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Comparative Evaluation of the Proximate Composition and Amino Acid Profile in Fresh and Traditionally Sun-dried Fish Species, *Puntius sophore* (Hamilton, 1822) and *Amblypharyngodon mola* (Hamilton, 1822)

Karabi Kalita [a++] and Devajit Basumatari [a#*]

[a] Department of Zoology, Cotton University, Guwahati-781001, Assam, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author KK originated the research idea, drafted the manuscript, analyzed and interpreted the data. Author DB did idea formulation, data analysis, reviewed and edited the manuscript. Both authors read and approved the final manuscript.

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[++] Research Scholar;
[#] Associate Professor;
**Corresponding author: Email: devajitbasumatari@cottonuniversity.ac.in;*

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ABSTRACT

Fish is one of the most widely consumed sources of animal protein in the world. However, they are subjected to countless post-harvest changes, so technologies such as sun-drying are employed to preserve fish for a longer time. Currently, sun-dried fish also have a major place in terms of nutrients alongside fresh fish. The present study has been conducted to determine the proximate composition and amino acid content of fish species namely, *Puntius sophore* (Puthi), and *Amblypharyngodon mola* (Moa) in fresh and sun-dried conditions to enunciate the nutritional benefits of consuming sun-dried fishes together with fresh fishes. During the study period, fresh fish samples collected were divided into two batches. In one batch all the analyses were carried out under fresh conditions. In the corresponding batch, all the tests were carried out in sun-dried conditions. Proximate composition and amino acid analysis were carried out as per the guidelines of AOAC (2015). The moisture, crude protein, crude fat, ash, and carbohydrate content of the fresh and sun-dried *P. sophore* and *A. mola* revealed significantly higher moisture content in the fresh fish samples than in the sun-dried samples. Significantly higher crude protein, crude fat, ash, and carbohydrate contents were obtained in the sun-dried samples compared to the samples in fresh condition. The Essential Amino Acid (EAA) predominant in fresh and sun-dried samples of both the species were found to be Lysine and Histidine respectively. The predominant Non-essential Amino Acid (NEAA) in fresh and sun-dried samples of both species was Glutamic acid. The analysis of nutritional parameters reveals that sun-dried fish can provide comparable nutrition to fresh fish. This study is therefore designed to promote the consumption of sun-dried fish among a broader segment of society, highlighting its dietary significance.

Keywords: Fish; sun-dried fish; proximate composition; amino acid composition.

1. INTRODUCTION

India is one of the mega biodiversity countries in the world occupying ninth position in terms of freshwater mega biodiversity [1]. In this context the Northeastern region of India is considered to be one of the hotspots of freshwater fish biodiversity in the world, bestowed with the mighty river Brahmaputra. A variety of freshwater fishes are the Small Indigenous Species (SIS), which grow to a size of 25 cm or 9 inches in mature stages of their lifecycle. Around 216 species of Small Indigenous fishes have been found in Northeast India [2]. These SISs are rich in macronutrients such as protein (including essential amino acids), polyunsaturated fatty acid (importantly EPA and DHA), ash, carbohydrate contents, and micronutrients such as vitamins and minerals. These small indigenous fishes are also affordable sources of nutrition thus serving as an important part of food security, mainly among poor people in developing and underdeveloped countries, thus conferring fulfillment of two (2) among the seventeen (17) Sustainable Development Goals viz., 'ZERO HUNGER' AND GOOD HEALTH AND WELL-BEING'. However, fish species are highly perishable and different activities such as autolysis, rancidity, and lipolysis result in fish spoilage that render them unfit for consumption. As such, a variety of post-harvest technologies are employed to preserve fish for a longer duration of time that focuses mainly on increasing its shelf life. Amongst all, traditional sun-drying is one of the oldest and most common methods of fish preservation in Northeast India. The North Eastern area of India experiences an abundance of different fish species during the monsoon season. Thus, fishermen, including locals, capture fish in bulk amounts of which the surplus is often preserved using processes such as sun-drying. However, apart from being considered just a delicacy the dried fishes are noted with the presence of considerable nutritional components such as protein, fat, ash, amino acid, mineral contents, etc. The nutritional significance of dried fish is further explained by the fact that the crude protein levels in sun-dried fish are nearly twice as high as those in fresh fish, if not in quality but in quantity [3]. The prevalence of dry fish in the NE part of India is marked by the presence of Asia's largest dry fish market "Jagiroad dried fish market" in the district of Morigaon, Assam.

