Amino acid and fatty acid profile of whole-dried and minced-dried fish discards (*Leiognathus* **sp.) from shrimp trawl fishery in Jaffna, Sri Lanka**

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Abstract – The by-catch and discards from shrimp trawl fishery have increased during the last few years. *Leiognathus* sp. is one of the most discarded fish species in the shrimp trawling fishery in Jaffna, Sri Lanka. Lack of information was found regarding the nutrient composition of *Leiognathus* sp. and therefore the present study was carried out to assess the presence of amino acids and fatty acids in whole-dried and mince-dried fish which are beneficial for human health. The total sum of amino acids was estimated to be 229.8 and 174.4 mg/g raw protein percentages in whole-dried fish and minceddried fish, respectively. The protein content of fish is mainly explained by the sum of the total amino acids identified. The total lipid was 5.5% and 7.3% in whole-dried fish and minced-dried fish, respectively. Proline and glycine were found to be the abundant amino acids in both whole-dried and minced-dried fish. A total of 13 fatty acids in whole-dried fish and 14 fatty acids in minced-dried fish were identified in the present study. The n-3 polyunsaturated fatty acids were found to be one of the highly abundant compounds in this fish and docosahexaenoic acid is also considerably higher in amount. In the sense of higher availability of protein, storage of whole-dried fish will be a better option than minced-dried fish. Drying is one of the best and easiest methods to preserve this fish and dried fish products could be supplied to the local population at a low cost to overcome the nutrient deficiency among them. Alternatively, utilizing these bycatch fish is an effective method to reduce marine pollution as well.

Keywords: Amino acids, Discards, Fatty acids, *Leiognathus* sp., Shrimp trawling

1. INTRODUCTION

Marine fisheries are of considerable social and economic importance around the entire 1770 km of Sri Lanka's coastline. Fishing is an ancient, traditional occupation for the people in Sri Lanka especially in Jaffna. Gurunagar is one of Jaffna's major fish landing centers, and many fishers are involved in shrimp trawl fishing. According to the Department of Fisheries and Aquatic Resources, Jaffna, about 390 trawlers are in operation in Jaffna district.

Shrimp trawls are unfortunately one of the least selective fishing gears, catching large quantities of non-targeted species (Hall *et al*., 2000; Nnelly, 1995). This by-catch is often discarded at sea and this has increased during the last few years (Sivanthan *et al*., 2016). Discarding at sea indicates an unsustainable use of marine resources and efforts should be made to utilize this by-catch by bringing it to the shore and preferably utilizing it in any possible way (Tsukamoto *et al*., 2008). People do not pay much attention to the nutrient composition of discarded fish species. Therefore, in order to make consumers more attentive to the nutritional content of discards, information on nutrient value must be made available (Nurnadia *et al*., 2011). The proximate composition and nutritional value should therefore be investigated to assess the possible uses. This could increase the availability of low-cost fishery products to rural people and reduce malnutrition among poor people (Thompson and Amoroso, 2014).

Figure 1: *Leiognathus* sp. caught as by-catch during shrimp trawling and landed in Gurunagar

The fish selected for the present study is *Leiognathus* sp. This fish should be considered important because these are caught in large quantities in shrimp trawls compared to other discarded fish. *Leiognathus* sp., called "pony fish", is a small fish caught as a by-catch in shrimp fisheries (Figure 1).

Fish comprises protein, moisture, and fat as major nutrient components and carbohydrates, vitamins, and minerals as minor components (Begum *et al*., 2012). The protein content of fish is more important to consumers (Boran and Karacam, 2011) because protein is necessary for key body functions including the development and maintenance of muscles and the provision of essential amino acids (Mohanty *et al*., 2014). Proteins are versatile molecules that are made up of amino acids held together by a peptide bond (Ahmed *et al*., 2022). Fish is also considered a better food source for good quality fat (Chandrashekar and Deosthale, 1993). The fat content in fish is highly variable and depends on species, fish diet, age, spawning season, and muscle type [\(Gehring](https://scialert.net/fulltextmobile/?doi=ijbc.2011.161.169#615996_ja) *et al*., 2009).

