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ORIGINAL ARTICLE

Extraction and Purification of Fish Oil from Marine And Freshwater Fish Waste and Assessment of Nutritional Parameters on Solid By-Products

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ABSTRACT
Fishery processing industries generate huge quantity of post-harvest waste and environmental issues associated with waste disposal. Considering the above issue, the present study, crude fish oil was extraction from marine and freshwater waste samples in three different extraction procedures such as Direct streaming method, Bligh & Dryer and Folch method were adopted. The extracted cured oils were purified by adapting relevant techniques. In addition, the essential proximate composition and nutritional properties were estimated from fish solid by-product waste of fish oil extraction processes. The maximum yield of 1000g freshwater fish waste noticed 100.6 ± 0.9 ml of crude fish oil by DS method. The high yield of marine water fish waste production of 92.7±0.8ml was recorded in similar DS method. The percentage of oils on crude oil removed as impurity in each step was calculated as 17.9%, 11.3%, 2.3%, and 8.3% in degumming, neutralization process bleaching and deodorization process respectively. A total number of eight different fatty acids were recorded from both marine and freshwater fish sample. The samples of marine and freshwater fish solid wastes were determined the essential amino acids (EAA) of Aspartic acid, Glutamic acid, Asparagine, Serine, Gultamine, Glycine, Alanine, Cystine and Tyrosine were noticed. A total number of five essential macro minerals composition and two trace metals were recorded. In the macro-mineral composition, the calcium was recorded in maximum percentage from the marine solid waste. The present study in utilization of waste into higher value-added products will be immense source of fishmeal and fish oil in solving the upcoming demands.
Keywords: Essential amino acids (EAA), Fish Species, Freshwater Fish Waste

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INTRODUCTION

Fish oil is a resourceful product of many applications in the food and technical products all over the world. Fish oil is the lipid fraction removed from fish and fish-by-products. It has significant constituent of pharmaceuticals, industries and animal feeds; contributing with essential fatty acids needed for normal growth, health and reproduction. Fish oil is also rich resource of omega 3 fatty acids, which represent over 30% of the total fatty acids present. India is producing considerable quantity of fishmeal by several small and medium industries. There are essential amino and fatty acids that are present in fish meal but not present in tissue from terrestrial plants or animals and has high ash content especially when made mainly from fish bones [1].

Recent studies have identified a number of bioactive compounds such as fish muscle protein, collagen and gelatine, fish oil, fish bone, internal organs and crustacean shells from marine by-products [2].These wastes have high content of nutritive compounds like protein which is the substrate of fishmeal production [3]. Several methods have been proposed for extraction of fish oil from experiment to commercial production levels. Following the detail literature review of oil extraction from fish waste, present study has adapted three different methods of extraction and four phase purification processes.

MATERIAL AND METHODS

The study samples were collected from fish landing centres and processing fish waste at Parangipetai and Cuddalore. Simultaneously, the freshwater fish wastes were also collected from fish market. The samples were divided and stored in deep freezer at -20°C for further analysis. For the crude fish oil extraction from marine and freshwater samples three different extraction procedures such as Direct streaming method, Bligh & Dryer and Folch method were adopted [4-6]. The extracted crude fish oils were purified by Degumming, Neutralisation, Bleaching and Deodorisation processes [7-10].

In the present study, the oil extraction process of direct streaming and Bligh & Dyer have produced maximum yield of fish-oil and adequate quantity of remaining fish solids. Therefore, the solid product wastes of above two methods were taken for nutritional analysis. The total protein, carbohydrate, lipid, ash, moisture composition, mineral, amino acids and fatty acids content were determined by the eminence procedures. [10-14]

RESULT AND DISCUSSION

The crude fish oils were extracted from marine and freshwater waste fish products by using three different methods vis, Direct Streaming, Bligh & Dyer and Folch method. Among the three methods, higher yield was obtained from the samples of freshwater fish waste using the Direct Steaming (DS) method. The maximum yield of 1000g freshwater fish waste noticed 100.6 ± 0.9 ml of crude fish oil by DS method. The high yield of marine water fish waste production of 92.7±0.8ml was recorded in similar DS method (Table.1).