Sun-dried fish species also hold a significant place in the field of ethnomedicine. Different ethnic communities of NE India consume sun-dried fish as a cure for different ailments. Thus, traditional knowledge regarding the medicinal uses of bioresources, such as dry fish, has greatly influenced the health care system of

indigenous people. However ethnic population in Assam accounts for only 12.47 percent of the total population and thus majority of people overlook the consumption of sun-dried fish, believing them to be less relevant in terms of nutrition. Also proper scientific validation linking ethnomedicinal significance of these fishes to nutrition is very scarce. The present study is thereafter designed to evaluate the nutritional aspects of sun-dried fishes and thus enunciate the health benefits associated with its routine consumption on a larger scale.

2. MATERIALS AND METHODS

2.1 Study Area

Guwahati is the capital city of the state of Assam in North-East India and is located on the south bank of the river Brahmaputra. Guwahati metro lies on the geographical coordinates of 26° 11' 0" N, 91° 44' 0" E and is a significant riverine port city.

2.2 Sample Collection

Fresh fish samples of *Puntius sophore* and *Amblypharyngodon mola* were collected from Uzanbazar landing site of Guwahati city. The samples collected were transported to the laboratory for further analysis in polythene bags with ice packs to restrict microbial contamination and growth.

2.3 Sample Preparation

The fresh fish samples collected were washed with clean tap water, eviscerated and scales and fins removed. The samples were then again washed thoroughly under running tap water. The prepared samples were divided into two equal batches. In one batch all the analysis were carried out under fresh condition. In the corresponding batch all the test were carried out in sun-dried condition.

2.3.1 Preparation of sun-dried samples

The sun-dried samples were prepared as per procedures by [4]. The eviscerated and cleaned samples were laid upon bamboo trays (locally called ‘Saloni’) and kept under the sun for 7-10 days from morning 9am to evening 4pm until the samples were dried. The average temperature noted was between 28-32°C.

