retention time, the selected fatty acids were identified.

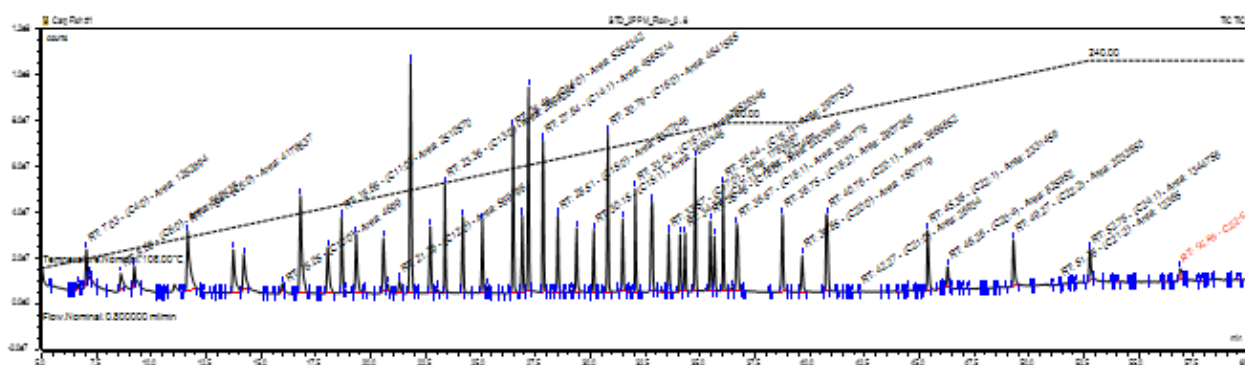


Figure 1: Standard curve of fatty acid with retention time.

Poa Fish (*Poa poa*):

The Poa fish (*Poa poa*) sample had six different types of fatty acids (figure 1.1), including Butyric acid (C4:0), Octanoic acid (C8:0), Lauric acid (C12:0), Myristic acid (C14:0), Octadecanoic acid (C18:0), Tetracosanoic acid (C24:0). This figure presents that there were no unsaturated fatty acids; all the fatty acids were saturated. The concentration of Myristic acid (C14:0) was higher in content than all of other fatty acids (greater than 0.6g/100g) and the concentration of Lauric acid (C12:0) was lower than all of other fatty acid (similar to 0.1g/100g).

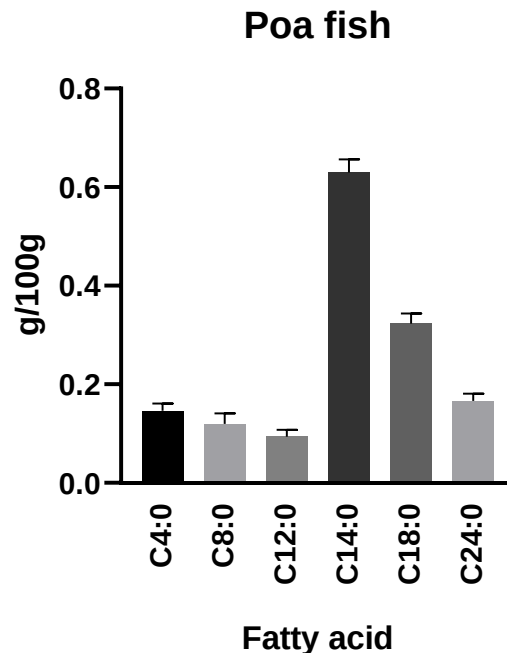


Figure 1.1 Concentration of fatty acid content (g/100g) in Poa fish. Y-axis depicts for the concentration of the fatty acids and X-axis is the different fatty acid in the fish. Myristic acid was higher in content and Lauric acid was lower in content which were showed in black and gray color. Here, n = 3.

Rupchanda Fish (*Chinese pomfret*):

Rupchanda fish sample figure had shown several types of fatty acid. Two polyunsaturated fatty acids had been identified; other fatty acids were saturated. Rupchanda fish (*Chinese pomfret*) had 5 different types of fatty acids, including Butyric acid (C4:0), Hexanoic acid (C6:0), Pentadecanoic acid (C15:0), Eicotetraenoic acid (C20:4), (12Z, 15Z)-heneicosadienoic acid (C21:2). Butyric acid (C4:0), Hexanoic acid (C6:0), Pentadecanoic acid (C15:0) are saturated fatty acid and Eicotetraenoic acid (C20:4), 12Z, 15Z - heneicosadienoic acid (C21:2) are poly unsaturated fatty acid. The concentration of Pentadecanoic acid (C15:0) was higher than any other fatty acids (almost similar to 0.6g/100g). And the concentration of Butyric acid (C4:0) was lower than any other fatty acids (below than 0.1g/100g).

