Hindawi
Journal of Food Biochemistry
Volume 2024, Article ID 7410065, 11 pages
https://doi.org/10.1155/2024/7410065

Research Article

Preparation, Characterization, and Nutritional Analysis of *Napham*: An Indian Traditional Smoke-Dried-Fermented Fish Paste

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Received 22 September 2023; Revised 1 January 2024; Accepted 11 January 2024; Published 25 January 2024

Academic Editor: Anurag Singh

Napham, a unique smoked-dried-fermented fish paste, is a traditional delicacy prepared by the Assamese Bodo people. This research focuses on the process of *Napham* preparation and explores its nutritional benefits. To make *Napham*, sundried and smoked trash fishes, along with *Alocasia macrorrhiza* stems, are ground and stuffed into immature bamboo tubes, where they ferment for 2-3 months. Although *Napham* possesses a distinct taste and texture that may not appeal to everyone, the fermentation process significantly enhances its nutritional value by increasing its vitamin content, improving fatty acid profiles, and enhancing amino acid profiles. The study reveals that *Napham* is a remarkable source of protein (63.65 ± 0.83 g/100 g), potassium (667.87 ± 3.48 mg/kg), sodium (531.48 ± 3.43 mg/kg), and magnesium (56.23 ± 1.53 mg/kg). *Napham* contains both omega-3 and omega-6 fatty acids, including linoleic acid (10.10 ± 0.002%), α-linolenic acid (6.47 ± 0.062%), arachidonic acid (4.65 ± 0.031%), eicosapentaenoic acid (1.08 ± 0.007%), and docosahexaenoic acid (3.44 ± 0.036%). Furthermore, *Napham* is rich in essential amino acids such as methionine (227.407 μmol/L), isoleucine (478.525 μmol/L), leucine (797.944 μmol/L), valine (640.867 μmol/L), phenylalanine (320.573 μmol/L), lysine (1066.557 μmol/L), and histidine (104.525 μmol/L), complementing its nutritional profile.

1. Introduction

Fish and fish products play a crucial role in maintaining a balanced diet due to their high-quality protein, healthy fats, minerals, and vitamins. The significant presence of unsaturated fatty acids, primarily found in fish fat, further contributes to their nutritional value [1]. These products account for approximately 20% of the animal protein consumed daily by the world's 3.1 billion people, emphasizing their importance in the human diet [2]. Moreover, the consumption of fish and fish products has been positively linked to increased life expectancy and improved health outcomes [3]. Notably, Japan, a country with the longest lifespan and a lower prevalence of obesity and cardiovascular diseases, demonstrates higher consumption of aquatic protein and aquatic fat, as indicated by Tacon and Metian [4]. Conversely, the United States of America faces significant concerns regarding obesity and cardiovascular diseases. In contrast to various other food categories, fish tend to have a lower calorie content while being abundant in valuable omega-3 fatty acids (n-3 PUFA). Scientific studies have demonstrated that n-3 PUFA can alleviate symptoms associated with metabolic syndrome, diabetes, weight gain, and arterial disease and even contribute to brain development [3].

There is a significant worldwide appetite for fish and products derived from them; however, various challenges exist due to factors like susceptibility to corruption, oxidation, and seasonal availability. As a result, preservation techniques have been adopted to address these obstacles [5–7]. Among these techniques, fermentation has emerged as the most widely utilized method for fish preservation, surpassing freezing, salting, and smoking. Fermentation offers a convenient way to preserve fish over an extended period without necessitating significant space or energy requirements [5].

Fermentation is a cheap and time-tested process of food preservation [1]. It involves different probiotic microorganisms for its process of transformation. In the process of fermenting fish, the bioactive and volatile chemicals are biotransformed into new edible forms with increased flavours and fragrances [8]. Both naturally occurring and introduced microorganisms (starter cultures) contribute to the chemical and textural alterations in fermented fishes [9]. Because the fermentation of fish involves a variety of bacteria, it is possible that people have been inadvertently consuming microbes through their ethnic foods [10]. Humans have been eating fermented fish since time immemorial. Although they were most likely created for the purpose of preservation, it would have been obvious that these meals had other desirable qualities. Fermented fish have definite tastes, textures, appearances, and functions when judged against the unprocessed components from which they are produced. Fermented rations were purposely made many millennia ago, far before nutrition research, as a regular supply of vitamins, minerals, calories, and supplementary nutrients [11].

Fermented fish in various ways is a delicacy in many cuisines throughout the globe. The fermentation process varies from place to place, based on the type of fish being processed and the preferences of local people. In different regions of south and southeast Asia, these products are almost a regular cuisine, typically served as condiment with rice [11]. In India, especially the people of north-eastern region make their cuisine strikingly akin to that of the countries of Southeast Asia. Together with rice, more than 98% of the population in the northeastern part of India relies on fish as their primary source of animal protein. This can be in the form of fresh fish or preserved fish products [12]. In point of fact, fish and its product have been connected to the social and economic life of people living in Northeast India from the beginning of history. This area comprises a diverse population consisting of more than 100 tribes and communities, each of which has cultivated its own distinctive ethnic cuisine over many generations. The communities of different states of Northeast India put up with their own methods of preparing fish-based different traditional ethnic products from time immemorial. These products are prepared either through the methods of drying, smoking, and fermentation or the combining methods of drying, smoking, and fermentation together [13].

While there are records of various fermented fish products from the region, the scientific potential of *Napham*, a traditional smoke-dried fermented fish paste by the Bodo tribe, has not been fully explored. *Napham* holds great significance and popularity among the Assamese Bodo people, serving as a cherished delicacy [14]. However, the specific technique for preparing *Napham* and its nutritional composition have yet to be documented. Consequently, this study aims to document the traditional process of *Napham* preparation and evaluate its nutritional profile. The goal is to generate interest in this traditional Northeast Indian food item and encourage further comprehensive investigations into its properties.