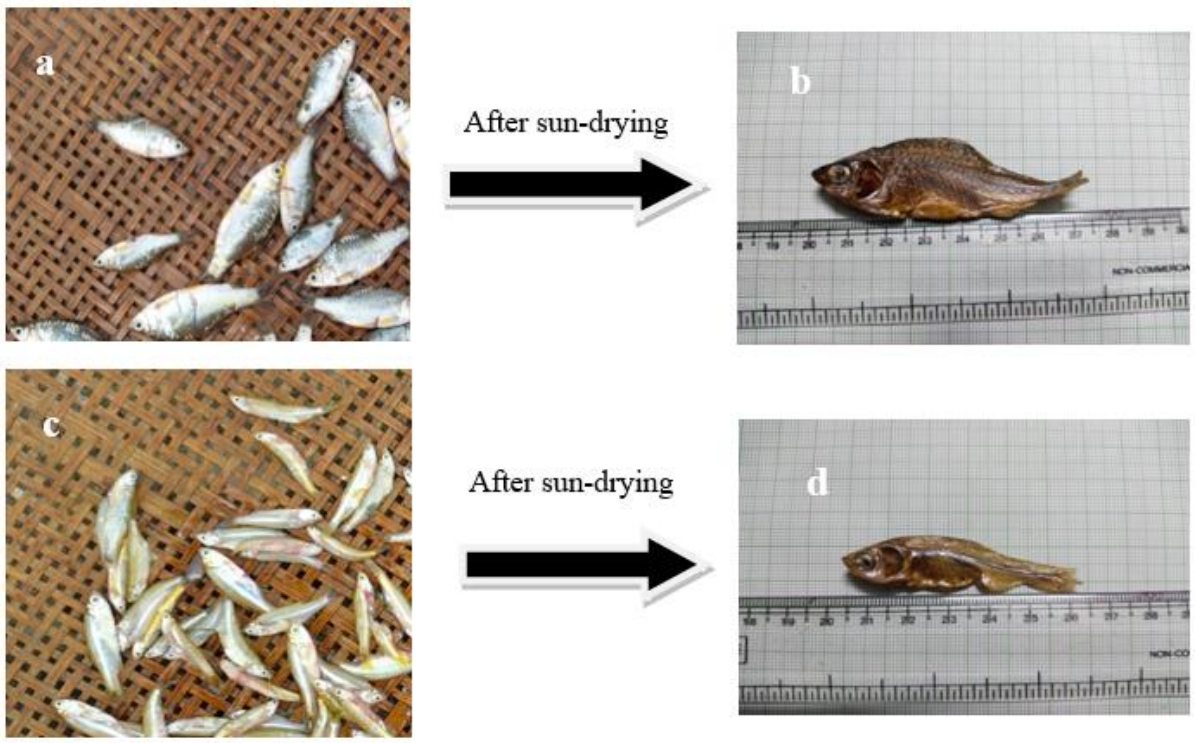

Fig. 1. Preparation of sun-dried samples of fish species: in fresh condition *Puntius sophore* (a), *Amblypharyngodon mola* (c); in sun-dried condition *Puntius sophore* (b), *Amblypharyngodon mola* (d)

2.4 Proximate Analysis

Proximate analysis was done by using standard methods given in Association of Official Analytical Chemists [5]. All types of analysis were carried out in triplicate. Electronic balance was used for weighing purpose.

2.4.1 Moisture content

The moisture content of the samples were determined by taking a known weight of the sample in a glass petridish of 125cm and drying it in a hot air oven at 100-105°C till a constant weight was achieved. The difference in weight of the sample indicated the moisture content, which was calculated by using the following formula:

$$\text{Moisture content}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight of sample taken}} \times 100$$

2.4.2 Crude protein content

The protein content of the fish samples were determined by micro-kjeldahl method. 2 g of the sample was digested in digestion unit for 45 minutes by concentrated H_2SO_4. The digested sample was then distilled in distillation unit. Finally it was titrated with 0.1 N HCl and the reading value was noted. Crude protein was obtained by multiplying the total nitrogen by a conversion factor of 6.25 (Jone's factor).

$$\text{N}(\%) = \frac{(\text{Titration reading} - \text{blank reading}) \times \text{strength of acid} \times 14 \times 100}{\text{Weight of the sample}} \times 100$$

$$\text{Crude Protein content } (\%) = \text{N } (\%) \times 6.25$$

2.4.3 Crude fat

Fat content was estimated using Soxhlet method. For the estimation of fat content, the dried samples left after moisture determination were finely grinded. About 2 g of sample was kept in a thimble and placed in extraction apparatus. Extraction thimble was placed in extraction jars and fat was extracted using non-polar solvent, diethyl ether. The % fat was calculated using standard formula:

$$\text{Crude lipid } (\%) = \frac{\text{Weight of the residue}}{\text{Sample weight}} \times 100$$

2.4.4 Ash

4g of fish sample was weighed into an empty pre-weighed crucible and kept in a muffle furnace which was then ignited at 550°C till the residue become white. The furnace was turned off to cool and then the sample was weighted again. The ash content was calculated as follows:

$$\% \text{ Ash} = \frac{\text{Weight of crucible plus sample after ashing} - \text{Empty weight of crucible}}{\text{weight of the sample before adding}} \times 100$$

2.4.5 Carbohydrate

The percentage of carbohydrate was determined by simply subtracts the total percentage of moisture, protein, fat and ash from 100. The following equation was used to estimate the amount of carbohydrate [6].

Carbohydrate (%) = 100 – (% moisture + % protein + % fat + % ash)

2.5 Amino Acid Analysis

High Performance Liquid Chromatography (HPLC) (method QA.16.5.10/AOAC 19th edition) was employed for determination of amino acid contents of the fish samples.