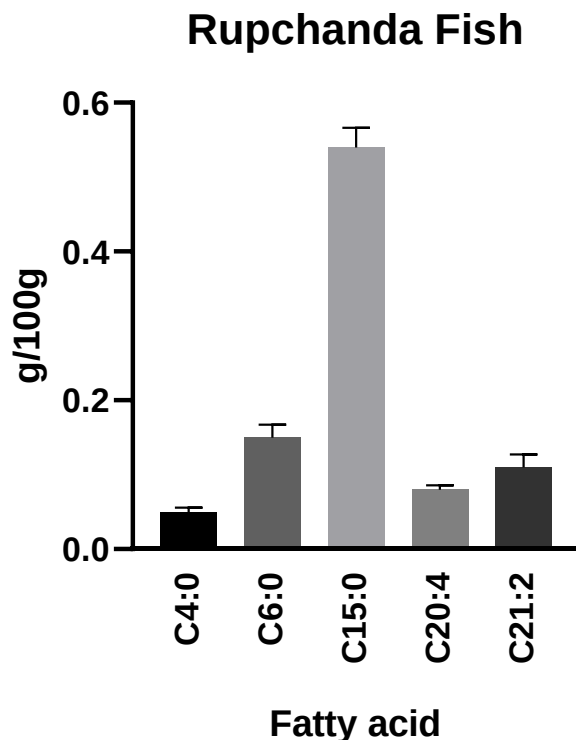


Figure: 1.2 Concentration of fatty acid content (g/100g) in Rupchanda fish. Y and X axis depicts for the concentration and different fatty acids in the fish as the pentadecanoic (C15:0) acid was higher and Butyric acid (C4:0) was lower in content as shown in different color. Here, n = 3.

Hilsha Fish (*Tenualosa ilisha*):

Hilsha fish sample figure had shown several types of fatty acid. Two monounsaturated fatty acids had been identified; other fatty acids were saturated. Hilsha fish (*Tenualosa ilisha*) had five different types of fatty acid, including Butyric acid (C4:0), Hexanoic acid (C6:0), Penta-decanoic acid (C15:0), Palmitoleic acid (C16:1), Oleic acid (C18:1). Butyric acid (C4:0), Hexanoic acid (C6:0), Penta-decanoic acid (C15:0) are saturated fatty acid. Palmitoleic acid (C16:1) and Oleic acid (C18:1) are omega-7 and omega-9 monounsaturated fatty acid respectively. The concentration of Penta-decanoic acid (C15:0) was higher than any other fatty acids (approximately 0.4g/100g). And the concentration of Butyric acid (C4:0) was lower than any other fatty acids (less than 0.1g/100g).

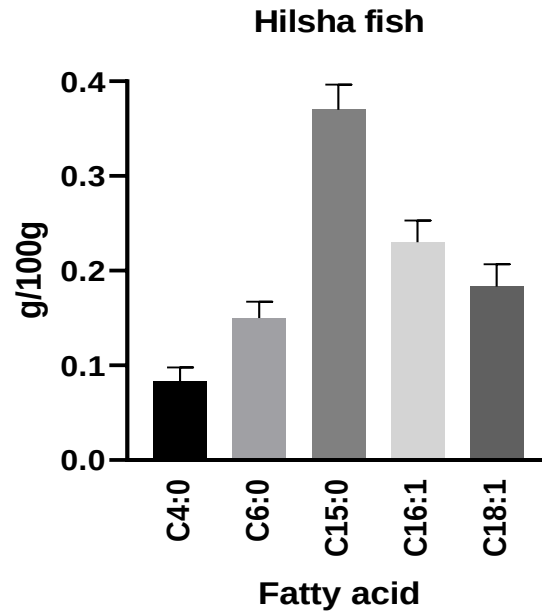


Figure: 1.3 Concentration of fatty acid content (g/100g) in Hilsha fish. Y and X axis depicts for the concentration and different fatty acids in different color shades. Pentadecanoic acid (C15:0) was in higher content and Butyric acids (C4:0) was lackluster in substance. Here, n = 3.

