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RESEARCH ARTICLE

Extraction and Evaluation of Fish Body Oil from Lesser Sardines Employing Different Extraction Methods

M. Pravinkumar, L. Xavier Eugien, C. Viswanathan, G. Ponnusamy and S. M. Raffi*

Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai - 608 502, Tamilnadu, India

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ABSTRACT

To study the extraction of fish oil from the tissues of *S. fimbriata, S. gibbosa* and *S. albella* employing four different extraction methods, Bligh & Dyer, Modified Bligh & Dyer, Mcgill & Moffat and Direct Steaming. 1000g of fish tissues of S. fimbriata produced 91±3.7 ml, 94±3.2 ml, 80±2.5 ml and 116± 6.1 ml of crude fish oil by Bligh & Dyer (B&D), Modified Bligh & Dyer (MB&D), Mcgill & Moffat (M&M) and Direct Steaming (DS) methods respectively. An average of 1000g of *S. gibbosa* fish tissues produced 78±4 ml, 75±2.1 ml, 67±1.5 ml and 93±4.3 ml of crude fish oil by B&D, MB&D, M&M and DS methods respectively. An average of 1000g *S. albella* fish tissues produced 63±1.5 ml, 65±1.5 ml, 53±1.6 ml and 84±3.5 ml of crude fish oil by B&D, MB&D, M&M and DS methods respectively. Among the four methods, higher yield were obtained from samples of *S. fimbriata* followed by *S. gibbosa* and *S. albella*.

Key words: Fish oil, *S. fimbriata, S. gibbosa, S. albella* and Different methods.

1. INTRODUCTION

Fish oil is an excellent dietary sources, rich in essential fatty acids, especially Polyunsaturated Fatty Acid (PUFA) in the form of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) [1]. Essential Fatty Acids (EFAs) are those which are not synthesized in human body, namely ω-3 (n-3) and ω-6 (n-6). Some fishes such as herring, mackerel, salmon, sardines and tuna have a fairly good quantity of these compounds [2]. Production of fish oil from low value fishes has gained increased momentum in recent past because of its wider application prospects. Fish oil when compared to terrestrial animal and vegetable oil, is characterised by a complex nature of saturated, unsaturated and polyunsaturated fatty acids [3,4]. Production of high and pure grade fish oil acquired greater importance as it is considered as one of the main natural repository of omega-3 polyunsaturated fatty acids (PUFAs); which provides tremendous benefits to human health [5].

The production of the fish oil deals with the separation of lipids from other constituents of the fish. Fish oil is produced by several methods, including physical fractionation [6], low temperature solvent fractionation [7] and supercritical fluid extraction [8]. Various processing methods have been adopted for the extraction of fish oil from the liver and whole body, such as Soxhlet method, Bligh and Dyer method [9], direct steaming method, solar extraction method [10,3] and Mc Gill and Moffat method [11].

The lesser sardine fishery along Indian coast is represented by 10 species among which seven species formed the bulk of its fishery, the remaining occurs sporadically and in stray numbers at certain fish landing centres [12]. Among this group, *Sardinella fimbriata, Sardinella gibbosa* and *Sardinella albella* are arrived in significant proportions than that of other species of lesser sardines. Commercial level production of fish oil are largely confined to economically important fishes like cod fishes and little effort has been directed towards extraction of fish oil from low value fishes such as lesser sardines. Extensive studies were carried on the importance of fish oil in health point of view. Little work has been done pertain to the extraction of fish oil from low value fishes. In this backdrop the present study was planned to extract fish body oil from low value fishes such as lesser sardines,

***Corresponding Author:** S. M. Raffi, **Email:** raffi_cas@yahoo.co.in, **Phone No:** +91-9443880121

namely, *Sardinella fimbriata, Sardinella gibbosa* and *Sardinella albella* employing four different extraction methods so as to check the yield of fish oil obtained between the species.

2. MATERIALS AND METHODS

Lesser sardines such as *S. fimbriata, S. gibbosa* and *S. albella* were collected from fish landings of Muttom, Kanyakumari district, Tamil Nadu, southwest coast of India (lat. $8^0$7' N; long. $77^0$19'E) for a period of one year (October 2011 - September 2012). Since these species are landed only during certain corners of the year, specimens of uniform size (at maximum length) from all three species were alone taken into consideration for the production of fish oil. The fishes were identified with the help of FAO (Fish and Agricultural Organisation) Species Identification Guide for Fishery Purposes.

Extraction of Oil

The fishes were washed thoroughly in running water for the removal of sand and external debris. Scales, head, fins, spines, digestive system and excretory system were removed and the tissues alone were taken for extraction of oil. The tissues were subjected for extraction of oil by different methods as follows (a) Bligh and Dyer method [9], (b) Modified Bligh and Dyer method **(**i.e 1:1.5 V/V methanol:chloroform**)**, (c) McGill and Moffat method [11] and **(**d**)** Direct steaming method.