Preparation of hydrolyzed amino acid Sample: About 100 mg of homogenized fish mince was weighed in to a test tube filled with nitrogen and digested at 120 º C for 24 hrs in an oven. The contents of the test tube were cooled and filtered using Whatman No 1 filter paper. The filtrate was then evaporated in a vacuum flash evaporator. The contents were made acid free by repeated washing with distilled water and subsequent evaporation.

HPLC analysis: 20 µL of the hydrolyzed sample was injected in HPLC (1260 Infinity) equipped with a C18 reverse phase (RP) column and a fluorescence detector. The amino acids were identified the concentration of each type of Amino acids were calculated using the formula

$$\frac{\text{Area of Spl x Std. Conc. x Vol. x Dil x P}}{\text{Wt of Sample x Area of Std x } 1000000}$$

2.6 Statistical Analysis

Results are represented as Mean±SD. All the experiments are done in triplicates. t-test was performed to find out significant differences between the results obtained. The statistical analysis was performed in PAST 4.13 Software.

3. RESULTS AND DISCUSSION

In the present study an attempt has been made to compare the proximate composition and amino acid profile of selected fish species in both fresh and sun-dried condition such that an

analysis can be put forwarded to explain the changes in nutritional composition if any.

3.1 Proximate Composition

As per the nutritional value, quality, sensory, and physical attributes of fish and fisheries products are mostly determined by their proximate components, which include moisture, protein, fat, and ash. However, a given species' composition varies from habitat to habitat and from season to season. These compositional shifts are usually caused by changes in the amount and quality of food that fish eat as well as how much movement they do. In the present study, the results of proximate analysis are denoted in percentage (g/100g) and expressed as Mean±SD.

3.1.1 Moisture

The moisture content of the fresh fish samples were found to be in the range of to 71.46±0.73% and 75.21±0.53% respectively for *P. sophore* and *A. mola* (Tables 1, 2) where the highest moisture content was found in *A. mola*. Similar results in moisture content were obtained by [7] where the moisture content was found to be 72.65±0.33% for *P. sophore*; 73.68±0.09% for *A. mola*. The moisture content of the sun-dried fishes in the present study was found to be in the range of 7.11±0.43% (*P. sophore*) to 8.95±0.57% (*A. mola*) (Tables 1, 2) which is supported by similar findings of [8] where the moisture content of 10 sun-dried samples was found ranged from 2.772% to 7.818%. The reduction in moisture content in the sun-dried fish samples in comparison to the fish samples in fresh condition was basically due to the evaporation of moisture whilst being dried under the sun.

3.1.2 Crude protein

The crude protein content was found to be highest in *Puntius sophore* (16.56±0.61%), followed by *Amblypharyngodon mola* (15.84±0.38%) (Tables 1,2) which coincides with the findings as stated by [7] where the protein content was found to range between 14.44±0.29% to 17.75±0.12% respectively for *P. sophore* and *A. mola*. The protein content of the sun-dried fish species was found to be highest in *P. sophore* (53.91±0.61%) followed by *A. mola* (52.71±0.70%) (Tables 1,2). According to [9], the sun-dried fishes normally contain 60 to 80% protein. Reference [8] shows also studied the protein content of ten selected dried fishes where the protein varied from 27.46% to 56.84%. The crude protein content was found to be significantly higher in the sun-dried fishes which indicated that the protein nitrogen content did not degrade during the sun-drying process. This interpretation is in agreement with the results noted by [10].

3.1.3 Crude fat

The crude fat content in the present study was found to be 3.91±0.51 in *P. sophore* and 3.38±0.33% in *A. mola* (Tables 1, 2). Crude fat values of the two species as reported by [7] were higher than values of the present study in case of *P. sophore* but lower for *A. mola*. The crude fat content was found in the range of 9.67±0.29% and 7.90±0.42% in the sun-dried fish species; where the highest fat content was found in *P. sophore* (Tables 1, 2). A study by [11] reveals similar findings where the crude fat content was found to range between 4.08 ± 1.29% to 8.92 ± 1.98 %, with the highest value obtained in *P. sophore*. Increase in crude fat content in the sun-dried samples might be due to dehydration i.e., reduction in moisture content resulting from drying under the sun. The increase of crude fat content during sun-drying confirms with the findings as noted by [10].