Loita Fish (*Harpadon nehereus*):

Loita fish (*Harpadon nehereus*) had 6 different types of fatty acids, including Octanoic acid (C8:0), Tridecanoic acid (C13:0), Pentadecanoic acid (C15:0), Pentadecanoic acid (cis-10) (C15:1), Arachidic acid (C20:0), Heneicosanoic acid (C21:0). Octanoic acid (C8:0), Tridecanoic acid (C13:0), Pentadecanoic acid (C15:0), Arachidic acid (C20:0), Heneicosanoic acid (C21:0) are saturated fatty acid and Pentadecanoic acid (cis-10) (C15:1) is unsaturated fatty acid. The concentration of Pentadecanoic acid (C15:0) was higher than any other fatty acids (approximately greater than 1g/100g). And the concentration of Octanoic acid (C8:0) was lower than any other fatty acids (approximately 0.1g/100g).

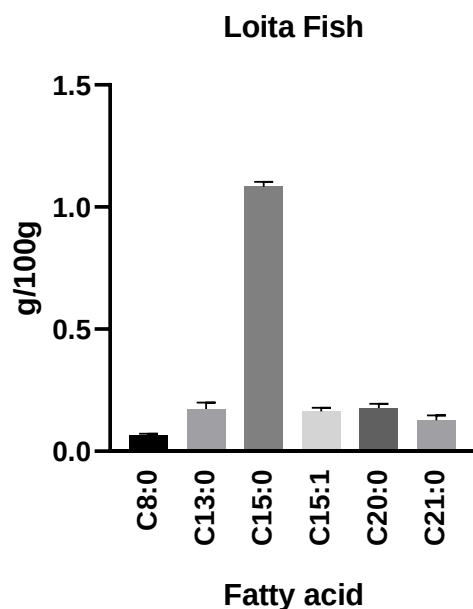


Figure: 1.4 Concentration of fatty acid content (g/100g) Loita fish. X-axis and Y-axis illustrates for the concentration and variety of fatty acids. In Loita fish 6 different fatty acids was found that shown in different color. Pentadecanoic acid (C15:0) was found much higher in content than other fatty acids. Here, n = 3.

Baila Fish (*Hemibagrus menoda*):

Baila fish (*Hemibagrus menoda*) had 7 different types of fatty acid, including Hexanoic acid (C6:0), Octanoic acid (C8:0), Myristic acid (C14:0), Pentadecanoic acid (C15:0), Heptadecanoic acid (C17:0), 12Z,15Z - heneicosadienoic acid (C21:2), Cis - 13,16 - docosadienoic acid (C22:2). Hexanoic acid (C6:0), Octanoic acid (C8:0), Myristic acid (C14:0), Pentadecanoic acid (C15:0), Heptadecanoic acid (C17:0) are saturated fatty acid. 12Z, 15Z - heneicosadienoic acid (C21:2), Cis - 13, 16 - docosadienoic acid (C22:2) are unsaturated fatty acid. The concentration of Pentadecanoic acid (C15:0) was higher than any other fatty acids (approximately 1g/100g) and the concentration of Myristic acid (C14:0) was lower than any other fatty acids (approximately 0.1g/100g).

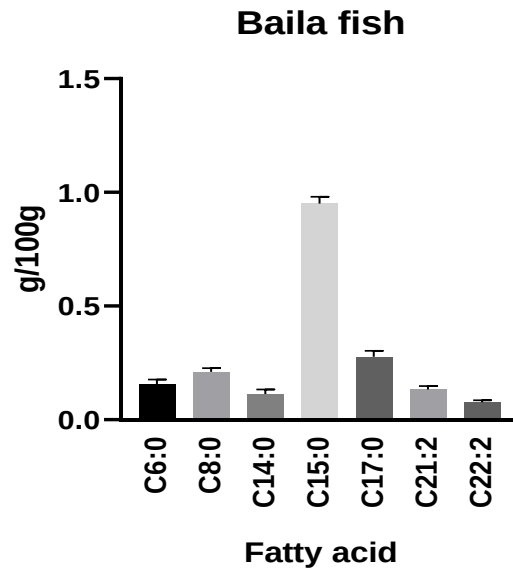


Figure: 1.5: Concentration of fatty acid content (g/100g) in Baila fish. There were found seven fatty acids in that Pentadecanoic (C15:0) acid was found in greater in content than other fatty acids. Here, n = 3.

