Characterization of fish oils and Fatty acid investigation from *Mugil parsia* fish.

Abstract: This paper reports the isolation and characterization of oil of *Mugil parsia* fish. The oil was extracted from dried *Mugil parsia* fish collected from the Rupsha River in Khulna with n-hexane using Soxhlet apparatus. The oil contained of *Mugil parsia* fish was 17± 4.0 %. Some physico-chemical properties of raw fish like moisture, ash content and protein content were determined and were found to be 75.45 %, 1.258 % and 20.00 % respectively. Some physico-chemical properties of the extracted oil like color, specific gravity, peroxide value, saponification value, free fatty acid (FFA) and iodine value were determined and were found to be yellowish brown, 0.913±0.31, 9.9±0.1 meq/kg, 295.4±mg KOH/g, 11.9±2.0%, 5.7±0.11mg KOH/g, 150.3±0.12 mg/100g respectively. Identification of fatty acid composition by GC-MS analysis of the *Mugil parsia* fish oil, showed the Phthalic acid, mono-(2-ethylhexyl) ester, palmitic acid, oleic acid, stearic acid, icosanoic anhydride, glycidyl stearate were identified as the major constituents.

Key words: *Mugil parsia*, Soxhlet apparatus, peroxide value, iodine value, GC-MS analysis.

Introduction

Mugil parsia (Synonym *Liza parsia* Hamilton 1822), English name 'Gold spot mullet'occurs in the area, along the coasts of Sri Lanka, west India, Pakistan, Bangladesh and the Andaman Islands. In shallow coastal waters, estuaries, lagoons and sometimes entering tidal rivers, there occurs a schooling species. In ponds with 87% salinity, they can survive, Spawning takes place at sea.

Many studies have been conducted on fish flesh and its oil. Fish flesh is composed of high-quality proteins and lipids (oils) that are high in monounsaturated and polyunsaturated fatty acids [1]. Fish oil has been used to relieve muscular pain as well as arthritis. Various reports on the study and identification of fish oil having high pharmacological activity potential as a hypoglycemic [2], hypolipidemic agent [3−5], antiarthritic agent [6−8] and preventing an agent renal damage [9−11], inspired us to undertake the present study. This investigation deals with the fatty acid and lipid characterization of fish oils from marine fish *Mugil parsia* available in the coastal waters of Bangladesh.

1.1 Materials and methods

1.2.1 Sample Preparation

Mugil parsia (Synonym *Liza parsia* Hamilton 1822), samples were collected from the Rupsha River in Khulna at Rajshahi University in May, 2016. The average weights of Gold spot mullets were1.5 kg. The head, scales, bones, fins, viscera and gills were removed and cut the meat in to small pieces and dried for an hour to reduce the moisture content and then crushed well to almost paste form by a hand crusher. All fish samples were frozen and stored at -20^{o} C until use.

1.2.2 Physico-chemical analyses of fish

1.2.2.1 Determination of moisture content

Moisture content of seed meal was determined by the AOCS method [12]. Five grams of test portion was taken in dish container and dried it in an oven at 130°C for 2h. Heated portion was allowed to cool in a desiccator to room temperature and loss of weight determined.

1.2.2.2 Determination of ash content

Fish samples about 0.5 g was ignited and incinerates at 550 ^{o}C for about 12 h in muffle furnace, and then ash content determined according to AOCS standard method [12].

1.2.2.3 Determination of protein content

Water-soluble protein were determined spectrophotometrically by Lowry method [13].

1.2.2.4 Extraction of oil

About 100g of dried *Mugil parsia* fish was then weighed into a thimble and plugged with fat-free cotton and then placed into Soxhlet apparatus for approximately 16 h. Anhydrous n-hexane was used for the extraction of fish oil. Then the oil was freeze-dried by freeze dryer and stored at -80^{o} C until use.

1.2.2.5 Physico-chemical analyses of extracted fish oil

1.2.2.5.1 Determination of fish oil color

Colour of absolute oil was noted from physical appearance.

1.2.2.5.2 Determination of Specific gravity

The specific gravity of the oil was determined by using the specific gravity bottle.

1.2.2.5.3 Determination of Peroxide values:

The Peroxide values (PV) of fish oil were determined according to AOAC method [14]. Oilsample (5 g) was weighed into a 200 ml conical flask and mixed with 300 ml of glacial acetic acid and chloroform (3:1) and mixed thoroughly by swirling the flask. Saturated potassiumiodide (0.5 ml) was then added and the mixture was left in the dark for 1 min with occasionalswirling, followed with further addition of 30 ml distilled water. The mixture was titrated with0.1 N sodium thiosulphate solution with 1 ml of 1.0 % soluble starch as indicator until the bluecolour disappeared. A blank sample titration was also carried out in the same manner but withno oil added.

1.2.2.5.4 Determination of saponification value :

The saponification value (SV) of the fish oil was determined following procedures described inAOCS method [15]. Oil sample (1 g) was dissolved in 12.5 ml of 0.5 N ethanolic potassiumhydroxide. The mixture was refluxed for 30 min until oil droplets disappeared and was left tocool to room temperature. Phenolphthalein indicator was then added and the hot soap solutionwas titrated with 0.5 N HCl until the pink colour disappeared. A blank titration was also carriedout in the same manner except no oil was added.

