Analysis of Minerals, Amino acids and Fatty acids in Fermented Fish *Napham* traditionally prepared by Bodo ethnic group of Kokrajhar, Assam

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Abstract:

Napham is the traditional fermented fish product of Bodo ethnic community of Assam. The present work tries to estimate the mineral composition, amino acid, fatty acid and microbial composition of *Napham*. The mineral content shows that *Napham* is good source of Ca (68.90±0.16 µg/ml), Na (67.26±0.150 µg/ml), Mg (32.20±0.9 µg/ml), K (10.68±0.74 µg/ml) and Fe (9.10±0.30 µg/ml). Analysis of amino acid revealed that *Napham* consisted of 32% essential amino acids, 40% non-essential amino acid and others included 28% out of total 25 amino acids. The fatty acid profile shows that amongst saturated fatty acid composition, Pentadecanoic acid (C15:0) contribute 50% of the total fatty acid followed by Tetradecanoic acid (C14:0)24.67% and Tridecanoic acid (C12:0) about15.04%. Amongst monoenoic acid 4-Octadecenoic acid (C18:1n-14) contributes about 3.65 %.

Key words: ***Fermentation,Fermented fish, Napham, minerals, fatty acids, amino acid***

Introduction:

Fermented fish products are very popular in most parts of South-East Asia, generally as a condiment for rice dishes. The NE region of India is bestowed with many fermented fish products. These traditionally preserved fish products are: *gnuchi* and *suka ko maacha*, *sidra* and *sukuti* of Darjeeling hills, and Sikkim; *ngari* and *hentak* of Manipur; *tungtap* of Meghalaya; *karati*, *bordia*, and *lashim* of Assam; and *shidal* of Tripura (Thapa.N., 2016). Fish consist of important sources of proteins, lipids, minerals and vitamins which have high nutritional value that give several benefits to human health. Trial and error experiments contribute to develop many indigenous techniques and practices for processing and preserving foods at community level. Traditionally fishes are preserved by techniques like smoking, drying, salting, fermentation, marinating etc. Most of these processes extend the shelf life by retarding the microbial activity due to reduction in moisture content, changing the pH or other physiological parameters. Fermentation have evolved as the preferred method of preservation by the local ethnic communities of North East due to high incidence of rainfall in the region that makes other process of preservation like drying difficult in the region. Indigenous methods and solutions applied are culturally acceptable, economically practicable, and more appropriate for the local environment and conditions than modern techniques and solutions suggested by scientific experts. Fermented fish products are special because of their ability to provide a certain unique characteristic, especially in terms of aroma, flavor, and texture. This may be credited to the ability of micro-organisms or their enzymes to transform organic materials encountered in fish muscle tissue into compounds which are simpler during the fermentation process (Beddows, 1998). The specific flavor generated can induce appetite and because of this characteristic fermented fish products are liked by its consumers.

Napham is a fermented fish product prepared by the Bodo tribe of Assam. Like all cultures of world Bodos too have its own ethnicity and food habits. This fermented fish product is mostly prepared by the womenfolk of this tribal populace. The raw materials used in preparation of *napham* are small locally available fishes, tender shoots of Arum (*Colocasia esculanta*), hollow cylinder of matured *Bambusa balcoa* stem, kharai, and banana leaf/lemon leaf. Small fishes are firstly gutted, cleaned and then dried in sun for about two hours till the water is completely drained and dehydrated. The semi dried fishes are then smoked under low flame by burning dry chaff of rice grain till they are fully dried. This dried fish is then pounded with the help of mortar and pestle known as *uwal* and *gaihen* in local *Bodo* dialect. (Narzary, et al, 2016). At the time of pounding, the stems of arum are also added. During documentation it was also discovered that sometimes vegetables like kumra (Ash gourd), maitha (*Hibiscus sabdariffa*), cabbage, radish leaves etc. are also added. This vegetables or plant products are added to add moisture to the fish paste. After the mixture is ready, the paste is transferred to the container made of hollow bamboo stem that is open from one side and closed from other side by inter-node. The fish paste is then covered with khar (*Musa*) leaf and the opening of the bamboo container is sealed with clay paste prepared by mixing with straw. It is sealed tightly to ensure anaerobic condition and the whole preparation is then kept for 2to 3 months for fermentation. *Napham* is one of the lesser known fermented fish product of Assam which can equate to other similar and more popular products like *shidol & Chutki mash* etc. The present work tries to estimate the amino acid, fatty acid and mineral composition of *Napham*.