Chapila Fish (*Gudusia chapra*):

Chapila fish sample figure has shown several types of fatty acid. Two monounsaturated fatty acids had been identified; other fatty acids were saturated. Chapila fish (*Gudusia chapra*) had 5 different types of fatty acid, including Hexanoic acid (C6:0), Pentadecanoic acid (C15:0), Cis-10 pentadecanoic acid (C15:1), Palmitic acid (C16:0), Cis-15-tetracosenoic acid (C24:1). Hexanoic acid (C6:0), Pentadecanoic acid (C15:0), Palmitic acid (C16:0) are saturated fatty acid. Cis-10 pentadecanoic acid (C15:1), Cis-15-tetracosenoic acid (C24:1) are monounsaturated fatty acid. The concentration of Pentadecanoic acid (C15:0) was higher than any other fatty acids (Approximately 0.9g/100g). And the concentration of palmitic acid (C16:0) was lower than any other fatty acids (around 0.1g/100).

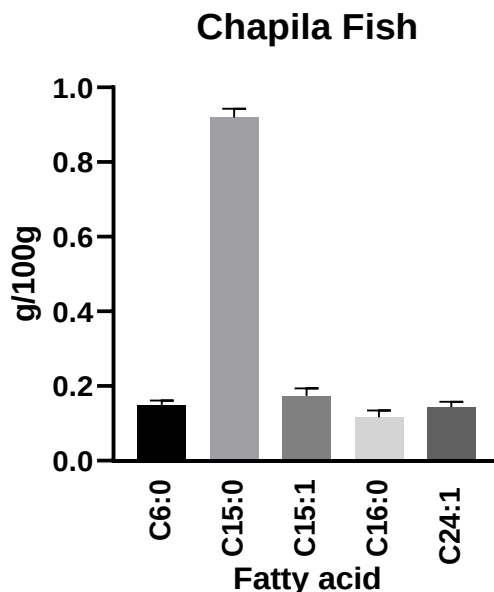


Figure 1.6: Concentration of fatty acid content (g/100g) in Chapila fish. X-axis and Y-axis illustrates for the concentration and variety of fatty acids. Five different fatty acids had found in Chapila fish, in that Pentadecanoic acids (C15:0) was found in higher. Here, n = 3.

DISCUSSION

In six kinds of marine fishes, we have identified several different kinds of fatty acids, including saturated, monounsaturated and polyunsaturated varieties among others. In this study, we have identified 18 specific fatty acids from 6 marine fish species including Butyric acid (C4:0), Hexanoic acid (C6:0), Octanoic acid (C8:0), Dodecanoic acid (C12:0), Tridecanoic acid (C13:0), Myristic acid (C14:0), Pentadecanoic acid (C15:0), Cis-10 pentadecanoic acid (C15:1), Palmitic acid (C16:0), Palmitoleic acid (C16:1), Heptadecanoic acid (C17:0), Octadecanoic acid (C18:0), Octadecenoic acid (C18:1), Arachidic acid (C20:0), Arachidonic acid (C20:4), Heneicosanoic acid (C21:0), (12Z,15Z)-heneicosadienoic acid (C21:2), Cis-13,16-docosadienoic acid (C22:2), Tetracosanoic acid (C24:0) and Cis-15 tetracosenoic acid (C24:1).

In Poa fish (*Poa poa*), there were only saturated fatty acids; there were no unsaturated fatty acids. Figure 1.1 showed that Myristic acid (C14:0) had the highest concentration of all fatty acids (more than 0.6g/100g). Lauric acid (C12:0), which had the lowest concentration of all the fatty acids, with a concentration of 0.1g/100g. The long-chain saturated fatty acid myristic acid was one of the most abundant fatty acids in Poa fish (14:0). The Rupchanda fish (*Chinese pomfret*) had saturated fatty acids included butyric (C4:0), hexanoic (C6:0), and pentadecanoic (C15:0), while polyunsaturated fatty acids included eicosapentaenoic acid (C20:4) and 12Z, 15Z- heneicosadienoic acid (C21:2). Pentadecanoic acid (C15:0) exhibited the greatest concentration of all fatty acids (about 0.6g/100g), as seen in Figure 1.2. Butyric acid also had the lowest concentration of any fatty acid (less than 0.1g/100g). The Hilsha fish (*Tenualosa ilisha*) had saturated fatty acids include butyric acid (C4:0), hexanoic acid (C6:0), and pentadecanoic acid (C15:0). Oleic acid (C18:1) is an omega-9 monounsaturated fatty acid. Palmitoleic acid (C16:1) is an omega-7 monounsaturated fatty acid. Figure 1.3 showed that Penta-decanoic acid (C15:0) had the highest concentration of all fatty acids (about 0.4g/100g). Also, butyric acid (C4:0) had the lowest amount (less than 0.1g/100g) of all the fatty acids. In the Loita fish