1.2.2.5.5 Determination of FFA value and acid value:

Free fatty acids (FFA) value was determined according to the method describe in AOCS method [15]. An amount of 5 g oil sample was mixed with 75 ml of 95 % neutral ethyl alcoholand swirled. Phenolphthalein was added as indicator. The solution was titrated with 0.1 Nsodium hydroxide until pinkish colour was observed at end point. FFA values were determinedfrom equation given in the method.

From the FFA value, acid value (AV) was also calculated.

1.2.2.5.6 Determination of Iodine value :

The amount of iodine consumed is determined by titrating the iodine released (after adding KI) with a standard Thiosulphate.

0.3 g of fats was weighed into a small weighing dish and placed in a 250 cm^3 conical flask 10 cm^3 of carbon tetrachloride was added to the samples. To all the flask an equal quantity of about 25 cm^3 wigits reagents was added using a burette, this was mixed well and kept in the dark for an hour, after that it was titrated with standard 0.1M sodium thiosulphate solution while adding 15cm^3 of 10 % potassium iodide solution and 100 cm^3 of distilled water using starch as an indicator.

All the experiments were carried out in triplicate and the data is reported as mean ± standard deviation (SD) [16]. During all the experiments, calibrated glassware and chemicals of analytical grade were used.

1.2.2.6 Gas chromatography–mass spectrometry (GC-MS) analysis of *Mugil parsia* fish oil

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC-Mass spectroscopy was conducted (GCMSQP5050, Shimadzu, Japan) to characterize bioactive compound. A large number of constituents have been identified by GC-MS analysis of the crude ethanol extract. The quantification and the identification of compounds in the crude extract and active bands isolated by preparative TLC were accomplished using GC-MS analysis.

1.2.2.6.1 GC-MS analysis of fatty acid composition of *Mugil parsia* fish

The chemical compositions of fish oil were analysed by using GC–MS technique and the fragmentation analysis was performed. Fatty acid Compositions of the fish oil were separated and identified by gas chromatography–mass spectrometry (GC–MS) agilent 6890N gas chromatography hooked to agilent 5973N mass selective detector. They equipped with a flame ionization detector and capillary column with HP-5MS (30 m×0.25 mm×0.25μm). The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and ramped at 10° C $min^{-1}$to 180° C for 1 min and then ramped at 20° C min^{-1} to

280 °C for 15 min. The injector temperature was maintained at 270° C. The samples (1µL) were injected neat, with a split ratio of 1: 10. The carrier gas was helium at flow rate of 1.0mL min $^{-1}$. Spectra were scanned from 20 to 550 *m/z* at 2 scans s $^{-1}$. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in database.

Statistical Analysis: All the experiments were carried out in triplicate and the data is reported as mean ± standard deviation (SD) [16]. During all the experiments, calibrated glassware and chemicals of analytical grade were used.

1.2.3 Result and Discussion

Physico-chemical properties of Mugil parsiafish oil extracts

In order to determine the stability and quality of fish oil extracts, some quality assessment was conducted. These results are shown in Table 1, Table 2 and Table 3. In Table 1, the moisture, ash and protein content were 56.45± 4.0, 1.258± 0.2 and 35± 0.23 respectively. The oil contained of *Mugil parsia* fish was 17± 4.0. The oil obtained from *Mugil parsia* fish had a yellowish-brown color. The oil was showed the specific gravity 0.913±0.31. As shown in table 2, Young [17] had reported that peroxide value (PV) of crude fish oil was between 3 and 20 meq/kg. In this study, the PV was found to be 9.9 ± 0.1 meq/kg, which is well below acceptable limit of 20 meq /kg oil. This indicated that the fish oil extracted had low lipidoxidation rate [18]. The values of acid value (AV) and free fatty acids (FFA) in extracted fish oil were found to be 5.7 ± 0.11 mg KOH/g and 11.9 ± 2.0 %, respectively. The acceptable limit for AV was reported to be 7-8 mg KOH/g [19]. Chantachum [20] had reported that high heating temperature during oil extraction deactivated the enzyme and the release of free fatty acids by the lipase activity thus lowered the FFA value. Ashraduzzaman et al [21] had reported that acid value of seed oil was between 1.3-1.6 mg KOH/g. Due to low temperature used during the extraction of oil in this study, enzyme lipase present may not have been deactivated and thus more free fatty acids could be release by lipase activity. Thus, caused

high fatty acid value in this study which was also probably due to enhanced oxygen transfer which led to increased lipid oxidation, as propounded by Dauksas et al. [22]. Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali treatment. The SV of fish oil obtained in this study was higher (295.4 ± 3.0 mg KOH/g) than standard value for fish oil (180-200 mg KOH/g), given by AOCS [15]. Bimbo &Crowther [23] reported that crude oil contains minor amount of non-triglyceride substances. Thus, it is possible that high SV was due to impurities present in crude fish oil. Additionally, higher saponification value may be contributed by the unsaponifiable matter present in the leaching wastes materials such as sterols, glyceryl ethers, hydrocarbons, fatty alcohols and some minor quantities of pigments and vitamins.