2. Methodology:

2.1 Collection of material and analysis of minerals:

Samples were collected from markets of Kokrajhar town, Debargaon, Dotma Bongshigaon, Bongaigaon market, Jornagri area of Kokrajhar district and adjoin area and brought in the laboratory. The samples were stored in ambient temperature. Trace elements was detected by Graphite Furnace-Atomic Absorption Spectrometer (GF-ASS) Model: Analytic Jena Vario-6

2.2 Analysis of amino acid:

The sample was hydrolyzed by 6N hydrochloric acid hydrolysis. For the analysis of amino acid in10 ml of the hydrolyzed sample 40 ml of methanol was added and incubated overnight at -20˚ C. After overnight incubation sample was centrifuged and the supernatant was taken for evaporation. The sample was evaporated completely under N_2 gas at 60˚C using dry bath. 70 µL of PITC reagent was added to the sample, vortexed and placed on thermo mixer for 1 hour at 45˚ C. Then the samples were vacuum dried. To the pellet 200 µL of buffer A (10Mm sodium acetate p^H: 6.4) was added and centrifuged. The supernatant was collected and filtered using syringe filters. 20 µl of the sample was loaded into HPLC for the analysis. AGILENT (make) reverse phase HPLC with the model 1200 series.

2.3 Analysis for fatty acid profiles:

Lipids were extracted from the sample by the method of Bligh and Dyers (1959) and methylated as described in AOAC (2001). Lipid samples were derivatized using BF3 Methanol reagent, followed by vortexing and heating at 60˚C for 20min. The sample was cooled on ice and was extracted within 5minutes using chloroform. The extraction was repeated thrice and the chloroform layer was pooled. The resultant is then reduced to dryness in vacuum. The samples were re-dissolved in chloroform and 1µl is loaded on to the column.

2.3.1 Instrumentation:

The GC-MS system consisted of an AOC-20i auto injector (SHIMADZU), a GC -2010 gas chromatograph, and GCMS-QP 2010 quadruple mass spectrometer (SHIMADZU). GC was performed on a DB-5 column with 30m length, 0.25 mm i.d. and 0.25 m film thickness (Supelco). Injection temperature was 200C, the interface set to 280C, and the ion source adjusted to 200C. The carrier gas used was helium set at a constant flow rate of 1 ml/ min. The temperature program was oven temperature to 100 C, hold for 4min. Temperature is increased at a rate of 5C, till reaches 250C and hold for 10min.Gradually increased at a rate of 5C, till reaches 280C and hold for 4min. Mass spectra were recorded at two scans per second with a mass-to-charge ratio 50 to 600 scanning range.

3. Result and discussion:

3.1 Mineral composition:

The mineral content shows that *Napham* is good source of Ca 68.90±0.16 µg/ml, Na 67.26±0.150 µg/ml, Mg 32.20±0.94 µg/ml, K10.68±0.74 µg/ml and Fe 9.10±0.30 µg/ml. The quantitative composition of other 12 minerals are given in table no.1

Table1: Mineral composition of *Napham*

Sl no.	Elements	Concentration in µg/ml
1	Fe	9.10±0.30
2	Co	0.21±0.006
3	Cu	4.71±0.02
4	K	10.68±0.74
5	Mn	2.85±0.001
6	Cr	0.19±0.001
7	Ni	0.13±0.09
8	Ca	68.90±0.16
9	Zn	3.18±0.004
10	Ag	0.07±0.001
11	As	ND
12	Mo	0.08±0.006
13	Na	67.26±0.150
14	Cd	0.079±0.009
15	Pb	0.16±0.07
16	Mg	32.20±0.94

3.2 Amino acid composition:

Analysis of amino acid revealed that *Napham* consisted of 32% essential amino acids,40% non-essential amino acid and others included 28% out of total 25 amino acids.(Fig1)The essential amino acids (Munro and Crim ,1988) found in *Napham* were Tryphtophan (0.03%), Valine3-Methyl histidine (0.9%), Lysine(0.91%), Leucine (0.97%), Isoleucine (1.04%), Methionine (1.10%), Histidine (14.24%).Out of these the essential amino acid found in considerable amount were histidine with 23µg/ml followed by Methionine with 1.8 µg/ml and Isoleucine with1.7 µg/ml. The non essential amino acid found were Arginine(0.62%), Aspargine (1.26%), Glycine(1.60%), Alanine (1.90%), Glutamic acid (2.39%), Tyrosine (2.95%), Carnosine (3.1%), Aspartic acid(5.07%), Proline OH(32.17%), Proline(11.29%).The non essential amino acid as the highest composition