Furthermore, some nutritional components of fish have functional effects on human health for example amino acids, fatty acids, vitamins, and minerals (Boran and Karacam, 2011). Amino acids play a significant role in protein synthesis with other essential functions such as transportation of structural proteins, vitamins, oxygen, carbon dioxygen, and enzymes (Chalamaiah *et al*., 2012). Essential amino acids and non-essential amino acids are commonly found in many fish species abundantly (Mohanty *et al*., 2014).

Fish consumption is strongly recommended by health authorities, not only for its high-quality protein content and certain micronutrients but also for being a good source of fatty acids (FAs) considered highly beneficial for human health, in particular the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) (Lund, 2013; Kris-Etherton *et al*., 2009; Rimm *et al*., 2018). Fat and Fatty acids (FA) play important roles in human nutrition and health, and EPA and DHA are important precursors of fatty acid hormones such as eicosanoids and resolvins and components of cell membranes. Therefore, it is not surprising that there is a higher demand for fish with a growing concern for the health aspects of the diet. Fatty acids in general are also important energy sources for both the fish and humans consuming the fish. Ghaeni *et al*., (2013) analyzed the fatty acid composition of *Leiognathus bindus,* however, they only investigated the muscle of the fish. Due to the small size of the fish, filleting prior to consumption is not a realistic processing method.

Whole fish is even richer in nutrients than muscle (fillet) because the bones and the viscera are particularly rich in certain micronutrients such as calcium, phosphate, and vitamins like A, D, and B. In addition, the viscera (liver) and belly flaps may have a higher fat content than the muscle. So from a nutritional point of view, whole small fish are of better value than fish fillets (Gencbay and Turhan, 2016).

In the concept of malnutrition, protein-calorie malnutrition (PCM) is the most lethal form, and the extreme conditions of PCM, mostly observed in children in developing countries, are kwashiorkor and marasmus, which are caused by chronic deficiency of protein and energy, respectively (Koethe and Von Reyn, 2016; Zhang *et al*., 1999). Fish is an important and cheap source of animal protein. To prevent malnutrition in some developing countries, the consumption of fish products is strongly recommended (FAO, 2018; Abbey *et al*., 2017). Therefore, there is a need to identify the nutritional properties of fish, especially the protein composition of locally available, lowcost fish species. Fish is a highly perishable food characterized by a short shelf-life. Therefore, fish should be preserved as soon as possible after the catch (Adeyeye and Oyewole, 2016; Tavares *et al*., 2021). One simple way to preserve small fish like *Leiognathus* is sun drying or drying by more expensive technological methods (Doe and Alley, 2020).

There is little information on the proximate composition of *Leiognathus* species, especially, information on the fatty acid composition and amino acid composition (Mohanty *et al*., 2014; Rahayu *et al*., 2014; Suganthi *et al*., 2015). Therefore, the present study aimed to investigate protein, lipid, total and free amino acids, and fatty acid composition of dried whole and minced *Leiognathus* sp. landed as by-catch during shrimp trawling in Jaffna, Sri Lanka.

2. MATERIALS AND METHODS

2.1 Sample collection

Samples (discard fish) were collected from shrimp trawler fishermen immediately after landing and brought directly to the laboratory, Department of Fisheries, Faculty of Science, University of Jaffna. Samples were randomly divided into two portions, and one portion was minced using a cleaned grinder. The whole and minced fish were dried in an oven at 80 °C until obtaining a constant weight, and then the dried samples were packed separately into polypropylene bags. The samples were carefully transferred to the laboratory of the Norwegian College of Fishery Science, UiT - The Arctic University of Norway. Samples were blended separately using a motor and pestle for proximate analysis.