Earlier studies on extraction of oil from the *R. kanagurta* was evident that 100 ml of crude fish oil was registered from 1000 g of fresh fish by direct steaming process [15]. The oil yield from liver of trash fish *Odonus niger* was assessed by four different methods: Soxhlet, Bligh and Dyer, direct steaming and solar extraction. The percentage of oil yielded by Soxhlet method was noticed the maximum (67.78%), followed by direct steaming (42.58%), Bligh and Dyer (54.3%) and solar extraction process (32.08%) [4].

Further, the application of three different oil extraction methods i.e., wet reduction, acetone and Bligh and Dryer for seafood processing waste. The yield of fish oil obtained (1.6%) was found to be highest with chloroform/methanol/water (2:4:1) [16]. The mechanical devise for extraction from various fish waste yield was noticed about 11% of total weight of fish-wastes [17].

The oil extraction of freshwater tropical fish, *Monopterus albus* head and body parts were studied adapting the method of Bligh and Dryer. The results showed that the lipid content varied between 0.5 and 1.06 g / 100 g body tissues, and between 0.40 and 0.78 g / 100 g of head tissue. The major fatty acids share of palmitic, oleic, arachidonic and docosahexaenoic acids were identified [18]. The comparative assessment of fish oil extraction from three methods (Soxhlet, Bligh & Dryer and Pressing method) have been applied in *Clarias macrocephalus* fish. The Soxhlet method showed the highest yield of lipid (36.71%) content, followed by Bligh and Dryer (26%) and pressing method (17%). The Bligh and Dryer method delivered the lipid content with 92.7% purity [19]. In general, *Clarias spp.* have rich fat depositions in their body potions of abdomen and head sections.

The estimation of oil production qualitative properties in four size groups of *Sardinella longiceps* were examined by direct steaming method [20]. The size groups included Group I (size range of 7.1–10.0 cm), Group II (size range of 10.1–13.0 cm), Group III (size range of 13.1–16.0 cm) and Group IV (size range of 16.1–19.0 cm). The four groups showed different yield of fish oil quantities. The group IV recorded the highest values of 165.00 ± 1.00 ml/kg followed by group III (145.66 ± 1.15 ml/kg), group II (129.33 ± 0.58 m/kg), whereas group I. The lowest values of 78.33 ± 0.58 ml/ kg, and the average yield was recorded (180.0 ± 4.9) ml/kg fish tissues.

The fish oil investigation on production of fresh and marine water fish oil were carried out using Soxhlet apparatus and n-Hexane as the solvent method. Five different species (*Mormyrops deliciousus, Bagrus docmac niger, Tilapia dagati, Clarias anguilloris, Titus*) were used for the extraction experiment. The result showed that the freshwater fish *Mormyrups deliciousus* and marine fish *Titus* have an adequate percentage of oil content of about 30.22% and 24.02% respectively, while *Clarias anguilloris*, *Tilapia dagati* and *Bagrus docmac niger* fishes noticed in poor harvest of 6.72, 14.52 and 17.92, respectively [21].

Fish oil yield of various species were measured by using a solvent system (chemical assay) with appropriate techniques. The obtained results showed that the overall percentage yield of the oil edible muscle portion was 3.23±0.41 g/100g in wet tissue. The maximum yield of oil content (4.27±0.25%) was registered in *Labeobarbus spp*. followed by *Clarius gariepinus* (3.37±0.32%) and *Oreochromis niloticus* (2.93±0.35)[22]. The Bligh and Dyer (B&D) method is considered as one of the best for lipid extraction from fish and serves as a benchmark for comparison between other solvent removal methods [23-25]. The electrolysed cathode water method of extraction also proved that the yield in better lipid quantities

than Bligh and Dyer method [26]. Bligh and Dyer method provides higher yields only in the abundant presence of polar lipids [27].