consist of OH Proline with 52 µg/ml followed by Proline with 18.28 µg/ml and Aspartic acid with 8.203 µg/ml. Other intermediate compounds includes Phoshoserine (0.86%), Cystathionine (1.36%), Ornithine (0.4%), Taurine (1.70%), Amino adipic acid (12.21%), Anserine (0.22%), Phosphoenolamine (1.05%). In related product *Ngari*, a fermented fish of Manipur, NE India, glycine, proline, aspartic acid and the essential amino acids phenylalanine, leucine, lysine (4.95, 3.15, 3.64, 3.23, 2.46 and 3.00%dry weight respectively) were detected. Where as in *hentaak* glycine, alanine, proline, aspartic acids, glutamic acid and the essential amino acids phenylalanine, lysine, leucine (5.72, 4.09, 4.45, 3.84, 3.35, 4.91, 3.81 and 4.79 % dry weight respectively) were reported (Majumdar R K. et al,2015). In shidal, Majumdar et al (2009) reported that it contained more of non-essential amino acids such as aspartic acid, glutamic acid, alanine (7.71, 14.15, 7.55 g/100 g protein respectively) as compared to essential amino acids like valine, isoleucine, leucine, phenylalanine and lysine (4.51, 4.19, 6.81, 4.14 and 6.16 g/100 g protein respectively). The composition of amino acid in *Napham* is given in table no.2.

Amino acid	**Types**	**µg/ml of sample**
Arginine	Non-essential	0.999
Aspargine	Non-essential	2.046
Glycine	Non-essential	2.583
Alanine	Non-essential	3.069
Glutamic acid	Non-essential	3.876
Tyrosine	Non-essential	4.780
Carnosine	Non-essential	5.024
Aspartic acid	Non-essential	8.203
Proline	Non-essential	18.286
OH Proline	Non-essential	52.089
Total Non-essential amino acid=100.95 µg/ml		
Anserine	Others	0.363
Ornithine	Others	0.477
Phoshoserine	Others	1.391
Phosphoenolamine	Others	1.697
Cystathionine	Others	2.198
Taurine	Others	2.759
Amino adipic acid	Others	19.767
Others =28.65 µg/ml		
Tryphtophan	Essential	0.0491
Valine	Essential	1.228
3-Methyl histidine	Essential	1.464
Lysine	Essential	1.481
Leucine	Essential	1.572
Isoleucine	Essential	1.681
Methionine	Essential	1.786
Histidine	Essential	23.066
Total Essential amino acid=32.33 µg/ml		