2. Materials and Methods

2.1. Collection of Napham and Its Identification. *Napham* is a smoke-dried-fermented fish paste prepared by the *Bodo* community of Assam, India. It is prepared in the traditional manner, with all feasible hygienic conditions maintained during the production procedure (Figure 1). *Napham* was collected from Udalguri district of Bodoland Territorial Region (BTR), Assam, India, during mid of August, 2022 (Figure 2). Udalguri district (92° 06′ 7.74″ E longitude and 26° 45′ 13.21″ N latitude) is located in the North Brahmaputra Valley region of Assam with a total geographical area of 1852.16 km^2. The process of making *Napham* was documented through personal conversations with the locals, who have expertise in its preparation. The gathered *Napham* samples were then transported to the laboratory of the Department of Food Engineering and Technology at Tezpur University in Assam, India. To maintain their freshness, the samples were kept in a refrigerated environment conditions (0–4°C) refrigerated until further testing and analysis could be conducted.

2.2. Method of Preparation of Napham. Any species of trash fishes that is readily available during the rainy season was selected to prepare *Napham* along with the petiole of arum (*Alocasia macrorrhiza*). The fishes were cleaned for use as raw material by having their gills, scales, guts, etc. removed and then washed in tape water. Likewise, the petioles of arum were also peeled off and cut into small pieces using knife. To remove the moisture from the raw materials, the dressed fishes and cut pieces of arum petiole were sundried (28–32°C) separately for a day. During sun drying attention was paid to avoid contamination from dirt, flies, mosquito, and other insect pests. After being partially dried, the fish was spread out over a hearth at modest fire and allowed to further dry and smoke for a night until they become hard. Smoking facilitates attractive colour, taste, and smell to the fish and has antibacterial properties. When the fishes had smoked and dried properly, they were combined with pieces of semidried petioles in a 3 : 1 weight ratio. The mixture was then grounded using a traditional wooden hand pound or huller until they become coarse paste. Some *Bodo* people use 2-3 teaspoons (2–2.5 mL) of local alkali (*khar*) to the concoction for faster fermentation. The coarse concoction was then tightly stuffed in hollow immature bamboo tube having single internode, leaving 6–9 cm headspace for consequent sealing. Clean, dry banana leaves are the primary sealing

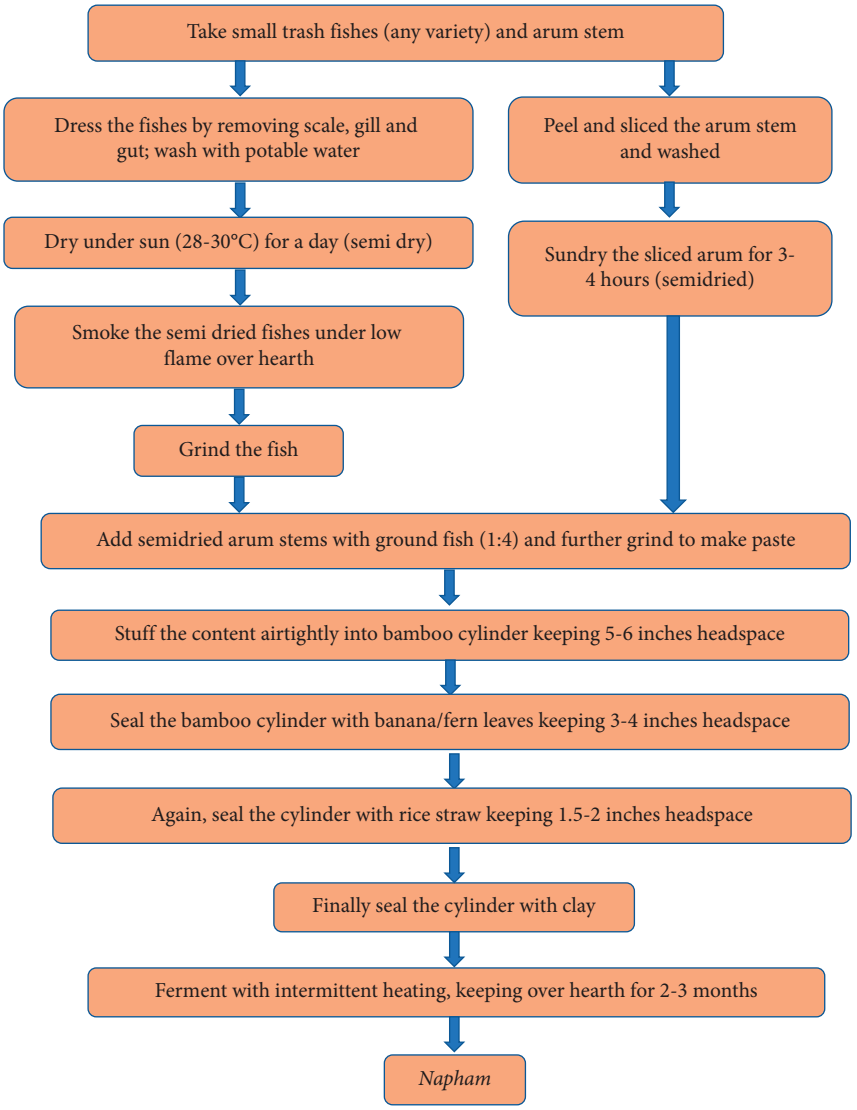
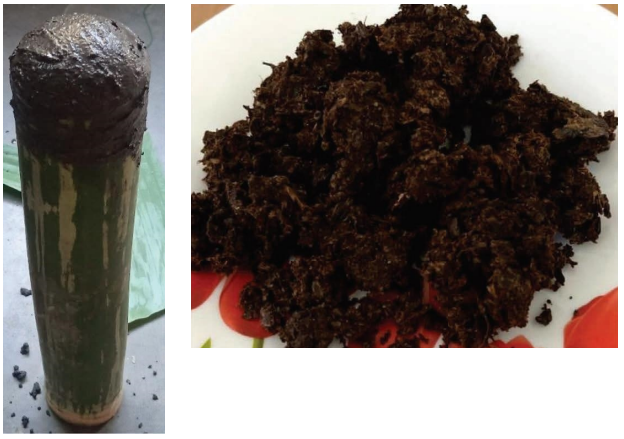


Figure 1: Flowchart of preparation of *Napham*.