The oil extraction study, 1000g of marine fish waste sample produced at 92.7 ± 0.8 ml, 75.6 ± 0.8 ml and 33.3 ± 1.2 ml of crude oil by Direct Steaming (DS), Bligh & Dyer (B&D) and Folch methods respectively (Fig.1).Similarly,1000g of freshwater fish waste sample produced at 100.6±0.9 ml, 81.3±0.8ml and 42.7±2.5ml of crude fish oil by Direct Steaming (DS), Bligh & Dyer (B&D) and Folch methods respectively . Comparatively, the maximum yield of 1000g freshwater fish waste noticed 100.6±0.9ml, of crude fish oil by DS method. The minimum of 42.7±2.5 was recorded in Folch method. The high yield of marine water fish waste production of 92.7 ± 0.8ml was recorded in DS method. The minimum of 33.3 ± 1.2 oil production was recorded in Folch method from marine fish waste.

In the present study, the percentage of oils on crude oil removed as impurity in each step was calculated as 17.9%, 11.3%, 2.3%, and 8.3% in degumming, neutralization process bleaching and deodorization process respectively. In general, the total recovery of oil from the initial to final stage of purification process was 90.05 % of refined oil was obtained from 100 ml of crude freshwater fish oils (Table. 2).The percentage of marine fish waste loss in crude oil removed as impurities on each step of purification was calculated. The impurities were calculated in maximum of 18.6% was noticed process in the degumming process, followed by neutralization (12.3%) and deodorization (11.3%). The bleaching procedure recorded the minimum of 3.3%. The total recovery of oil from the initial to final stage of purification process was recorded in 88.63 % of refined oil was obtained from 100 ml crude fish oil (Table.2).

The purification technique of *Sardinella fimbriata* fish quantity of fish oil produced after each step of purification, namely degumming, neutralization, bleaching and deodourization were worked out individually [28]. The crude fish oil of 100 ml was subjected to degumming produced 83 ± 0.5 ml, 100ml of degummed oil subjected to neutralization produced 87 ± 0.5 ml, 100ml of neutralized oil subjected to bleaching produced 95 ± 1.0 ml oil and from 100ml bleached oil subjected to deodourization yielded 82 ± 0.5 ml of purified oil. During the process of purification, the high average weight loss of oil was observed during the degumming process that resulted in a loss of 18.94% followed by 15.79% neutralization, 10.69% deodourization and 14.99% in bleaching respectively.

The purification of 100 ml of crude fish oil was reduced to 90.098 g after deodourization process have collected fish oil soap stock from discarded fish products[29]. The crude fish oil was further pre-treated to remove impurities including fish residue, water and saline compound to obtain 85 wt. % refined fish oils. The weight of the oil progressively reduced as it is descending through various steps of purification, which might be due to the periodic elimination of FFA's and other impurities. The reduction in weight of the oil at a constant volume during various refining process was primarily due to the removal of impurities, minerals, other suspended solids, etc., in each step. Crude fish oil is expected to contain certain amount of minerals among which phospholipids plays a major quantum [30].

The proximate composition of remaining solid fish waste of Bligh & Dyer and Direct streaming method estimation showed narrow and wide range of variations. The maximum of 47.33±0.02 was noticed in Direct streaming method from marine solid waste. The fat values were showed least concentrations from all four solid wastes. The carbohydrate moisture and ash contents were noticed at moderate levels of percentages between the methods and samples (Table.3).In the present study, the samples of marine and freshwater fish solid wastes were determined the essential amino acids and non essential amino acids. Essential amino acids (EAA) of Aspartic acid, Glutamic acid, Asparagine, Serine, Gultamine, Glycine, Alanine, Cystine and Tyrosine were noticed. The total of EAA were recorded in 20.81 ± 0.16, 27.87 ± 0.15, 3.45 ± 0.08 and 6.09 ± 0.13 in samples of B&D and DS method solid sample of marine and freshwater fish. Among the EAA, Glutamic acid (8.25±0.02 %, 10.13±0.02 %) and Glycine (1.00±0.02 %, 1.19±0.01 %) were major components in marine and freshwater fish solid waste. Several components like Cystine, Glutamic acid, Gultamine and Alanine were not found in B&D method solid waste samples.Among the total non-essential amino acids (NEAA) of marine and freshwater fish solid wasteswere found to be as 16.97 ± 0.17 %, 29.35 ± 0.14 % and 4.35 ± 0.11 %, 8.22 ± 0.13 % in processed B&D and DS method solid waste respectively. The NEAA, Arginine (3.25 ± 0.01 %, 4.02 ± 0.01 %) and Proline (0.84±0.01 %) and Iso-leucine (1.07±0.01 %) as major components in marine and freshwater fish solid waste. However, the components of Histidine and Tryptophan were not identified in the NEAA estimation of freshwater solid waste (Table. 4).

