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Impact of Freezing and Drying on Fatty Acid and Microelement Content of Small Indigenous Fishes of Assam, India

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Abstract: Small indigenous fishes are good source of micronutrients and omega 3 fatty acids for human being. They are harvested at a large amount, but since they are extremely perishable food item, a small fraction of it is sold at fresh condition and rest has to be preserved i.e., either by freezing or drying and sold at low price. The ethnic people of North east India, consume them in both their fresh and preserved form, without having much knowledge about its nutritional status. Hence, an investigation was done to find out the nutritional change in terms of mineral content and polyunsaturated fatty acids during their preservation period. The fatty acid and micronutrient content was estimated in six small indigenous fishes altogether in fresh, frozen and sun-dried state. The sample fishes were– *Amblypharyngodon mola*, *Chanda nama*, *Esomus danricus*, *Mystus tengara*, *Puntius sophore* and *Trichogaster fasciata*. In all the three states, fatty acid content was estimated by GCMS and micronutrient content was estimated by AAS. Results showed that there is definite impact of preservation in the nutrient values of small indigenous fishes. Freezing increased the value of certain essential fatty acids i.e. EPA, DHA, Arachidonic acid, Vaccenic acid and Oleic acid. In case of micronutrients the impact of freezing was negligible, since values decreased insignificantly. The sun-dried fishes showed higher nutritional value in both the micronutrient content and fatty acid content than the fresh and frozen fishes. It was observed that fatty acid content and microenutrient do not get deteriorated during a short term freezing period however, sun dried conditions enhances the nutritional content in terms of fatty acids and micronutrient quality of the small indigenous fishes of Assam.

Keywords: Small Indigenous Fishes, Fatty acid, Micronutrients, Frozen fish, Sun dried fish

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Introduction

Fish is one of the major sources of nutrients, including the proteins, lipids, carbohydrates, vitamins and minerals needed to maintain a healthy human body (Sarkar *et al*., 2017). Compared to other sources of animal proteins, fish is readily available and less expensive in tropical

nations (Mohanty *et al*., 2019, 2022; Sinha *et al*., 2022). It is essential for improving nutritional status, food security, cardiovascular health and other health-related disorders (Bezbaruah and Deka, 2021). A fish meal, in addition to providing essential nutrients, contains significant amounts of fatty acids, amino acids and some of the most important vitamins and minerals, which serve as a source of energy for healthy living and are sometimes referred to as 'rich food for poor people' (Balami *et al*., 2019; Bezbaruah and Deka, 2021). Asserting a high content of poly-unsaturated fatty acids, shreds of evidence suggest that fish flesh and fish oil are helpful in reducing the serum cholesterol level in human as well (Stansby, 1985). Hence, it aids in the maintenance of good health like prevention and treatment of cardiovascular, inflammatory and neurological diseases (Li *et al.*, 2019; Jan *et al.*, 2021). Fish consumption has gained popularity in recent years (Supartini *et al*., 2018; Lee and Nam, 2019; Krittanawong *et al*., 2021; Krešić *et al*., 2022) due to its high level of polyunsaturated fatty acid, particularly omega-3 fatty acids such as docosahexaenoic acid (C22:6) and eicosapentae-noic acid (C20:5), as they cannot be synthesized by human body (Alam *et al.*, 2016).

The North East part of India is enriched with various freshwater bodies that have a wide range of fish species including Small Indigenous Fishes (SIFs). SIFs are defined as fishes which grow to the maximal size of 25-30 cm. in mature or adult stage of their life cycle (Felts *et al*., 1996). This region being an ideal habitat for various endemic small fish, supports as many as 216 species out of total 450 SIF species found in India (Mayanglambam *et al*., 2022; Pegu *et al.*,2023).