3.1.4 Ash content

The ash content in the fresh fish samples was found to range between 3.72±0.44% and 3.30±0.38% where the highest value was found in *P. sophore* (Tables 1, 2). The result of the present study coincides with the findings of [7]. The ash content of the sun-dried fish samples was found to be highest in *P. sophore* (11.57±0.31%), followed by *A. mola* (10.34±0.35%) (Tables 1,2). These results are higher than the findings recorded by [11,12]. Increase in ash content in the sun-dried fishes may result due to different drying conditions. Fishes dried under the sun are exposed to dust being carried by wind, insects which results in increase in organic matter. Also increase in ash content can be explained due to the reduction of moisture content in the sun-dried samples which is in accordance to the explanations by [12,13].

3.1.5 Carbohydrate content

The carbohydrate content of the fresh fish sample species was found to be highest in *P. sophore* (4.35±0.57%) and lowest in *A. mola* (2.27±0.41%) (Tables 1,2). Study by [7] recorded carbohydrate content as 2.79±0.17% in *A. mola* to 4.48±0.15% in *P. sophore* which is almost similar to the present study. The carbohydrate

content in the sun-dried samples was found to be in the range of 17.74±0.41% in *P. sophore* and 20.10±0.51% in *A. mola* (Tables 1, 2). Reference [12] revealed similar values of carbohydrate content (19.23± 1.19%) in sun-dried *P. sophore*. The carbohydrate content of the two sun-dried species in the present study is almost in correspondence to the values recorded by [11].

In comparison to the proximate composition of the fresh fish species, the crude protein, crude fat, ash and carbohydrate contents were found to be significantly higher in sun-dried fishes. The higher concentration of these proximate parameters in the sun-dried samples is basically due to the reduction in moisture which results in a significant increase in the other proximate parameters. Reduced moisture in the sun-dried fish species also results in restricted microbial growth thereby increasing the shelf life for about 6 months to 1 year in room temperature. The results of the proximate composition analysis revealed that sun-dried fish are rich in protein, fat, ash, and carbohydrate content, making them a potentially effective alternative food source for addressing hunger issues such as Protein-Calorie Malnutrition (PCM).

3.2 Amino Acid Profile

In terms of amino acid composition, the present study revealed the presence of both essential and non-essential amino acids in both fresh and sun-dried condition. In the present study for *P. sophore* in fresh condition the predominant essential and non-essential amino acid (g/100g) was found to be Lysine (4.09±0.03 g/100g) and Glutamic acid (7.15±0.02 g/100g) respectively (Table 3). Also in the fresh fish samples of *A. mola* the predominant essential and nonessential amino acid was Lysine (4.12±0.01) and Glutamic acid (8.23±0.01) respectively (Table 4). Reference [14] recorded the amino acid content of 27 fish species including *P. sophore* and *A. mola* where the predominant essential and non-essential amino acid was recorded to be Histidine (1.4 ± 0.3 and 2.8 ± 0.4 g/100g) and Aspartic acid (1.2 ± 0.2 and 1.4 ± 0.1 g/100g) in both the fish species respectively. Another study by Bhalerao [15], recorded the amino acid content of *P. sophore* in two different sites and revealed Lysine (3.5±0.22 and 3.34±0.08 g/100g) and Glutamic acid (6.34±0.23 and 5.61±0.41 g/100g) as the predominant essential and non-essential amino acids respectively which corresponds to the present study.