(a) (b)

Figure 2: *Napham*: (a) sealed container and (b) the product.

material, and they are stuffed air tightly into the bamboo tube, leaving a headspace of 3–5 cm. The bamboo tube is finally sealed off using the clay material (the leftover of filtering local alkali) so that it prevents the entry of insects and air. Fermentation improves the palatability by softening the bones and enhancing the flavor and texture of fish. The sealed bamboo tubes are then place over hearth (a bamboo made rising platform over a kitchen oven) for 2-3 months to facilitate fermentation. The product is ready for consumption after this fermentation period (Figure 2). The sealed *Napham* can be stored for 6–8 months while store at the hearth itself or at any clean places in the room.

The authors found that several phases and features of the manufacture of *Napham* were not in accordance with the scientific principles of fish processing technology. Mechanization of the whole preparation process of *Napham* in a single unit without deviating from the original appearance, colour, taste, and smell may provide the conformity of proper hygiene and sanitation.

2.3. Proximate Analysis. Using the standard procedure of the Association of Official Analytical Chemists, the samples were analysed for their protein, fat, moisture, ash, and total carbohydrate compositions [15].

2.4. Determination of Colour. The colours of the sample were measured with a colorimeter (ColourFlex, Hunter Lab Reston, VA, USA) and recorded in lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) mode of the CIE system [16].

2.5. Estimation Minerals and Trace Elements

2.5.1. Sample Digestion. Moisture-free *Napham* (6 g) was combusted in a muffle furnace at 650°C for 6 hours to obtain white ash [15]. This white ash was further used for the analysis of mineral contents. For that, the digestion of the sample (white ash) was carried out through Kjeldahl digestion system (Kel Plus KES 08L E). One gram of sample was weighed in a dry Kjeldahl digestion tube. 10 mL of concentrated acid (3 : 1 sulfuric acid: nitric acid) and about 3 g of digestion mixture or digestion activator (copper sulfate and potassium sulfate at a ratio of 1 : 1.5 by weight) were added. Mixture was digested by heating first slowly and then vigorously between 360°C and 410°C till the colour turns into light yellow. After that, the digested samples were allowed to cool down, followed by made up of volume to 100 mL with Milli-Q water. In all the steps, not a single piece of glassware (flask, pipette, etc.) was used. All plastic containers were disinfected with 10% ultra-pure grade HNO_3, followed by thoroughly rinsed with ultra-pure water [17].

2.5.2. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Analysis. Analysis of minerals and trace elements (Fe, K, Mn, Ca, Zn, Na, and Mg) employing inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed at the Sophisticated Analytical Instrumentation Centre (SAIC) in Tezpur University, Assam, India. Digested samples were analysed using an Avio 220 Max ICP-OES system (PerkinElmer). Argon was employed as a plasma gas and nitrogen as a collision gas. Each analysis was conducted in triplicate [18].

2.6. Estimation of Vitamin Contents

2.6.1. Water-Soluble Vitamins. In this investigation, water-soluble vitamins were extracted using a technique adapted from that published by Sami et al. [19]. 2 g of dried powder of *Napham* was mixed with 25 mL of 0.1 N sulfuric acid and incubated at 121°C for 30 minutes. When it reached room temperature, the pH was adjusted to 4.5, then 50 mg of α-amylase was added, and kept for overnight at 35°C. The concoction was then filtered through Whatman No. 1 filter paper, followed by adding Milli-Q water (50 mL), and filtered once more via a syringe membrane filter (0.22 μ). That filtrate (20 μL) was then put into the HPLC apparatus (Waters, 2690, US), and the quantification of the water-soluble vitamins was accomplished by the results being compared to standards. Separation via chromatography was accomplished by using a RP-HPLC column (C18) in combination with mobile phase A: methanol (33%), B: 0.023 M orthophosphoric acid, pH = 3.54 (67%) at a 0.5 mL/min flow rate. At room temperature, UV absorption at 270 nm was noted [19].

2.6.2. Fat-Soluble Vitamins. Five gram powdered sample of *Napham* was taken into a 250 mL conical flask following the addition of 20 mL of warm water and stirred well with a magnetic stirrer. The mixtures were added with 1 g of α-amylase and put for 30 minutes at 60°C in water bath. Following that, 30 mL of ethanol, 20 mL of 50% aqueous KOH, 1 g of ascorbic acid, and 100 mg of BHT were added. To change the vitamin esters into their alcohol forms, the samples were blended once more and again put for 30 minutes at 80°C in water bath of saponification. Following the saponification procedure, the flasks were put right away in an ice bath to quickly cool to ambient temperature. The solutions were the transferred into separating funnel and stirred well by adding 30 mL of Milli-Q water and 50 mL of petroleum ether. The upper organic phases were once again poured into a new separating funnel and three times rinsed with 100 mL of Milli-Q water. The cleaned ether layer was filtered into a round flask (250 mL) by passing it through 3 g of anhydrous sodium sulfate, with the lower aqueous phase being discarded. A rotary evaporator was used to evaporate the organic layer at 40°C for up to 2 mL and then subjected to further evaporation under steam from nitrogen. The dried extracts were then reconstituted with methanol (HPLC grade) and injected into the HPLC system with appropriate filtration through a syringe filter (0.22 μ).