2.2 Lipid analyses

The total lipid of each sample was determined gravimetrically (Folch *et al*., 1957). A 19 ml of dichloromethane/methanol (2:1, v/v) was added into 1 g of sample, mixed for 20 minutes using a shaker (Heidolph Multireax) and the solution

Figure 2: (a) Whole-dried, (b) Minced-dried *Leiognathus* fish samples

was filtered using a folding filter. Thereafter, 4 ml of 0.9% sodium chloride (NaCl) was added, mixed, and then centrifuged at 2000 rpm for 10 minutes. The lower phase was transferred into a pre-weighed glass tube and evaporated to dryness using nitrogen gas, from the final weight, the amount of lipid in the sample was calculated.

2.3 Total amino acid composition

For total amino acid determination, 40 mg of dried sample were mixed with 0.7 ml of distilled water and 0.5 ml of internal standard (N-leo 20 mM). Thereafter, 1.2 ml of concentrated HCl (37%) was added to that solution. Samples were flushed with N_2 gas for 10-15 minutes and kept in a heating cabinet at 110 ºC for 22-24 hours. The samples were cooled down and centrifuged at 1300 rpm for 5 minutes, 100 µl of the sample was transferred to assay tubes and evaporated to dryness with N_2 gas. Thereafter sample was dissolved in 1 ml of loading buffer (Lithium citrate buffer, pH-2.2), and the sample was analyzed using an amino acid analyzer.

2.4 Free amino acid composition

The free amino acid was determined by adding 1 ml of nor-Leucine (20 mM) and 9 ml of distilled water into 200 mg of the sample. Then samples were homogenized using ultrarax (15) seconds) and 1ml of 35% SSA (5-Sulfosalicylic acid) was added to remove proteins and large peptides. The samples were centrifuged at 6000 rpm for 10 minutes (4 °C) , 800 µl of loading buffer was added into 200 µl of the sample (supernatant) and the sample was analyzed.

The samples were analyzed using a Biochrom + Amino Acid Analyzer with a lithium citrateequilibrated column (at 37 ºC) and post-column derivatization with ninhydrin. The signals were analyzed with Chromeleon Software. Identification and quantification of the amino acids were done by comparing them with the standard curve prepared with physiological amino acid standards, Supelco A6407 (Acids and neutrals) and Supelco A6282 (Bases).

2.5 Fatty acid composition

Fatty acid compositions were determined by direct methylation (Dulavik *et al*., 1998). Samples (60 mg) were mixed with 2.4 ml of 2 M HCl in methanol (with 0.05% BHT) and placed in a heating block (100 ºC) for two hours. Thereafter, 2.4 ml of $H₂O$ and 12 ml of heptane were added, and mixed well. The upper phase was pipetted into new tubes and dried under nitrogen gas and the residue was dissolved in 50 µl of heptane. An Agilent 6890 N equipped with a 7683 B auto-injection and a flame ionization detector (Agilent Technologies Inc., Santa Clara, CA, USA) was used to perform gas chromatography (GC). Helium gas was used as a carrier gas in a Variance CP7419 capillary column (50 μ m \times 250 μ m nominal) (Variance Ing., Middelburg, The Netherlands). The injector and detector temperature program was 60 ºC for 1 minute, 130 ºC for 3.3 minutes then 195 ºC for 53.3 minutes followed by 240 ºC for 64.8 minutes. Fatty acids were determined according to the FA standards; polyunsaturated fatty acids (PUFA), no 1, PUFA no 2, and PUFA no 3 (Sigma Chemicals Co). By using internal standards, the total amount of FA and individual FA in the fish was determined (Table 2). Three replicate GC analyses were performed and the results were expressed in percentage of FA area in chromatogram as mean values \pm standard deviation.

2.6 Statistical analysis

For statistical analysis, the Wilcoxon rank sum test was performed to test for significance (p<0.05) between minced and whole-dried fish using RStudio (version 1.2.5001).