Bligh and Dyer Method

100g of homogenised fish tissues were weighed into beaker (Capacity 1 litre) to this 10ml of distilled water was added and mixed. Methanol:chloroform was added at the ratio of 1:2V/V and the mixture was thoroughly homogenized. The mixture was centrifuged at 2000rpm for 20 minutes at room temperature. The resultant aqueous layer was removed with the help of separating funnel. The chloroform fraction was evaporated using Rotatory evaporator and finally the yield of obtained oil was recorded [9].

Modified Bligh and Dyer Method

Bligh and Dyer Method was modified and carried out for oil extraction, i.e 1:1.5 V/V Methanol: chloroform was added, mixed and the above mentioned procedure was repeated and the yield was recorded.

McGill and Moffat Method

30g of anhydrous sodium sulphate was added to 100g of homogenized fish tissues. The mixture was homogenized for 3 minutes and centrifuged at 2000rpm for 20 minutes at room temperature. The resulting oil was separated from the aqueous layer using separating funnel and the yield was recorded [11].

Direct Steaming method

About 1000 g of homogenized fish tissues was taken in a muslin bag and kept in steam boiler (Sakthi Instrument) at 70-80 °C for 30 minutes. The boiled fish tissues were then pressed with the aid of Fish Oil Extractor (designed in our laboratory and about to be patented), so as to remove the liquid content from the tissues (containing oil and water). Then the oil was separated from the water by centrifuging at 2000 rpm (REMI, C 24BL Cooling Centrifuge) for 15 minutes and further by using separating funnel. The filtered oil was stored separately in an opaque dark bottle and placed in deep freezer at -20 °C. These was repeated for 5 times and the average yield was calculated, which is expressed in percentage. The filtered oil was stored separately in opaque dark bottle and placed in deep freezer at -20°C.

Quality Assessment of Fish Oil

The oil was subjected for the determination of specific gravity by the method outlined [3], Refractive index by Hollow prism method, moisture content by ISI method, Free Fatty Acids (FFA) [13], iodine value (IV) [14], peroxide value (PV) [13], saponification value (SV) [14], The colour of the purified fish oil was observed by placing against luminescent light.

3. RESULTS

Yield of fish oil extracted from *Sardinella fimbriata, Sardinella gibbosa* and *Sardinella albella* employing various extraction methods

The fish body oil was extracted from the tissues of *S. fimbriata, S. gibbosa* and *S. albella* employing four different extraction methods, namely Bligh & Dyer, Modified Bligh & Dyer, Mcgill & Moffat and Direct Steaming. Between fish species, there was significant difference in the yield of fish oil. Among the four methods, higher yield were obtained from samples of *S. fimbriata*, followed by *S. gibbosa* and *S. albella*. This disparity was largely due to the difference in proximate composition between fish species. 1000g of fish tissues of *S. fimbriata* produced 91±3.7 ml, 94±3.2 ml, 80±2.5 ml and 116± 6.1 ml of crude fish oil by Bligh & Dyer (B&D), Modified Bligh & Dyer (MB&D), Mcgill & Moffat (M&M) and Direct Steaming (DS) methods respectively (**Fig 1**). An average of 1000g of *S. gibbosa* fish tissues produced 78±4 ml, 75±2.1 ml, 67±1.5 ml and 93±4.3 ml of crude fish oil by B&D, MB&D,

M&M and DS methods respectively (**Fig 2**). An average of 1000g *S. albella* fish tissues produced 63±1.5 ml, 65±1.5 ml, 53±1.6 ml and 84±3.5 ml of crude fish oil by B&D, MB&D, M&M and DS methods respectively (**Fig 3**). The average yield of oil extracted using different extraction techniques are presented in (**Table 1**). Analysis of variance (two way) showed significant variation between the species and various methods (**Table 2**). In general, the yields of fish oil obtained from tissues of different species of lesser sardines among all the methods are expressed in the following order of descend as follows:

Sardinella fimbriata > *Sardinella gibbosa* > *Sardinella albella*

Among the different extraction procedures, the highest yield was obtained in Direct Steaming in all the three species. In the case of *S. fimbriata* and *S. gibbosa* the yield was placed in the order of descend as follows:

Direct Steaming > Bligh & Dyer > Modified Bligh & Dyer > Mcgill & Moffat

Whereas, in the case of *S. albella* the yield was placed in the order of descend as

Direct Steaming > Modified Bligh & Dyer > Bligh & Dyer > Mcgill & Moffat

Since higher yield of fish oil was obtained in *S. fimbriata* than that of other species; the qualitative analysis of *S. fimbriata* was alone recorded here so as to avoid redundancy . All the analytical values are well within the acceptable standard values for all the methods. The moisture content values (0.831± 0.12) and free fatty acid value (1.56 ± 0.16) in modified Bligh and Dyer method were found to be superior to that of other methods. It is important to note that the results of various analytical parameters did not exhibit profound variation between the methods.