RP-HPLC analysis was conducted using an HPLC system that featured a diode array detector. UV detection of α-tocopherol (vit E) was measured at 290 nm using Agilent Eclipse XDB-C18 column as the stationary phase, and methanol served as the solvent [20].

2.7. Quantification of Free Sugars. Analyses of oligosaccharides and free sugars were performed using HPLC [21]. The free sugars were extracted by mixing of sample (0.5 g) with 0.08 M phosphate buffer (10 mL, pH 6.0), followed by vortexed for 2 minutes, and then stored at 4°C overnight. In the next step, 2 mL of sample extract was combined with acetonitrile (2 mL), followed by again vortexed for 2 minutes, and kept at 4°C for 10 to 12 hours. After passing the extract via a 0.20 μ membrane syringe filter, it was injected into the HPLC system. The reference standards used were glucose, sucrose, fructose, and maltose.

2.8. Estimation of Fatty Acid Profile by Gas Chromatography with Flame-Ionization Detection (GC-FID). The fatty acid profile of Napham was determined according to the method of Jarukas et al. [22]. To produce fatty acid methyl esters (FAMEs), powdered samples (1 g) were directly transesterified with 2% H_2SO_4 in methanol. FAMEs were then sorted and quantified using gas chromatography attached with a flame ionisation detector (Agilent Series, 7890 Series, USA). As a way to determine the concentration of fatty acids present in the samples, the Supelco 37 FAME Mixture (Sigma Cat. No. 47885-U) was used as a standard against which area percentages were calculated.

2.9. Estimation of Amino Acid with Amino Acid Analyser. The hydrolyzed sample, which had been dried under vacuum, was reconstituted in a solution containing 100 mM HCl and subsequently filtered through a 0.22 μm syringe-driven filter. Each free amino acids were analysed with a Biochrom 30^+ Amino Acid Analyzer (DKSH India Private Limited) equipped with a visible detector in accordance with the manufacturer's instructions. This equipment uses ion exchange chromatography (IEC) to separate amino acids and a postcolumn detector to quantify ninhydrin (NIN) reactive chemicals. The NIN-derivatized individual amino acids were detected at 570 nm [23].

2.10. Statistical Analysis. The data were analysed with a one-way ANOVA (analysis of variance) in SPSS 16.0 (SPSS software for Windows, release 16.0, SPSS, Inc., USA) and presented as the mean standard deviation (SD) of triplicate samples.

3. Results and Discussion

3.1. Proximate Composition and Colour Analysis. Most of the time, *Napham* is prepared during the monsoon season, when trash fish and arum are easy to find in the locality. After fish, arum is the second most important ingredient in *Napham*, and it makes the product thicker and increases its viscosity as well. As arum is high in starch and hence serves as a carbohydrate supplement in *Napham*. Since fish has a very low carbohydrate content, this ingredient contributes to making up for it. The bamboo container used is an eco-friendly packaging material. The raw bamboo imparts a flavor similar to bamboo shoots to the product. This flavor increases the value of the product as bamboo shoots are a favorite dish in northeastern region of India. In addition, due to its extremely tough surface, bamboo prevents pests like insects, flies, mosquitos, rodents, and even cats from damaging the product. The moisture, ash, protein, fat, and carbohydrate content of *Napham* was found to be 3.52 ± 0.37, 13.95 ± 0.02, 63.65 ± 0.83, 12.12 ± 0.15, and 6.32 ± 0.24 g/100 g (Table 1). These results are relatively different from those of Majumdar et al. [1], who reported that the proportions of moisture, protein, and fat content in *hentaak*, a fermented fish paste of Manipur, India, were 35.0 ± 1.04, 37.63 ± 0.89, and 9.91 ± 0.17 g/100 g correspondingly. Potential causes for this deviation have included the fact that we showed results on a dry-matter basis or it could be because of the different maturation periods of different fish species used. The low moisture content could be attributable to the use of sundried fish in the production of *Napham*, or it could be linked to their fat content. Generally, fresh fish contain 15–22 g/100 g of protein content, but here, the increased protein content can be ascribed to microbial production of proteins from metabolic intermediates throughout their growth cycles. *Ngari*, a fermented fish product made from *Setipinna* species, was shown to be nutrient dense in a study conducted by Sarojnalini and Suchitra [24], with average values of 36.44% moisture, 36.25% total protein, 6.66% lipids, and 10.22% ash. According to a nutritional analysis performed by Kakati et al. [25], Mowashidal was found to be of exceptionally good grade. After 6 months of fermentation, the sample had a pH of 5.8–6.5, protein concentration of 31.28–31.70%, and an ash content of 9.95–11.11%.

Results from the colour analysis of the *Napham* demonstrated that its lightness (L^*) was 37.68 ± 0.54 while its a^* (redness) and b^* (yellowness) were 1.20 ± 0.27 and 7.13 ± 0.02, respectively (Table 1). The potential cause for low L^* value indicates the formation of a darker colour with browning pigments resulting from the occurrence of the Maillard reaction during the process of fermentation. The observed minimal values of a^* and b^* could perhaps be attributed to the degradation of carotenoids within the product. There is no publicly available information characterising the colour of *Napham*; however, research on *Nham*, a Thai-fermented sausage aged for 84 days, has revealed $L^*a^*b^*$ values of up to $55.09 + 0.97$, $7.80 + 0.21$, and $5.74 + 0.97$, respectively [26].