Table2: Amino acid composition of *Napham* µg/ml of sample

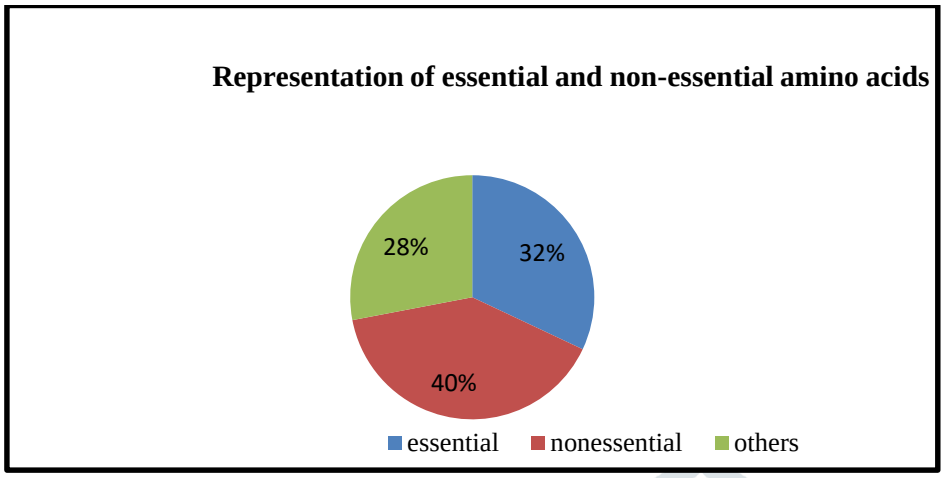

Figure1: Representation of essential and non-essential amino acid of *Napham*

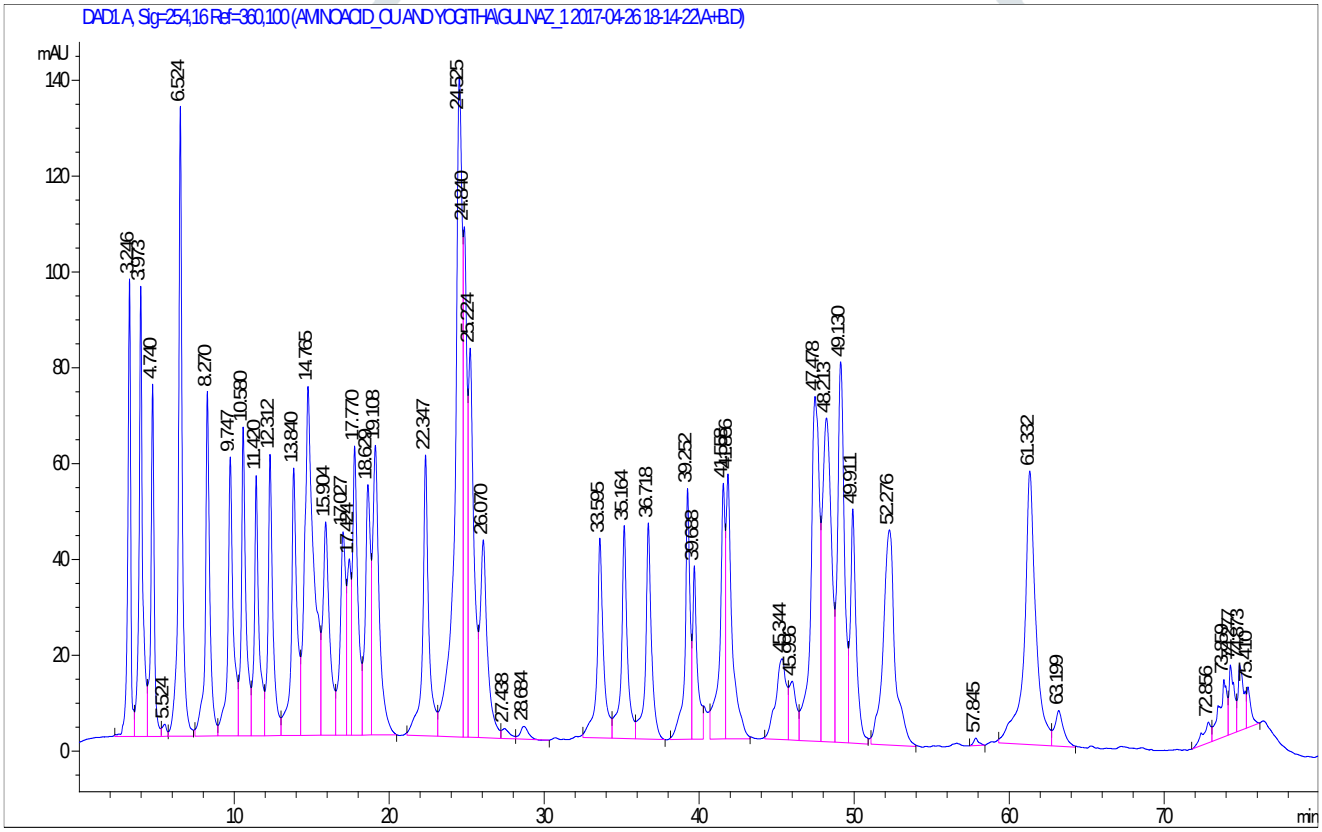

Figure2:HPLC Standard Chromatogram

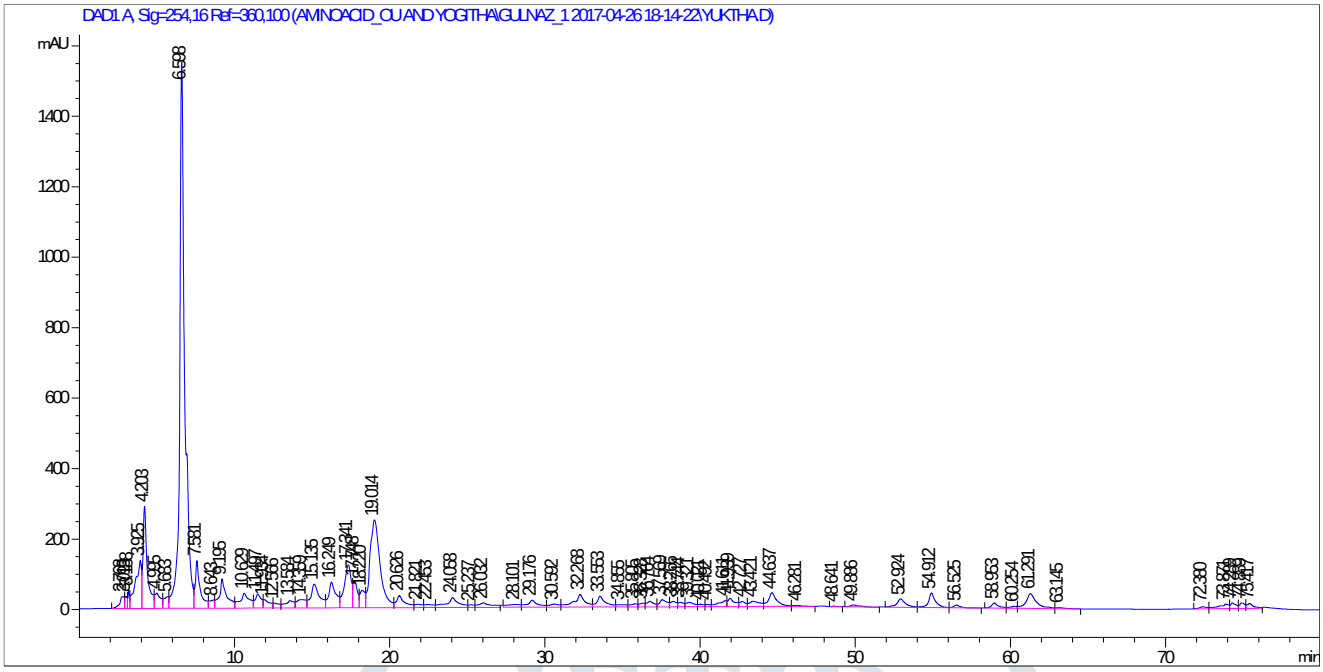

Figure3: HPLC Chromatogram of *Napham*

3.3 Fatty acid profile:

The quality and properties of natural fats are portrayed by the chemical structures of fatty acids. The fatty acids were expressed as percentages of total methyl esters (Fig2). The fatty acid profile shows that amongst saturated fatty acid composition Pentadecanoic acid (C15:0) contribute 50% of the total fatty acid followed by Tetradecanoic acid (C14:0)24.67% and Tridecanoic acid (C12:0) about15.04%. Amongst monoenoic acid 4-Octadecenoic acid (C18:1n-14) contributes about 3.65 %.

In *ngari* (C16:0) was found to be dominant and contributed about 7% of the total fatty acids; whereas, stearic acid (C18:0) was dominant in case of *hentaak* contributing to 25 % of the total fatty acids. Amongst the monoenoic fatty acids, vaccenic acid (C18:1n-7) contributed about one third (29.23 %) of the total fatty acids followed by oleic acid (23.58 %) in ngari; while, in case of hentaak, monoenoic fatty acids were dominated by C16:1n-5 which contributed 11.75 % of total fatty acid followed by oleic acid (11.40 %)(Majumdar R K. et al,2015).