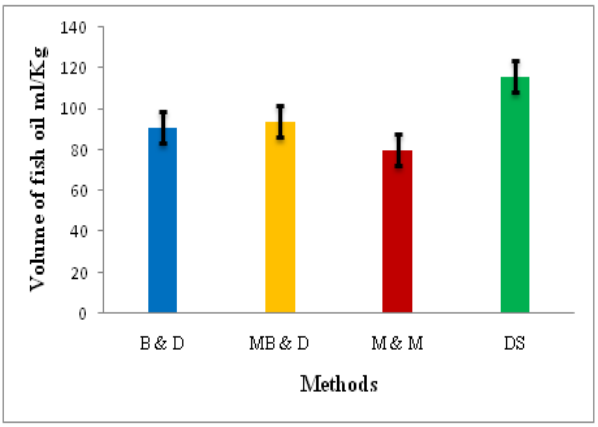

Fig 1: Yield of fish oil obtained from *S. fimbriata* employing different extraction methods

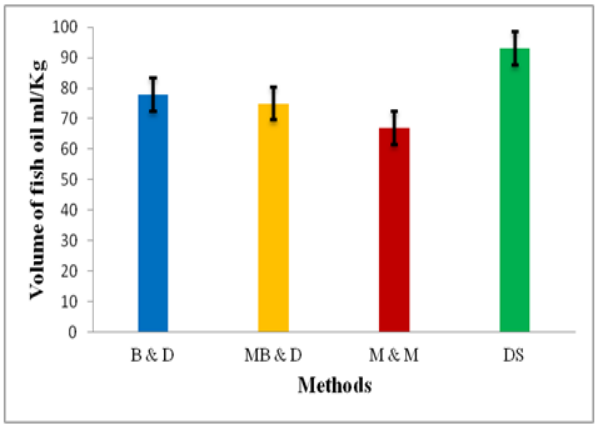

Fig 2: Yield of fish oil obtained from *S. gibbosa* employing different extraction methods

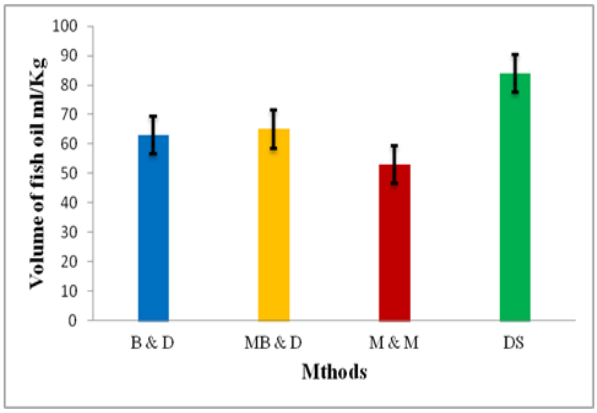

Fig 3: Yield of fish oil obtained from *S. albella* employing different extraction methods

Table 1: Average yield of fish oil produced by various methods in lesser sardines, *S. fimbriata, S. gibbosa and S. albella*

Species	Bligh & Dyer	Modified Bligh & Dyer	Mcgill & Moffet	Direct Steaming
S. fimbriata	91 ± 3.7	94 ± 3.2	80 ± 2.5	116 ± 6.1
S. gibbosa	78 ± 4	75 ± 2.1	67 ± 1.5	93 ± 4.3
S. albella	63 ± 1.5	65 ± 1.5	53 ± 1.6	84 ± 3.5

Table 2: Analysis of Variance (Two-Way ANOVA) in relation to oil extracted between species and different extraction methods

Source of variation	SS	df	MS	F	P-value	F-crit
Between species	1698.667	2	849.3333	136.5	9.9458	5.1432
Between methods	1502.917	3	500.9722	80.5133	3.0866	4.7570
Total	3238.917					

4. DISCUSSION

During the last two decades polyunsaturated fatty acids (PUFA) imposed greater interest among scientists due to its winsome medicinal and nutritional properties. Fish oil is being approved for human consumption as an important nutraceutical and chief ingredient in human diet. This warrants a great demand for fish oil round the world. Marine fishes, especially Clupeoid fishes serves as the major repository of fish oil. The yield of oil recovered from body and liver may vary from species to species and also in different fishing areas [15].

Several hygienic and scientific measures were employed, so as to improve the quality of fish oil,

in conventional meal plants and in other commercial processes, where fish oil is a byproduct. Great improvements has been achieved and are being made in maintaining the condition of raw materials and primary products so that fish oil does not have any major oxidation problems prior to extraction and refining, ensures safe for human and animal consumption. Although both extraction and refining technology is advancing rapidly, most of the fish oil being produced around the world is a byproduct of the conventional fish meal process, a wet rendering technique. Other processes of fish oil extraction are hydrolysis, silage production, solvent extraction, critical extraction and ion exchange.