GC-MS analysis:

The chemical composition of fish oil identified the aid of gas chromatography and decomposition products were characterized by mass analyzer detector GC/MS. The present study was carried out to identify fatty acid composition by GC-MS analysis of *Mugil parsia* fish oil (Table 4). Phthalic acid, mono-(2-ethylhexyl) ester, palmitic acid, Oleic acid, Stearic acid, Icosanoic anhydride, Glycidyl stearate were identified as the major constituents of *Mugil parsia* fish oil by GC-MS analysis. Oleic acid, a monounsaturated fatty acid. It decreased LDL cholesterol and increased HDL cholesterol cholesterol cholesterol [24]. It may also be responsible for the hypotensive (blood pressure reducing) effects. [25]. In previous study, it is revealed that stearic acid lowers LDL cholesterol [26]. It is reported that icosanoic acid (arachidonic acid) should be noted that for persons with chronic inflammatory disorder such as rheumatooid arthritis or inflammatory bowel disease. It is possible that icosanoic acid can exacerbate joint inflammation and pain [27].

Conclusion: The results of present study indicated that *Mugil parsia* fish contained significant amount of oil and protein. The presence of appreciable level of essential fatty acids and other favorable

physiochemical characteristic make the *Mugil parsia* fish oil nutritionally viable for human health. Unsaturated fatty acids content in fish oils may reduce the risk of chronic diseases.

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Table 1: Proximate composition of *Mugil parsia* fish.

Analysis	Fish oil
Moisture (%)	75.45± 4.0
Ash (%)	1.258± 0.2
Protein	20± 0.23
Oil (%)	17± 4.0
Color	yellowish brown

Specific gravity	0.913±0.31

Table 2: Physico-chemical analysis of extracted *Mugil parsia* fish oil.

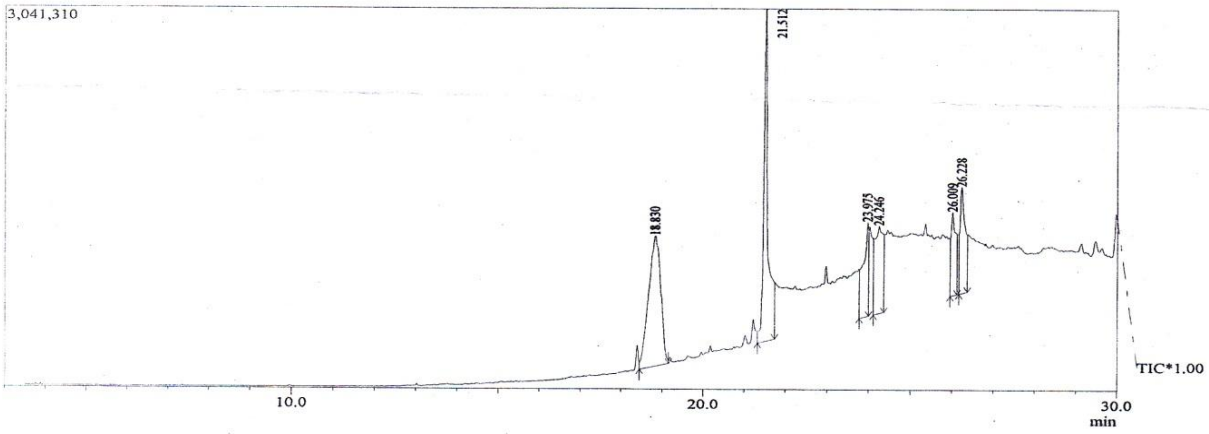
Analysis	Fish oil
Peroxide value (meq/kg)	9.9 ± 0.1
Saponification value (mg KOH/g	295.4 ± 3.0
Free fatty acid (%)	11.9 ± 2.0
Acid value (mg KOH/g)	5.7± 0.11
Iodine value(mg/100g)	150.3± 0.12

Table 3: Following compound are identified by GC-MS from *Mugil parsia* fish oil,

SI No	Name of the compound	Molecular weight	Concentration(Area %)
1	Phthalic acid, mono-(2-ethylhexyl) ester	278	30.42
2	Palmitic acid	256	27.24
3	Oleic acid,	282	9.72

4	Stearic acid	284	14.10
5	Icosanoic anhydride	606	7.67
6	Glycidyl stearate	340	10.85

Fig 1: Chromatogram of ***Mugil parsia*** **Fish oil**



Peak Report TIC

Peak#	Name	R.Time	Height	Area	Area%
1	Phthalic acid, mono-(2-ethylhexyl) ester	18.830	1024097	21047781	30.42
2	Palmitinic acid	21.512	2660523	18848497	27.24
3	Oleic acid	23.975	741328	6726926	9.72
4	Stearic acid	24.246	690768	9754487	14.10
5	Icosanoic anhydride	26.009	661952	5307467	7.67
6	Glycidyl stearate	26.228	845740	7511237	10.85
			6624408	69196395	100.00