3.2. Mineral Content. Mineral contents of *Napham* are presented in Table 2. Sodium levels were determined to be the maximum at 10642.40 ± 219.83 mg/kg, while iron was the lowest at 11.27 ± 0.40 mg/kg in the *Napham* sample. Calcium, potassium, magnesium, manganese, and zinc contents were found to be 8346.40 ± 182.80, 8302.00 ± 179.28, 251.84 ± 1.74, 40.88 ± 0.06, and 26.14 ± 0.35 mg/kg, respectively. The use of the entire fish, including the bones, in the fermentation process enhanced the ash content of the final product, which in turn contributed to a moderate amount of mineral content. The fact that it contains moderate levels of sodium, calcium, and potassium indicates that it is a good provider of dietary minerals. Low iron, zinc, manganese, and magnesium levels; conversely, this deficiency may be offset by other dietary sources that provide these crucial elements to the body.

Table 1: Proximate composition and colour of *Napham* (dry matter basis).

Proximate composition	Leaf	Unit
Moisture	3.52 ± 0.37	g/100 g
Ash	13.95 ± 0.02	g/100 g
Protein	63.65 ± 0.83	g/100 g
Crude fat	12.12 ± 0.15	g/100 g
Carbohydrate	6.32 ± 0.24	g/100 g
Colour		
Lightness	37.68 ± 0.54	L^*
Redness	1.20 ± 0.27	a^*
Yellowness	7.13 ± 0.02	b^*

Data presented are mean ± SD.

Table 2: Mineral content of *Napham*.

Minerals	Content (mg/kg)
Iron (Fe)	11.27 ± 0.40
Potassium (K)	8302.00 ± 179.28
Manganese (Mn)	40.88 ± 0.06
Calcium (Ca)	8346.40 ± 182.80
Zinc (Zn)	26.14 ± 0.35
Sodium (Na)	10642.40 ± 219.83
Magnesium (Mg)	251.84 ± 1.74

Values are presented as mean ± SD.

Hentak, a similar product to *Napham* was reported to have the highest content of calcium (472.11 ± 62.7 mg/100 g), followed by sodium (94.0 ± 12.78 mg/100 g), potassium (75.74 ± 6.62 mg/100 g), and magnesium (21.125 ± 3.78 mg/100 g), whereas iron, copper, and zinc were found to be less [1]. On the other hand, Giri et al. [27] observed that fermented fish pastes prepared by employing various koji moulds as starters had sodium potassium, calcium, magnesium, phosphorus, and iron concentrations of 3341 mg/100 g, 69 mg/100 g, 50 mg/100 g, 21 mg/100 g, 57 mg/100 g, and 1 mg/100 g correspondingly.

3.3. Vitamin Content. The HPLC chromatogram for water-soluble and fat-soluble vitamins of *Napham* is presented in Figure 3. Riboflavin level was found to be the highest among water-soluble vitamins at 78.22 mg/100 g, whereas thiamine content was determined to be minimal at 0.65 mg/100 g. The quantitative HPLC analysis of fat-soluble vitamins revealed that α-tocopherol was the most abundant, followed by calciferol, with detection limits of 9.36 mg/100 g and 6.66 mg/100 g, respectively (Table 3).

3.4. Composition of Free Sugars. Among the free sugars, sucrose content was found to be maximum with 347.35 ± 2.48 mg/100 g, followed by fructose (266.69 ± 26.73 mg/100 g), maltose (235.16 ± 26.44 mg/100 g), and glucose (9.40 ± 0.19 mg/100 g) (Table 4). Reference [28] similarly observed comparable outcomes with 74.144.4% glucose, 16.12.7% maltose, and 14.766.1% fructose in their soy paste.

3.5. Fatty Acid Profiling. The chromatogram of fatty acid composition of *Napham* is shown in Figure 4. The total saturated fatty acids content was 43.74 ± 0.136% with highest content of palmitic acid (30.20 ± 0.145%), followed by stearic acid (9.15 ± 0.081%), myristic acid (1.82 ± 0.019%), pentadecylic acid (1.12 ± 0.012%), arachidic acid (0.48 ± 0.027%), lauric acid (0.44 ± 0.008%), behenic acid (0.33 ± 0.032%), and lignoceric acid (0.19 ± 0.027%). Both oleic acid (22.02 ± 0.033%) and palmitoleic acid (6.31 ± 0.040%) were found to be the most abundant monounsaturated fatty acids. Among the polyunsaturated fatty acids (PUFAs) linoleic acid (10.10 ± 0.002%), α-linolenic acid (9.90 ± 0.012%), arachidonic acid (4.65 ± 0.031%), eicosapentaenoic acid (EPA) (1.08 ± 0.007%), and docosahexaenoic acid (DHA) (3.44 ± 0.036%) are dominated (Table 5). *Napham*'s nutritional value is attested to by the presence of omega-3 (α-linolenic acid, EPA, and DHA) and omega-6 fatty acids (linoleic acid); however, the product may lose some of these polyunsaturated fatty acids (PUFAs) during fermentation and afterward its exposure to air. Both lipolytic and proteolytic enzymes as well as microbes are involved in the fermentation of traditional fermented fish products [29]. According to earlier research, fermentation has a significant impact on the fatty acid content of fish [30]. For example, compared to raw fish, Mohamed [30] found that fermented tiger fish had a greater proportion of saturated fatty acid and a lower percentage of monounsaturated fatty acid and polyunsaturated fatty acid. In fermented Chinook salmon (*Oncorrhynchus tshawytscha*) roe saturated fatty acid (SFA) content decreased, monounsaturated fatty acid (MUFA) content did not change, whereas polyunsaturated fatty acid (PUFA) content dramatically increased during fermentation [31]. Majumdar et al. [1] reported a higher content of palmitic acid in *Ngari*, whereas stearic acid is reported to be higher in *hentaak*. *Ngari* was reported to have both omega-3 and omega-6 fatty acids, whereas *hentaak* was shown to contain exclusively omega-3 fatty acids. The n-6 polyunsaturated fatty acids detected in *Ngari* were linoleic acid (11.68%) and arachidonic acid (0.65%); however, *hentaak* solely contained arachidonic acid (8.54%).