A total number of eight different fatty acids were recorded from both marine and freshwater fish sample. It includes Myristic acid, Palmitic acid, Stearic acid, Palmitoleic acid, Octadecenoic acid, Linolenic acid, Alpha Linolenic acid and Docosahexaenoic acid (DHA). Among the fatty acid groups, three saturated fatty acids (SFA), two monounsaturated fatty acids (MUFA) and six polyunsaturated fatty acids (PUFA) were identified. In SFAs, major acids of Palmitic acid (C16:0) was found in 0.93 ± 0.02 % and 0.97 ± 0.02 % in the percentages marine and freshwater samples respectively produced through, DS method. In PUFA,

alpha linolenic was found in major acid 0.64 ± 0.02 % in freshwater solid waste from DS method (Table. 5).The total concentration of fatty acids group wise results were showed the moderate variation between the fatty acid groups of, marine and freshwater fish solid waste sample. The SFA, MUFA and PUFA contents were recorded 0.94±0.05 % 2.41±0.06 %, and 1.35±0.04 %, 2.41±0.05 %, 0.67±0.03 %,1.11±0.03 %, 0.57±0.02 %, 0.81±0.04 % and 0.29±0.02 %, 0.91±0.01 %, 0.65±0.02 %, 1.11±0.04 % in B&D and DS method respectively(Table. 5).

The mineral composition of remaining waste sample from B&D and DS method solid wastes of marine and freshwater fish were measured (Table. 6). A total number of five essential macro minerals composition and two trace metals were studied. In the macro-mineral composition the calcium was recorded in maximum percentage from the marine solid waste. The maximum quantity of calcium 489.3mg and 667.4 mg was recorded from marine solid wastes of BD and Ds methods respectively. The phosphorus content was registered next to the calcium with the range between 61.26 and 193.6 mg from the freshwater and marine samples respectively. The other mineral such as sodium, magnesium and potassium rests were exhibited at acceptable levels. The trace minerals exhibited the Iron (4.10 mg 5.71 mg, and 10.93mg, 13.03mg) and zinc (1.02 mg, 0.60 mg, 0.65mg, 0.54 mg) from marine and freshwater solid waste.

The proximate composition analysis of solid waste product by fish oil extraction process of Direct streaming and Bligh & Dyer method showed. The levels of protein in marine and freshwater fish solid waste showed the results of 45.31 and 42.71 respectively. In addition, the samples of marine and freshwater fish solid wastes were determined satisfactory levels of essential amino acids, fatty acids and minerals which are require for superior animal feed formulation. In the present study, the samples of marine and freshwater fish solid were documented all necessary essential amino acids. The essential amino acids (EAA) of Aspartic acid, Glutamic acid, Asparagine, Serine, Gultamine, Glycine, Alanine, Cystine and Tyrosine were noticed at acceptable levels, which are needed for commercial fishmeal. Several components like Cystine, Glutamic acid, Gultamine and Alanine were not found in B&D method solid waste samples

Fishmeal is the preferred animal protein supplement in the diets of farm animals and often the major source of protein in diets for fish and shrimp. Amino acids that cannot be synthesized by the animal, and therefore must be supplied in the diet, are classified as “essential”. The proximate compositions of commercial fishmeal produced from different species proximate study have revealed that the maximum proportion of crude protein in 60% followed by fat (29.5%) and ash content (28.2%) [31].