3. RESULTS AND DISCUSSION

While drying the fish using an oven, chemical and physical changes occurred and therefore, digestibility is increased due to protein denaturation; the amino acid composition might be changed and polyunsaturated fatty acids are often reduced. In general, drying has significant influences on the proximate compositions of fish (Chukwu and Shaba, 2009).

Table 1: Proximate composition of whole and minced-dried *Leiognathus* sp.

The total sum of amino acids ranges from 229.8 (22.98%) and 174 mg/g (17.4%) raw protein, indicating that the analyzed protein is mainly explained by the sum of the total amino acids identified. Mohanty *et al*. (2014) reported that 17.2% of crude protein was found in *Leiognathus splendens*. A similar result was found in minced-dried fish (Table 1) The protein percentage of *Leiognathus equulus* was 16.16% (Suganthi *et al*., 2015) which was lower than the results obtained for both whole-dried and minced-dried fish in the present study*.* The differences in protein content in whole-dried and minced-dried fish samples might be due to the functional properties of protein such as amino acids were decreased and protein denatures occurred in minced-dried fish than in wholedried fish during the storage period.

The lipid percentage of whole-dried fish and minced-dried fish are shown in Table 1. Rahayu *et al*. (2014) reported that the protein and lipid composition of *Leiognathus linolatus* was 16.67% and 1.26%, respectively which were lower than the results obtained in the present study. There was a high percentage of total lipid found in minced-dried fish than in whole-dried fish. This observation is due to the oxidative deterioration of fish oil in dried fish, the lipid level will be decreased after certain days of storage (Saito and Udagawa, 1992).

The percentage of total lipids is usually positively related to the total lipid level, especially when total lipids are greater than 5% and most of the lipid is stored as triglycerides when the total lipid is high, which contain 96% fatty acid (Weinrauch *et al*., 1977).

In the sense of the fat content of fish, storage of minced and dried fish is a better option than storage of dried whole fish. In contrast, when considering the higher availability of protein, whole-dried fish will be a better option than minced-dried fish.

The total amino acid composition of wholedried and minced-dried fish is presented in Table 2. The most abundant amino acids in both samples were proline and glycine. Lysine was the most abundant amino acid found in *Leiognathus splendens* sample followed by leucine, proline was not detected in *Leiognathus splendens* sample (Mohanty *et al*., 2014). The variation in amino acid composition might be attributed to variations in species composition, size, seasonal variation, and habitat (Gencbay and Turhan, 2016).

The high sum for whole-dried fish and minceddried fish can partly be explained by the relatively high levels of small-sized amino acids such as glycine (whole- 41 mg/g, minced- 35 mg/g), and proline (whole-37 mg/g, minced- 54 mg/g). Tryptophan is destroyed during acid hydrolysis.

It was surprising that there were differences in amino acids between both samples (Table 2). Some amino acids such as glutamine, serine, alanine, and methionine could not be observed in minced fish but were found in whole-dried fish. The differences may be due to some amino acids being destroyed during the storage period because the concentration and pattern of amino acids are very sensitive to the changes occurring in fish muscle during the storage period also the degradation of amino acid depend on pH,

Table 2: Total amino acid in whole-dried and minced-dried fish

temperature, and fish processing (Ciampa *et al*., 2012; Mukundan *et al*., 1986).

Leucine 23.93 ± 3.34 21

The essential amino acids such as threonine, valine, methionine, isoleucine, leucine, phenylalanine, lycine, and histidine were considered while calculating the total essential amino acids in this sample (Wu, 2010). No significant difference (p>0.05) was found in total essential amino acids for both whole-dried and minced-dried samples.