These fishes are valued for their high protein, fatty acid, vitamin and mineral content (Thilsted, 2012; Balami *et al*., 2019; Bezbaruah and Deka, 2021). The SIF provides better nutrition because they are frequently consumed whole, including the head, bones and eyes, utilizing all available nutrients, including micronutrients. It is a very important source of pre-formed vitamin A, vitamin D and vitamin E and also contains thiamine, riboflavin and niacin (vitamin B1, B2 and B3). SIFs are rich source of iron, calcium, magnesium, manganese, phosphorus, sodium, potassium and other important trace elements like zinc, iodine, selenium etc. Fish also contribute fatty acids that are necessary for the proper development of the brain and body, especially the Omega-3 fatty acids which are now considered important in prevention and treatment of cardiovascular, inflammatory and neurological diseases (Li *et al.*, 2019; Jan *et al.*, 2021). Different ethnic communities uses various indigenous fish species against different diseases such as ulcers, skin diseases, night blindness, general weakness, loss of appetite, anemia, tuberculosis etc (Neog and Konwor, 2023). All these qualities are in super condition in their fresh form i.e., till 7 h after its death. Fish is a highly perishable food which needs proper handling and preservation if it is to have a long shelf life and retain a desirable quality and nutritional value. Hence, needs preservation (storage), without destroying its nutritional status. Two best methods of fish preservation are freezing and drying and the people of this region are acquainted with both these methods, however, without detailed scientific information about the change in proximate composition of the fishes in their preserved states. Proximate composition helps in estimating the energy worth of fish and planning the most appropriate industrial and commercial processing. Therefore, it is relevant to evaluate the proximate composition (Hantoush *et al*., 2015) for their preservation in various forms. Meanwhile many of the original studies on the nutritional status of SIFs have well been recorded in the country including the northeast region (Mohanty *et al.,* 2010, 2013, 2019; Goswami *et al.,* 2012; Duarah and Das *et al.,* 2019; Mayanglambam *et al*., 2022; Pegu *et al.,* 2023). Since no such study on the small indigenous fishes has been done before to unveil their nutritional benefits under preserved conditions, this project has been designed to evaluate the impact of freezing and drying on the nutritional qualities, specially micronutrients and polyunsaturated fatty acids of

Table 1: Small indigenous fishes used in the experiments with their scientific names and local names in Assamese

S. No.	Genus	Species	Local Name
1	*Amblypharyngodon*	*mola*	Moa
2	*Chanda*	*nama*	Chanda
3	*Mystus*	*tengara*	Tingara
4	*Puntius*	*sophore*	Bhadi puthi
5	*Esomus*	*danricus*	Dorikona
6	*Trichogaster*	*fasciata*	Kholihona

the SIFs.

Materials and Methods

Collection of sample:

Raw fishes were collected directly from the Charan Beel located 25.3011^0 N latitude and 92.1517^0 E longitude (Charan beel is a freshwater perennial oxbow wetland, situated in the district of Morigaon, Assam) from the fishermen and then selected and sorted as specified in the Table 1. SIFs collected are dressed properly by removing the viscera to the best possible extent, washed with water and salt water followed by further washing.

The fishes were then divided in three parts each part containing all six species:

1. Fresh (FR): Freshly captured fishes.

2. Frozen (FZ): Parts kept in deep freezer for 7 days at -37°C.

3. Sun Dried (SN): Dried under sunlight for 10 days (till the moisture is completely removed). Open sun drying method were followed (Mustapha *et al*., 2014). In short, an unenclosed 0.61mx0,61 m bamboo sieve was used for open drying by exposure to direct sunlight. The intensity of light was measured by using Lux meter, Model LX 101A, which gave a reading of 31872±0.13 lumen/m^2.

The fresh, frozen and sun dried fishes (including their head, eyes and bones) were taken for further analysis.

(a) *Fatty acid analysis*:

For fatty acid analysis, the lipid was first extracted by Bligh and Dyer method (Bligh *et al*., 1959). In short, 2 g of crushed, homogenized sample was taken from the fresh fish sample. 5 ml of chloroform and 10 ml of methanol (1:2 ratio) was added to the sample and blended in a vortex mixture for about 2 min.

Another 5 ml of chloroform was added to the mixture and blended in a vortex mixture again for 30 sec. Then 4 ml of saturated NaCl solution was added and blended in a vortex mixture for 30 sec. The mixture was filtered through a pre methanol wetted filter paper and the solution was separated into two distinct layers. The upper chloroform layer is removed and lipid layer layer at the bottom was processed at the Central Instrumentation Facility (CIF) center of USTM for further fatty acid analysis by Gas Chromatography-Mass Spectrometry (GCMS) method (Model: TRACE 1300 ISQ 7000 ThermoFisher Scientific). The same process was done for the frozen and sun dried samples.