3.6. Free Amino Acids. Complete free amino acid profile of *Napham* was determined using an amino acid analyzer and presented in Table 6. Fifteen (15) free amino acids (asparagine, glutamic acid, glutamine, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, and histidine) were found in *Napham* (Figure 5). Amino acids have a crucial role as constituents of proteins, which are needed for the processes of growth, repair, and maintenance of tissues in the body. In the context of fermented fish products, the amino acid content might furnish insights about the nutritional significance of the product. The investigation of the amino acid composition of *Napham* indicated the presence of seven (7) essential amino acids and eight (8) nonessential amino acids. Out of the essential amino acids found in considerable amount were

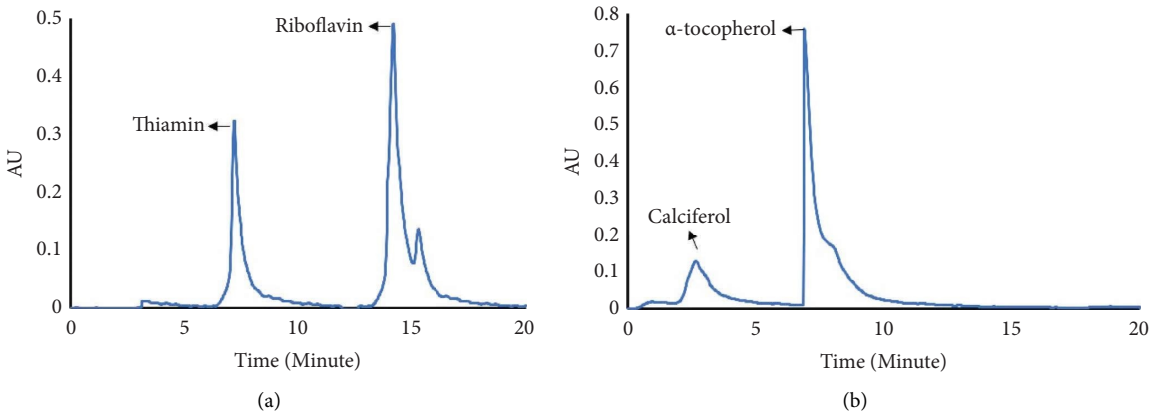


Figure 3: HPLC chromatograms of ethanolic extract of *Napham* for detection of vitamin content: (a) water-soluble vitamin and (b) fat-soluble vitamin.

Table 3: Vitamin content detected by HPLC in the extracts of *Napham*.

Sl. no.	Vitamin content	Retention time (min)	Concentration (mg/100 g)
1	Calciferol	2.684	6.66
2	α-Tocopherol	6.773	9.36
3	Thiamine	7.3	0.65
4	Riboflavin	14.372	78.22

*ND: not detected.

Table 4: Free sugar composition in *Napham*.

Carbohydrate composition	Content	Unit (g)
Glucose	9.40 ± 0.19	mg/100
Fructose	266.69 ± 26.73	mg/100
Sucrose	347.35 ± 2.48	mg/100
Maltose	235.16 ± 26.44	mg/100

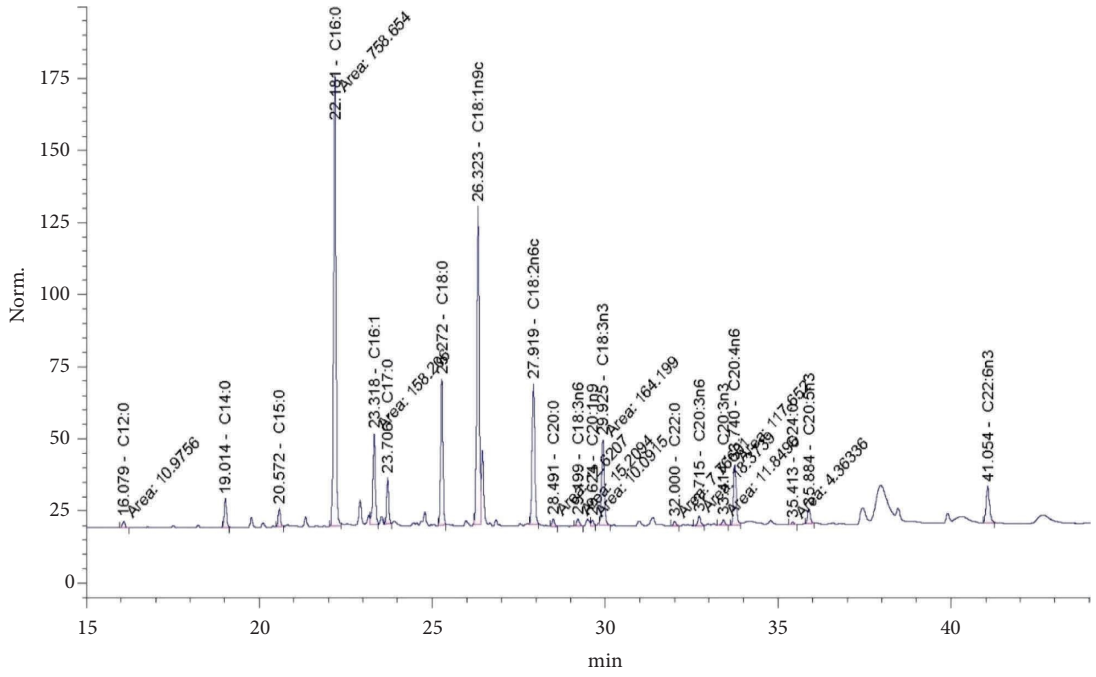


Figure 4: GC-FID chromatograms of *Napham*.