In case of sun-dried *P. sophore* for the present study the predominant essential and nonessential amino acid was found to be Histidine (3.84±0.02 g/100g) and Glutamic acid (9.03±0.03 g/100g) respectively(Table 3). While in case of sun-dried *A. mola* also the predominant essential and nonessential amino

Table 1. Proximate composition of fresh and sun-dried *P. sophore*

Proximate Composition	*P. sophore*(Fresh)	*P. sophore*(Sun-dried)
Moisture	71.46±0.73	7.11±0.43
Crude protein	16.56±0.61	53.91±0.61
Crude fat	3.91±0.51	9.67±0.29
Ash	3.72±0.44	11.57±0.31
Carbohydrate	4.35±0.57	17.74±0.41

Values are expressed as Mean±SD, n=3.
t-test was performed and there was a significant difference (P<0.05) between the means of individual proximate parameters in fresh and sun-dried conditions

Table 2. Proximate composition of fresh and sun-dried *A. mola*

Proximate Composition	*A. mola*(Fresh)	*A. mola*(Sun-dried)
Moisture	75.21±0.53	8.95±0.57
Crude protein	15.84±0.38	52.71±0.70
Crude fat	3.38±0.33	7.90±0.42
Ash	3.30±0.38	10.34±0.35
Carbohydrate	2.27±0.41	20.1±0.51

Values are expressed as Mean±SD, n=3.
t-test was performed and there was a significant difference (P<0.05) between the means of individual proximate parameters in fresh and sun-dried conditions

Table 3. Amino acid content of fresh and sun-dried, *P. sophore*

Essential amino acids	Fresh*P. sophore*	Sun-dried*P. sophore*
Histidine	3.23±0.01	3.84±0.02
Isoleucine	0.55±0.05	0.33±0.02
Leucine	2.31±0.02	1.68±0.01
Lysine	4.09±0.03	2.99±0.02
Methionine	0.98±0.01	0.87±0.02
Phenylalanine	1.22±0.02	0.35±0.01
Threonine	0.17±0.02	0.98±0.02
Tryptophan	0.11±0.02	0.33±0.02
Valine	1.45±0.03	1.47±0.01
Non-essential amino acids		
Alanine	4.13±0.02	2.41±0.02
Arginine	0.73±0.01	1.55±0.02
Aspartic acid	1.33±0.02	1.03±0.02
Asparigine	ND	ND
Cysteine	0.34±0.01	0.27±0.01
Glutamic acid	7.15±0.02	9.03±0.03
Glutamine	1.68±0.02	2.03±0.02
Glycine	5.91±0.01	4.53±0.01
Proline	5.81±0.01	4.21±0.01
Serine	1.42±0.02	1.23±0.06
Tyrosine	0.69±0.02	0.65±0.01

Values (in g/100g, dry weight) are expressed as Mean ±SD, n=3.
t-test was performed and there was a significant difference (P<0.05) between the means of individual AAs in fresh and sun-dried conditions

Table 4. Amino acid content of fresh and sun-dried, *A mola*

Essential amino acids	Fresh*A. mola*	Sun-dried*A. mola*
Histidine	3.85±0.04	4.65±0.02
Isoleucine	0.44±0.03	0.22±0.01
Leucine	2.71±0.01	2.06±0.01
Lysine	4.12±0.01	3.05±0.02
Methionine	1.13±0.06	0.99±0.01
Phenylalanine	1.67±0.01	1.15±0.05
Threonine	0.36±0.03	1.21±0.01
Tryptophan	0.27±0.01	0.47±0.01
Valine	2.16±0.05	2.51±0.01
Non-essential amino acids		
Alanine	3.78±0.05	1.93±0.01
Arginine	0.75±0.02	1.38±0.01
Aspartic acid	1.20±0.05	0.87±0.01
Asparigine	0.10±0.01	ND
Cysteine	0.55±0.02	0.41±0.02
Glutamic acid	8.23±0.01	10.05±0.01
Glutamine	1.57±0.01	1.88±0.02
Glycine	6.12±0.01	5.34±0.01
Proline	6.42±0.01	5.47±0.01
Serine	0.92±0.02	0.31±0.01
Tyrosine	0.54±0.02	0.62±0.02