Table 3: The essential amino acids and free amino acids found in whole-dried and minceddried fish

The total content of essential amino acids in whole-dried and minced-dried samples was 75.9 and 67.3 mg (Table 3). The most abundant essential amino acids were leucine and isoleucine in both samples followed by threonine. Methionine was found in low

quantities in whole-dried fish (5 mg), but it was not reported in minced-dried fish. Jiang and Lee (1985) found decreasing concentration of serine in frozen mackerel, mullet, carp, and amber fish samples during storage at the same time an increasing concentration of methionine. Herbert and Shewan (1975) also reported the increasing pattern of methionine in fish during storage at room temperature and frozen conditions. The variation in amino acid composition in wholedried and minced-dried fish might be occurred by the modifications in amino acid composition during the storage period (Ciampa *et al*., 2012).

No significant difference (p>0.05) was found in total free amino acids for both whole-dried and minced-dried samples. Free amino acids depend on how degraded the fish is. The pattern of free amino acid concentration depends on the fish species, storage time, and condition (Bramstedt, 1962). The total content of free amino acids in whole-dried and minced-dried samples was 7.3 and 13.7 mg. Alanine, glutamic acid, leucine, and lysine were the most abundant free amino acids in both samples, examined. Histidine was absent in both samples. Other free amino acids were reported considerably in small quantities.

The fatty acid compositions of whole and minced *Leiognathus* fish are shown in Table 4. A total of 13 fatty acids in whole fish and 14 fatty acids in minced fish were identified in this study. The results were expressed as a percentage of each fatty acid concerning total fatty acids.

The content of saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acid (PUFA), and long-chain polyunsaturated fatty acid (LL-PUFA n-3) in whole fish was found to be 42.3%, 24%, 33%, and 21% respectively, and in minced fish 42%, 23.3%, 32.7%, and 20.3% respectively. No significant difference (p>0.05) was observed in fatty acid composition between whole-dried fish and minced-dried fish.

Both whole and minced samples of *Leiognathus* showed a considerable amount of saturated and unsaturated fatty acids. The amount of **Table 4:** Fatty acid composition (% of total Fatty acids) in whole and minced samples Fatty acids Composition (%) Whole fish | Minced fish

unsaturated fatty acids and polyunsaturated fatty acids was higher than monounsaturated fatty acids, PUFA n-3 was found to be one of the highest compounds in this fish (Table 3). The n-3 polyunsaturated fatty acids are primarily ALA, DPA, EPA, and DHA, and among those, DHA is considerably higher in amount. As EPA and DHA are important precursors of hormones and components of cell membranes, this fish would be a better source of those fatty acids and play a considerable role in human health, especially in rural areas.

Long chain n-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) are closely associated with lower heart risk and lower sudden cardiac death risk (Rimm *et al*., 2018).

These findings proved that *Leiognathus* fish is a nutritionally rich fish variety, especially rich in omega – 3 fatty acids. However, this nutritionally valuable fish is discarded in large

quantities in the sea as they have very low demand in the fish market. Proper guidance and awareness regarding the nutrition of fish, especially this type of low-cost discarded fish is very important for coastal people. Therefore, it can be reduced the discarding the fish in the sea without any utilization. As the human body has a very low capacity to produce long-chain omega-3 fatty acids (EPA and DHA) they should come from fish through the diet.

The presence of DHA and EPA is quite high in this fish. As a low-cost and locally available fish, it would be affordable by most of the coastal and rural area consumers, thus consumption of this fish may show a significant impact on people in the health aspect.

4. CONCLUSION

Seafood is an important nutrient source for human health. This research provides basic nutritional information on *Leiognathus* sp, both whole-dried and minced-dried. *Leiognathus* fish is rich in protein and fatty acids. Omega-3 fatty acids reduce many cardiovascular risks. This investigation provides useful information on proximate composition, however much greater seasonal sampling of this fish is needed for establishing standards for composition. It is not surprising that consumption of *Leiognathus* sp. will be increased as a direct product or valueadded product in the future with a growing concern for the health aspect of the diet. From the nutritional point of view, it is a shame that this by-catch is discarded in most cases and not used for food. Preservation and consumption of this discarded fish could provide nutritionally valuable fish products with minimal cost that could be afforded by low-income people.

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