(*Harpadon nehereus*) there are two types of fatty acids, saturated ones like octanoic acid (C8:0), tridecanoic acid (C13:0), pentadecanoic acid (C15:0), arachidic acids (C20:0), eicosanoic acids (C21:0), and unsaturated ones like pentadecanoic acid (cis-10) (C15:1). Pentadecanoic acid (C15:0) exhibited the greatest concentration of all fatty acids (roughly higher than 1g/100g). And among the fatty acids, Octanoic acid (C8:0) had the lowest concentration (almost 0.1g/100g). The Baita fish (*Hemibagrus menoda*) had saturated fatty acids include hexanoic acid (C6:0), octanoic acid (C8:0), myristic acid (C14:0), pentadecanoic acid (C15:0), and heptadecanoic acid (C17:0) and unsaturated fatty acids include Cis - 13, 16 -docosadienoic acid (C22:2), 12Z, 15Z-heneicosadienoic acid (C21:2), and others. Of all the fatty acids, pentadecanoic acid (C15:0) had the greatest concentration (1g/100g). Myristic acid (C14:0), on the other hand, has the lowest concentration of any fatty acid (about 0.1g/100g). Chapila fish (*Gudusia chapra*) had saturated fatty acids like Hexanoic acid (C6:0), Pentadecanoic acid (C15:0), Palmitic acid (C16:0) and Cis-10 pentadecanoic acid (C15:1) and Cis-15 tetracosenoic acid (C24:1) are monounsaturated.

Pentadecanoic acid (C15:0) had the greatest concentration of all fatty acids (about 0.91 g per 100 grams). In addition, Palmitic acid (C16:0) has the lowest concentration of all fatty acids (about 0.1g/100g). The dispute over the health benefits of saturated vs unsaturated fatty acids has been a subject of study among the world's foremost nutritionists. Dietary consumption of unsaturated fats has been found to reduce atherosclerosis and coronary heart disease [31]. Long-term consumption of a diet high in monounsaturated fatty acids has been shown to lower plasma LDL cholesterol and platelet aggregation [32]. Rich in omega-3 polyunsaturated long-chain fatty acids, this fatty acid suppresses the production of adhesion molecules to avoid endothelial activation and increases the generation of nitric oxide in the arteries [33]. Unsaturated fatty acids are more susceptible to peroxidation than their saturated counterparts. Saturated fatty acids (SFAs) accounted for less than a quarter of total fatty acids, having palmitic acid (C16:0) being among the most common SFA. The range of fatty acid concentration was similar to prior research that used fish from a range of oceans and seas, from the Pacific to the Atlantic's southwestern shore [34]. Additionally, although being present in all of the investigated Pacific species, Palmitic acid (C16:0) was observed to be more abundant in the lean and low-fat species than in the fatty fish [35], Pentadecanoic acid (C15:0) was identified more abundant in our fishes and it is a dietary form of odd-chain saturated fatty acids (OCFAs). Higher circulation concentrations of OCFAs are linked to decreased mortality risks, as well as lower chances of becoming obese and developing a variety of cardiometabolic and liver disorders, according to multiple large-scale population studies carried out worldwide [27, 36- 37]. In fact, substituting polyunsaturated fatty acids for saturated fatty acids in the diet induces a statistically significant increase in liver and plasma lipid peroxidation [38-39].

CONCLUSION

Unsaturated fatty acids, such as monounsaturated and polyunsaturated fatty acids are often found in higher concentrations in marine fish. Palmitoleic acid (C16:1) Oleic acid (C18:1) are monounsaturated fatty acids of the omega-7 and omega-9 groups found in Hilsha fish. Eicotetraenoic acid (C20:4), 12Z, 15Z-heneicosadienoic acid (C21:2) are poly unsaturated fatty acids found in Rupchanda fish. The most abundant saturated fatty acid found in the marine fish species was pentadecanoic acid (C15:0). Through this investigation, we were able to uncover a wide range of significant fatty acids, which suggests that various categories of fish have a number of health advantages.

Acknowledgements

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Conflict of interests

The authors declare no conflict of interests.

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