(b) *Elemental analysis*:

For elemental analysis, sample preparation was done by wet ashing technique and analyzed by AAS (Atomic Absorption Spectroscopy). In short 5 g of the homogenized sample was heated in low flame in HNO_3 and perchloric acid (3:1) and the remaining solution after evaporation was diluted with deionized water and analyzed by AAS (Model: iCE 3500 AA System VP 100 Thermo Scientific).

Table 2: Fatty acids found in the fish samples

Short–chain Fatty Acid (SCFA)	Medium-chain Fatty Acid (MCFA)	Long-chain Fatty Acid (LCFA)
Acetic acid	Dodecanoic acid	Hexadecanoic acid
Butanoic acid	Undecanoic acid	Pentadecanoic acid
Hexanoic acid		Methyl stearate
Pentanoic acid		Heptadecanoic acid
Tridecanoic acid		Vaccenic acid
		Oleic acid
		Octadecanoic acid
		Arachidonic acid
		Eicosapentaenoic acid (EPA)
		Docosaehexaenoic acid (DHA)
		Tetradecanoic acid
		Octadecynoic acid
		3-Heptyl Hexanoate

Statistical Analysis:

All statistical analysis were done by using PAST 4.3 software.

Results and Discussion

Fatty acid profile:

The result of fatty acid estimation in fresh, frozen and sun dried fishes showed a wide range of essential and non-essential fatty acids in all the samples.

The fatty acid profiling of SIFs revealed the presence of short, medium and long chain fatty acids (Table 2). Amongst them the variations in some important omega fatty acids were studied in fresh, frozen and sun dried conditions (Table 3; Fig. 1). The one way ANOVA of the Table 3 data showed a significant variation with f = 5.739936 and p value = 0.011525 ($p<0.05$). It indicated that the content of the essential fatty acids changed significantly during their preservation period.

During frozen storage it was seen that the value of EPA, DHA, Arachidonic acid, Vaccenic acid and Oleic acid increased. Similar findings were obtained by Nicolae *et.al.* (2017), who found that deep freezing considerably impacts the essential fatty acids even for a short time of freezing specially Oleic acid which was found to increase with storage time. Oleic acid has a preventive properties for cancer, alzeimers disease, cardiovascular disease and skin repair (Helioswilton *et al*., 2013; Santa Maria *et al*., 2023). Morever, Tenyang *et al.* (2019) proved that Red Carp oil showed the best quality during its frozen storage uptil 3 months. The present findings also converge with the observations of Monika Zymon *et al.* (2007), who obtained a higher content of PUFA including oleic acid, linoleic acid, EPA and DHA, in the frozen calves meat as compared to fresh.

Again in dried conditions, the present investigation showed that the value of EPA, DHA, Arachidonic acid, Vaccenic acid and Oleic acid was the highest (Table 3). Similar findings were also obtained by Lyashenko *et al*. (2023), who found higher contents of Arachidonic acid, EPA and DHA in 29 species of salt dried fishes. Similarly Akintola *et al.* (2013) found that sun-dried giant tiger shrimps have higher content of polyunsaturated fatty acids mainly EPA and DHA than the fresh shrimps. The findings also coincides with Zarehgashti *et al*. (2018) who recorded that dried shrimp meat has higher fat content than the frozen meat. The dried shrimps showed highest

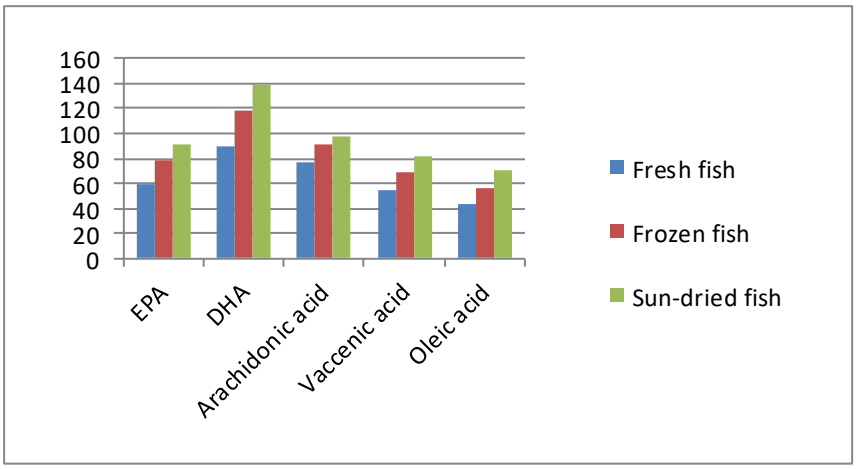

Fig. 1: Comparision of mass spectra of omega fatty acids of SIFs in fresh, frozen and sun dried conditions.