Table 5: Fatty acid composition (% of total fatty acids) of *Napham*.

Fatty acids	Content (%)
Lauric acid (C12 : 0)	0.44 ± 0.008
Myristic acid (C14 : 0)	1.82 ± 0.019
Pentadecylic acid (C15 : 0)	1.12 ± 0.012
Palmitic acid (C16 : 0)	30.20 ± 0.145
Stearic acid (C18 : 0)	9.15 ± 0.081
Arachidic acid (C20 : 0)	0.48 ± 0.027
Behenic acid (C22 : 0)	0.33 ± 0.032
Lignoceric acid (C24 : 0)	0.19 ± 0.027
Palmitoleic acid (C16 : 1)	6.31 ± 0.040
Oleic acid (C18 : 1n9c)	22.02 ± 0.033
Cetoleic acid (C20 : 1n9)	0.42 ± 0.025
Linoleic acid (C18 : 2n6c)	10.10 ± 0.002
Gamma linolenic acid (C18 : 3n6)	0.60 ± 0.010
Alpha linolenic acid (C18 : 3n3)	9.90 ± 0.012
Dihomo-gamma-linolenic acid (C20 : 3n6)	0.73 ± 0.002
Eicosatrienoic acid (C20 : 3n3)	0.46 ± 0.021
Arachidonic acid (C20 : 4n6)	4.65 ± 0.031
Eicosapentaenoic acid (C20 : 5n3)	1.08 ± 0.007
Docosahexaenoic acid (C22 : 6n3)	3.44 ± 0.036
TSFA	43.74 ± 0.136
TMUFA	28.74 ± 0.032
TPUFA	27.51 ± 0.168

*values are mean ± SD; TSFA = total saturated fatty acid; TMUFA = total monounsaturated fatty acid; TPUFA = total polyunsaturated fatty acid.

lysine (1066.557 μmol/L), followed by leucine (797.944 μmol/L), valine (640.867 μmol/L), isoleucine (478.525 μmol/L), phenylalanine (320.573 μmol/L), methionine (227.407 μmol/L), and histidine (104.525 μmol/L). The nonessential amino acid as the highest concentration found was alanine (1333.219 μmol/L), followed by glycine (1198.105 μmol/L), asparagine (1191.899 μmol/L), proline (830.086 μmol/L), glutamic acid (573.685 μmol/L), tyrosine (246.966 μmol/L), cysteine (39.242 μmol/L), and glutamine (11.402 μmol/L) (Table 6). The significance of these amino acids lies in their functions in diverse physiological processes within the human body, encompassing the synthesis of enzymes, hormones, and neurotransmitters. Hence, the presence of amino acids in fermented fish products can enhance their nutritional value and offer possible health advantages. Fish fermentation occurs as a result of microbial or enzymatic activity within the fish, leading to the decomposition of organic matter into simpler compounds such as peptides, amino acids, and various nitrogenous compounds [32]. Pla-ra, a fermented fish product of Thailand, contains 9 nonessential amino acids and 9 essential amino acids with glutamic acid having the largest percentage, followed by lysine and leucine, respectively [33]. Total amino acid content in the fermented Mackerel Sausage (*Rastrelliger kanagurta* Cuvier) was found to be 10,940.85 mg/100 g, which was higher than the total amino acid content in fresh mackerel [34]. *Ngari*, a traditional fermented fish delicacy originating from Northeast India, has a significant abundance of various amino acids, such as glycine, proline, and aspartic acid. In addition, it contains essential amino acids including phenylalanine, leucine, and lysine. In contrast, the analysis revealed that hentaak, a type of smoke-dried fermented fish product resembling *"Napham"*, contains a significant amount of glycine, alanine, proline, aspartic acid, glutamic acid, as well as essential amino acids such as phenylalanine, lysine, and leucine. Several nonessential amino acids, including glutamic acid, aspartic acid, and glycine, have been identified as contributing to the formation of taste attributes in fermented fish products. The *Ngari* and hentaak samples exhibited a respective proportion of 39.6% and 44.1% of essential amino acids in relation to the overall amino acid content [1]. The study conducted by Peralta et al. [32] revealed that the process of lengthy fermentation of prawn paste led to a significant increase in the overall concentration of free amino acids. This increase was observed mostly during the initial stages of fermentation, followed by a period of stability throughout the middle stages. However, as the fermentation process continued, there was a subsequent drop in the concentration of free amino acids. The decrease in the concentration of free amino acids may be attributed to their breakdown into amines, volatile acids, and other nitrogenous compounds as byproducts of bacterial metabolism or enzymatic degradation. The concentration of ammonia, which serves as an indicator of deterioration, has shown a clear increase throughout extended fermentation, as evidenced by the rise in odour intensity. The decrease in amino acids observed can potentially be attributed to the generation of Maillard reaction products, which would be evident by an increase in both brown colouration and fluorescence intensity. Shidal, another fermented fish product originating from Northeast India, has been identified as a significant reservoir of various amino acids, such as glutamic acid, aspartic acid, leucine, alanine, and lysine. Nevertheless, the presence of several amino acids, including tyrosine, histidine, arginine, and tryptophan, was observed in relatively limited quantities, whereas proline was not discovered. During the process of fermentation, it is conceivable that derivatives of amino acids, including amines and compounds involved in gluconeogenesis, may be generated [35]. Taste attributes of fermented fish and shellfish products have been associated with elevated levels of nonessential amino acids such as glutamic acid and glycine [1].