The protein composition of commercial fish samples (Herring, Capelin, Blue whiting) in different regions of Iran local fish market recorded that the mean values of protein content in Herring fish ranged from 70.5% to 73.1% and in blue Whiting and Capelin from 68.0% to 70.8% and 69.6% to 72.7% correspondingly. The fat content in herring meal ranged from 8.0 to 10.5 and capelin and blue whiting from 11.6 to 12.2 and 6.7 to 8.3 respectively [32].The nutritional parameters of three marine dry fishmeal such as *Har-podon nehereus, Johnius dussumieri* and *Lepturacanthus savala* study results described that the protein (6.35, 7.93 and 4.68), lipid (1.92, 0.67 and 1.13) and carbohydrate (1.70, 1.81 and 0.66) mean percentages respectively [33]. The amino acid profile of sardine commercial fishmeal from fish-processing waste in Alaska and the study indicated that the variation in quality of amino acids occurrences[34].The nutrient compositions of feed ingredients of industrial fishmeal and smoked fish waste meal showed the crude protein of 72.26 and 68.07 respectively [35].

Name of the Method	Marine fish waste (ml/kg)	Freshwater fish waste (ml/kg)
Direct streaming	92.7 ± 0.8	100.6 ± 0.9
Bligh & Dyer	75.6 ± 0.8	81.3 ± 0.8
Folch method	33.3 ± 1.2	42.7 ± 2.5

Table. 1. Extraction methods and levels of crude fish oil from marine and freshwater fish wastes.

Purification process	Crude Oil used (ml)	Purified fish oil obtained (ml)	
		Marine crude oil	Freshwater crude oil
Degumming	100	81.4±1.3	82.1±1.7
Neutralization	100	87.7±1.7	88.7±2. 5
Bleaching	100	96.7±0.9	97.7±0.5
Deodorization	100	89.7±1.3	91.7±1.3

Table. 2. purification methods and volume of fish oil from marine and freshwater fish waste.

Proximate	Marine fish soiled waste (%/g)		Freshwater fish soiled waste (%/g)	
	Bligh & Dyer	Direct streaming	Bligh & Dyer	Direct streaming
Protein	45.31±0.01	47.33±0.02	41.61±0.01	42.71.71±0.01
Fat	0.34±0.01	0.76±0.02	0.26±0.01	0.50±0.02
Carbohydrate	1.01±0.02	0.46±0.02	0.87±0.01	1.01±0.01
Moisture	1.46±0.01	2.11±0.02	0.98±0.01	1.67±0.01
Ash	6.36±0.02	7.58±0.01	14.47±0.01	12.69±0.02

Table. 3. show the proximate composition of marine and freshwater fish soiled wastes