Table 3: Omega fatty acids with their mass spectra (Mean±SD) in fresh and preserved fishes

Omega fatty acids	Fresh fish sample (m/z)	Frozen fish sample (m/z)	Sun-dried fish sample (m/z)
Eicosapentaenoic acid (EPA) – Omega 3	59.06±0.31	79.00±0.24	91.04±0.16
Docosahexaenoic acid (DHA) – Omega 3	90.02±0.18	119.05±0.34	139.08±0.41
Arachidonic acid – Omega 6	77.00±0.12	91.02±0.09	97.10±0.82
Vaccenic acid – Omega 7	55.00±0.06	69.00±0.43	81.09±0.72
Oleic acid – Omega 9	43.00±0.36	56.08±0.16	71.00±0.57

level of EPA and DHA.

Elemental analysis:

Elemental analysis of Zn, Cu, Ni, Cd, Cr, Fe, Mn, Ca, and P of small indigenous fishes in fresh, frozen and sun dried conditions were examined by Atomic Absorption Spectrophotometer. The results revealed significant variation as illustrated in Table 4 and Figure 2. The principal component analysis of the microelements i.e., Zn, Cu, Ni, Cd, Cr, Fe and Mn showed the highest correlation with the sun dried (SN) state. However, the values of the micronutrients in the fresh and the frozen fishes was negligibly different. The one way analysis showed p=4.34, with significance level $p<0.05$. The Fe and Zn showed the highest range variation but their values are distantly correlated with each other. The Cu, Cd and Cr values showed close correlation with each other.

The above data (Table 4) has significant variance as determined by one-way ANOVA with F = 191.1305 and P-value = 0.000159 ($P<0.05$).

In case of macronutrients, from the Ca and P level it was clearly seen that Ca and P increases significantly at the dried conditions (Fig. 3), as compared to the fresh and frozen states. The results coincides with the findings of Sroy *et al.* (2023), who found that Ca and P levels are significantly higher in dried fish powder of

Table 4: Values of microelements (mg/g) (Mean±SD) in SIFs in their fresh, frozen and sun dried conditions (FR=Fresh, FZ=frozen, SD=Sun Dried)

Sample	Zn	Cu	Ni	Cr	Fe	Mn	Cd
FR	2.87±0.03	1.51±0.07	0.12±0.05	0.56±0.01	5.5±0.04	5.03±0.06	0.04±0.02
FZ	2.82±0.08	1.11±0.02	0.09±0.01	0.54±0.05	4.68±0.03	4.86±0.02	0.01±0.05
SN	4.8±0.02	2.64±0.01	0.16±0.04	0.77±0.01	6.02±0.02	6.02±0.08	0.08±0.06