3.7. Antimicrobial Activity. Previous research indicates that probiotic bacteria isolated from the products similar to those of *Napham* have antimicrobial activities [36–38]. However, no overall research has been published suggesting that any kind of similar product to that of *Napham* possesses antimicrobial activities in its product properties. The authors are also currently focusing on validating the antimicrobial properties of the predominant bacterial isolates from *Napham*, as well as isolating and identifying probiotic bacteria associated with the fermentation process.

Table 6: Free amino acid content of *Napham*.

Sl. no.	Amino acid standards	Retention time (min)	Concentration (μmol/L)
Nonessential			
1	Asparagine	32.700	1191.899
2	Glutamic acid	33.900	573.685
3	Glutamine	36.167	11.402
4	Proline	45.433	830.086
5	Glycine	46.867	1198.105
6	Alanine	48.500	1333.219
7	Cysteine	59.600	39.242
8	Tyrosine	75.467	246.966
Essential			
9	Methionine	64.000	227.407
10	Isoleucine	69.333	478.525
11	Leucine	71.167	797.944
12	Valine	55.267	640.867
13	Phenylalanine	79.633	320.573
14	Lysine	104.800	1066.557
15	Histidine	109.067	104.525
	Total nonessential amino acid		5424.604
	Total essential amino acid		3636.398

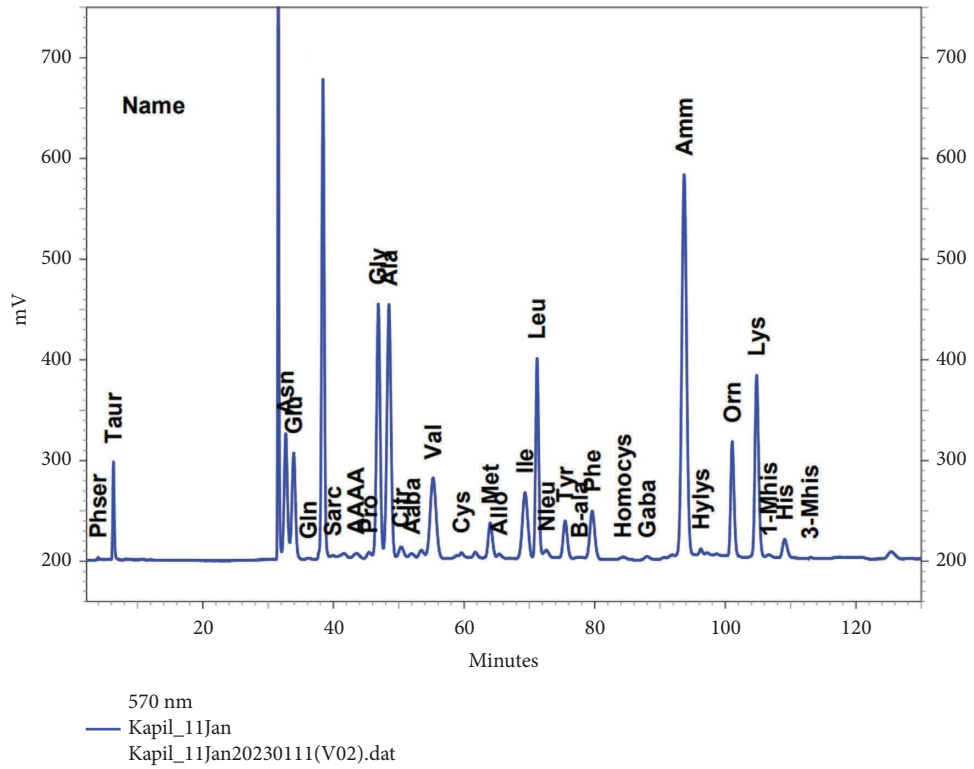


Figure 5: Amino acid analyzer chromatograms of *Napham*.

4. Conclusion

Fermentation resulted in higher nutritional quality (protein, fat, carbohydrates, and ash). However, as fermentation progressed, the pH and acceptability variables (colour, aroma, taste, and texture) dropped. Overall, the fermentation of *Napham* contributed to the increased composition of fatty acids and amino acids. The tribal communities in northeast India have a strong cultural attachment to traditional fermented fish products, which have long been recognized for their health benefits. Among the Bodo people in this region, *Napham*, a fermented fish paste, holds a special place in their diet. The unique flavor of *Napham* has become well-liked by the tribe, and its high nutritional value has contributed to positive health outcomes. The findings of this study demonstrate that *Napham* is highly nutritious due to its abundant protein, vitamins, minerals, free amino acids, and essential fatty acids. These bioactive components likely contribute to its widespread consumption within the indigenous community, serving both nutritional and medicinal purposes. While this research provides an initial understanding of *Napham*, further investigations are necessary to explore its medicinal benefits and elucidate the underlying mechanisms involved. This study aims to serve as a foundation for knowledge about *Napham* and encourage additional research into its potential value.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Kapil Deb Nath conceptualized the study, designed the experiments, contributed to the methodology, contributed to data curation, and wrote, reviewed, and edited the article. C. Nickhil performed investigation and wrote and edited the article. Debasish Borah performed investigation. R. Pandiselvam performed investigation, provided resources, wrote the original draft, and reviewed the article. Sankar Chandra Deka performed investigation and contributed to final checking of manuscript. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

This work was supported by the Assam Science Technology and Environment Council, Bigyan Bhawan, G. S. Road, Guwahati-781005, Assam, India.

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