Essential Amino Acids	Marine fish soiled waste(%/g)		Freshwater fish soiled waste(%/g)	
	Bligh & Dyer	Direct streaming	Bligh &Dyer	Direct streaming
Aspartic acid	5.05±0.02	7.15±0.02	0.25±0.01	1.01±0.02
Glutamic acid	8.25±0.02	10.13±0.02	-	0.51±0.01
Asparagine	0.24±0.02	0.97±0.01	0.68±0.01	0.81±0.01
Serine	0.71±0.02	1.03±0.02	0.50±0.01	0.63±0.02
Gultamine	0.78±0.01	1.02±0.01	-	0.48±0.01
Glycine	1.15±0.03	1.23±0.02	1.00±0.02	1.19±0.01
Alanine	3.01±0.02	3.71±0.02	-	0.14±0.02
Cystine	-	0.62±0.02	0.22±0.02	0.31±0.02
Tyrosine	1.62±0.02	2.00±0.02	0.80±0.01	1.02±0.02
Total	20.81±0.16	27.87±0.15	3.45±0.08	6.09±0.13
Non- Essential Amino acids				
Threonine	2.15±0.02	2.97±0.01	0.24±0.02	0.68±0.01
Arginine	3.25±0.01	4.02±0.01	0.21±0.02	0.42±0.02
Histidine	0.89±0.01	1.01±0.01	-	0.31±0.01
Valine	1.11±0.02	3.01±0.02	0.31±0.01	0.88±0.01
Methionine	0.23±0.02	0.93±0.02	0.25±0.01	0. 61±0.01
Iso-leucine	1.15±0.02	2.78±0.01	0.70±0.01	1.07±0.01
Phenyl alanine	1.05±0.02	1.91±0.01	0. 53±0.01	0.90±0.02
Leucine	2.77±0.01	4.07±0.01	0.47±0.01	0.92±0.01
Lysine	2.99±0.01	6.69±0.01	0.79±0.01	1.03±0.01
Proline	1.00±0.01	1.24±0.02	0.84±0.01	1.01±0.01
Tryptophan	0.38±0.02	0.71±0.01	-	0.39±0.02
Total	16.97±0.17	29.35±0.14	4.35±0.11	8.22±0.13

Table. 4. Essential and non-essential amino acids of marine and freshwater fish soiled wastes

Fatty acids	Carbon atom (n)	Marine fish soiled waste (%/g)		Freshwater fish soiled waste (%/g)	
		Bligh & Dyer	Direct streaming	Bligh & Dyer	Direct streaming
Saturated fatty acids(SFA)					
Myristic acid	C14:0	0.27±0.01	0.69±0.02	0.60±0.01	0.76±0.02
Palmitic acid	C16:0	0.40±0.03	0.93±0.02	0.49±0.01	0.97±0.02
Stearic acid	C18:0	0.27±0.01	0.79±0.02	0.26±0.02	0.68±0.01
∑ SFA		0.94±0.05	2.41±0.06	1.35±0.04	2.41±0.05
Mono Unsaturated fatty acids (MUFA)					
Palmitoleic acid	C16:1 n-6	0.40±0.01	0.79±0.01	0. 57±0.02	0.81±0.02
Octadecenoic acid	C18: n-9	0.27±0.02	0.32±0.02	-	-
∑ MUFA		0.67±0.03	1.11±0.03	0.57±0.02	0.81±0.04
Polyunsaturated fatty acids(PUFA)					
Linolenic acid	C18:2 n-3	-	-	0.38±0.01	0.64±0.02
Alpha Linolenic acid	C18:3 n-6	-	-	0.27±0.01	0.37±0.02
Docosahexaenoic acid	C22:6 n-3	0.29±0.02	0.91±0.01	-	-
∑ PUFA		**0.29±0.02**	**0.91±0.01**	**0.65±0.02**	**1.11±0.04**

Table.5. shows the fatty acid composition of marine and freshwater fish soiled waste

Minerals	Marine fish soiled waste (mg/g)		Freshwater fish soiled waste (mg/g)	
	Bligh&Dyer	Direct streaming	Bligh&Dyer	Direct streaming
Calcium	489.3	567.4	98.83	180.33
Sodium	34.4	25.7	25.93	17.83
Magnesium	12.4	12.4	8.43	9.33
Potassium	156.7	120.7	11.11	30.14
Phosphorus	193.6	245.8	61.24	101.02
Trace metals				
Iron	4.10	5.71	10.93	13.03
Zinc	1.02	0.60	0.65	0.54

Table. 6. Shows the results of mineral content in marine and freshwater fish soiled waste

CONCLUSION

The oil extraction process of direct streaming and Bligh & Dyer have produced maximum yield of fish-oil and adequate quantity of remaining quality solid by products. The proximate compositions of solid waste samples both marine and freshwater samples have determined satisfactory levels of essential amino acids, fatty acids and minerals which are essentially required for animal feed formulation. Realizing the source of the discarded waste fish, the present study of utilization the waste in producing higher value-added products which will be the immense source of fishmeal and fish oil in solving the upcoming demands.

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