Values (in g/100g, dry weight) are expressed as Mean ±SD, n=3.
t-test was performed and there was a significant difference (P<0.05) between the means of individual AAs in fresh and sun-dried conditions

acid was found to be Histidine (4.65±0.02 g/100g) and Glutamic acid (10.05±0.01 g/100g) (Table 4). Reference [11] studied the amino acid content of dried *P. sophore* and *A. mola* where the predominant essential amino acid was found to be Lysine (3.36 ± 0.15 and 3.64 ± 0.16 g/100g) followed by Leucine (3.00 ± 0.11 and 3.26 ± 0.10 g/100g) respectively for both the species and in case of sun-dried *P.* sophore the predominant non-essential amino acid was found to be Glutamic acid (5.76 ± 0.13 g/100g) followed by Glycine (3.22 ± 0.12 g/100g), while in case of *A. mola* the predominant non-essential amino acid was found to be Glutamic acid (6.20 ± 0.18 g/100g) followed by Aspartic acid (3.49 ± 0.12 g/100g).

In comparison to the fresh fish samples the amino acids namely Histidine, Threonine, Tryptophan, Valine, Arginine, Glutamic acid, Glutamine were found to be increasing in the sun-dried samples with a significant difference of $p<0.05$(Tables 3,4). Increase in Glutamic acid might be subjected to heat treatment during sun-drying. High values of glutamate in dried products also accounted for higher flavor and taste. However some amino acids were found to decrease in the sun-dried samples as compared to the fresh samples. Amino acids such as Isoleucine, Lysine, Leucine, Methionine, Phenylalanine, Alanine, Aspartic acid, Cysteine, Glycine, Proline, Serine, and Tyrosine were found to be decreasing in the sun-dried fish samples. In sun-drying process, heat is responsible for the reduction in the amino acid score for lysine [16]. Reference [17] have discussed the Maillard reaction in which the free epsilon amino group of lysine is susceptible to heat damage, forming additional compounds with non-protein compounds resulting in the reduction of lysine content. Decrease in amino acid content in the sun-dried samples might be due to the heating process that causes excessive denaturation of protein and destruction of amino acid. All the essential amino acids, including sulfur-containing amino acids like methionine and cysteine which are lacking in plant proteins [18] and methionine and lysine which are lacking in meat proteins from terrestrial sources are found in dried fish [19]. High-quality fish and sun-dried fish protein have enough of each essential amino acid needed for body building, maintaining lean muscle mass and an active metabolism, healing damaged tissues and warding off certain illnesses. Furthermore, the health benefits of fish and fish products' proteins, peptides, and amino acids have lately gained widespread recognition. Reference [18] reported that certain reduction in amino acids happened when fish was dried and stored. However, no evidence was recorded of any impact of the drying procedure on the amino acid content of fillets of grass carp (*Ctenopharyngodon della*) [20]. Different fish processing techniques such as sun-drying leads to the formation of different inter and intra-molecular bonds which results in unfolding of protein chains and exposure of free carboxylic and amino groups thus altering the content of amino acids Boziaris [21]. The present study reveals that sun-dried fishes contain all the essential and non-essential amino acids though changes in their composition has occurred as a result of sun-drying. Therefore, it is evident that little amounts of sun-dried fish can satisfy the body's requirement for different macro and micro-nutrients in a way that is beneficial to health.

5. CONCLUSION

As per 2023 reports of Global Hunger Index (GHI), India ranks 111 out of 125 countries, with a GHI score of 28.7, indicating a serious level of hunger. In light of this concerning scenario, researchers are continuously exploring alternative food sources that are both nutritionally significant and affordable. In this regard sun-dried fish emerges as a valuable option due to their high protein, crude fat, ash, and carbohydrate and amino acid contents. However, their consumption is largely limited to a specific section of the population, leaving their nutritional benefits underutilized by the wider society. This study aims to dispel misconceptions about the nutritional value of sun-dried fish and promote their inclusion as a viable source of animal protein, encouraging broader consumption across diverse communities.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

DECLARATION

Authors declared that the participated informants were apprised of the objectives of the research and the information was collected following a structured method.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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