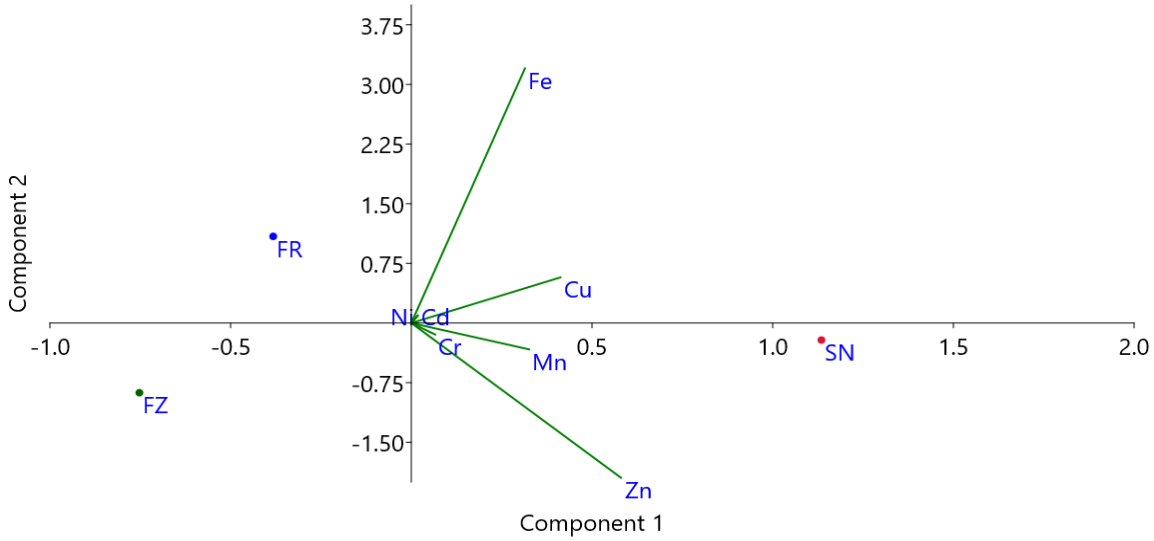

Fig. 2: Principal Component Analysis of microelements of SIFS in their fresh, frozen and sun dried conditions.

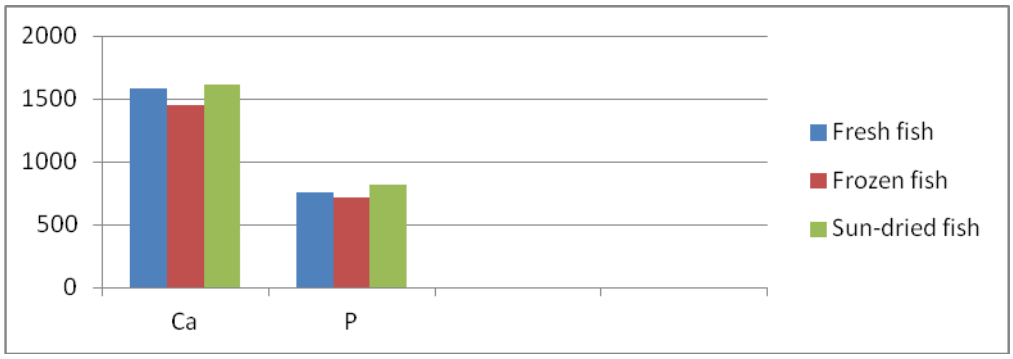

Fig. 3: Comparative values of macroelements (Ca and P) in mg/g (Mean±SD) in SIFs in their fresh, frozen and sun dried conditions.

Henicorhynchus siamensis as compared to fresh ones. Besides, they also found that Fe, Zn and Mnwere the predominant values in dried fish, which also supports the present findings. The results obtained may be because of removal of water while drying from the tissue and reduces the water activity, making the water inaccessible to microorganisms, extending the shelf life of dried products. This also makes the dried products edible for longer period and simultaneously increases other nutrients in the product that are beneficial for human health (Nowsad, 2005).

Comparatively, the small fish and their dried products are known to be a good source of minerals (Fawole *et al*., 2007; Roos *et al*., 2007; Jahan *et al.,* 2017). Dried fish also has higher lipid and protein contents than fresh fish, which is advantageous to fulfil daily nutritional requirements (Fitri *et al*., 2022). Rasul *et al*. (2021) mentioned that dried fish can be a good source of beneficial nutrients for human health, based on the proximate composition, amino acids, fatty acids profile and mineral content.

Many researchers found that the traditional sun-drying preservation of fishes is more beneficial than the fresh fishes. Small Indigenous Species (SIS) of fish can be used to encourage the consumption of micronutrient-rich fish species, particularly in vulnerable population groups such as young children, pregnant and lactating women, the sick and elderly people. To enhance the intake of animal-sourced meals by women and children, it is therefore immensely advantageous to include small fish species that are rich in micronutrients in the development and execution of agricultural policy decisions and programs (Islam *et al*., 2023).

Conclusion

Since the small indigenous fishes are eaten as a whole with their bones and head, they constitute a great source of nutrients to human health. In the present study, six common small indigenous fishes were taken altogether; because most of the time, people used to eat several species of SIFs altogether at a time.

The study can be concluded as the nutritional status of small indigenous fishes has not lost in terms of fatty acids and microelements in both of its short time preserved state – freezing and drying. The nutrient content in the frozen fishes were insignificantly variable as compared to the fresh fishes, while the sun-dried fishes showed a great variance in the nutrient content as compared to the fresh fishes. Therefore, sun-dried small indigenous fishes are more beneficial to human health for their nutritional value along with their medicinal value, since all the nutrient values were found higher in the dried state.

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