In the present study, profound variation was recorded in the yield of fish oil produced from three different species, namely, *S. fimbriata, S. gibbosa* and *S. albella* employing four different extraction methods, namely Bligh & Dyer, Modified Bligh & Dyer, Mcgill & Moffat and Direct Steaming. There was significant difference in the yield of oil extracted in such a way that *S. fimbriata* samples produced higher yields from all four extraction methods than that of *S. gibbosa* and *S. albella* samples. The dissimilarity in the yield between species was mainly due to variation in texture and proximate composition coupled with other factors such as gender and age, location; species origin characteristics such as spawning and migration seasons, seasonal variation in composition of plankton and also some environmental conditions such as temperature [16-19]. The yield of oil recovered from body and liver may vary from species to species and also in different fishing areas [15].

The Bligh and Dyer method, using chloroform & methanol, is generally considered to be the best method for polar lipid extraction. A minor modification were employed in Bligh and Dyer by altering the ratio of the solvents and is termed as Modified Bligh and Dyer method, was also commonly used to evaluate the difference in the lipid level [20-22]. Mcgill and Moffat method for extraction lipids and triglycerides is also a popularly used technique for extraction of body oil [11]. Direct steaming is considered as a good old traditional and economic technique for extraction of fish oil. The present study was undertaken to analyse the proficiency in yield between the above said methods, proved that oil extracted by Direct Steaming ensured higher yield than that of other methods. The present experiment supports the suggestions of [23] that oil extraction by steaming is easier, cheaper, quicker and is affordable to laymen and rural communities. It has been reported that solvent extraction methods are not employed for the preparation of oil from fish, because the equipment itself is expensive and the recovery of the solvent is not satisfactory [24]. The direct steaming at 80-85ºC is a simple and economical technique that ensures viable results [25]. From the results of the present study it is concluded that the conventional method of extraction (direct steaming method) is considered as the finest extraction process due to its winsome qualities such as higher yield, economic viability, less laborious and less time consumption etc. Other methods of extraction are comparatively more time consuming, laborious, costly and are critical due to the off odour and flavour of solvents.

Bligh & Dyer method was so far reported as the effective method for extraction of lipids [26] and in the present study, the yield from Bligh and Dyer method is 91±3.7 ml/Kg from *S. fimbriata*, 78± 4 ml/Kg from *S. gibbosa* and 63±1.5 ml/Kg from *S. albella.* Whereas, Direct Steaming method gave better results than the other three methods with an yield of 116±6.1 ml/Kg from *S. fimbriata,* 93±4.3 ml/Kg from *S. gibbosa* and 84±3.5 ml/Kg from *S. albella.* In the present study the oil was prepared from the fish muscles, in which the breakdown of cell wall requires only less environmental shock which was easily afforded by direct steaming. Steaming will coagulate the protein of fish, so that liquids and solids can be mechanically separated and fat cells are also disrupted, releasing oil into the liquid phase [27].

Bligh and Dyer method and other chemical extraction methods will definitely give higher yield than the conventional methods, when fish liver was chosen, since rupture of the liver cells requires more shocks, where chemical methods may be the better choice [3]. There are evidences which supports our study proving that there also various methods which will yield in better lipid quantities other than Bligh and Dyer method like electrolysed cathode water method [28]. Bligh and Dyer method provides higher yields only in the abundant presence of polar lipids [29,30]. Since the yield of Bligh and Dyer method is not high in the present study, which provides the information of the presence of low polar lipids in all the selected species of fishes. Described [31] a modified [32], using chloroform: methanol (2:1,v/v) solvent system, similarly Modified Bligh and Dyer method was carried out in triplicate by using

methanol: chloroform (1:1.5, v/v) solvent system to extract lipids that resulted in better quantities of yield than the standard method due to the fact of presence of more non polar lipids. Mcgill and Moffat method resulted in lowest quantities of yield from all the three fishes since it is highly specific for extraction of lipids from fish liver, especially for triglycerides [33]. The oil extracted from *S. fimbriata* in the present study, which was almost double the quantity of oil extracted from *S. lemuru* [33] in Malaysian waters. The body oil extracted from *S. fimbriata* gave higher yield than the other two lesser sardine species.

The present study concludes with the fact that among lesser sardine of Kanyakumari waters, *S. fimbriata* produced higher yield of fish oil than *S. gibbosa* and *S. albella.* The results unravel the fact that Direct Steaming proved to be an efficient method than that of other methods. The results stand as baseline reference about extraction of fish body oil from lesser sardines employing different extraction methods.

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