Some odd carbon number fatty acids and volatile compounds were also found in the product. A total of 15 volatile compounds were identified, including ethers, acids, esters, hydrocarbons, pyrazines, phenols, benzenes and others which may be the intermediate compounds during fermentation process. There is possibility that such odd carbon chain fatty acids might be from the microbes involved in fermentation. Some of the dominant compounds are Propenoic acid (29.53%), Retalin (21.12%),Orixane (10.71%) and Octadecane, 1-Chloro- (7.84%).The unpleasant, pungent and rancid odour may be due to such compounds. In Chinese traditional fermented shrimp the pungent and rancid odour was reported due to propanoic acid, butanoic acid, furans and 2-hydroxy-3-pentanone.A total of 62 volatile compounds were identified, including alcohols, aldehydes, ketones, ethers, acids, esters, hydrocarbons, pyrazines, phenols, and other compounds (Fan, Y. et al 2017). Ritalin being one of the most commonly known, is a central nervous system (CNS) stimulant of the phenethylamine and piperidine classes that is used in the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy. (Markowitz JS, et al, 2003)

Sl no.	Name of the compound	RT	M/Z	AREA	SI	%
1	Undecanoic acid C11:0	11.919	74	491774	94	1.22%
2	Dodecanoic acidC12:0	15.901	74	1228064	76	3.04%
3	Eicosanoic acidC20:0	13.499	74	142894	68	0.35%

4	Tridecanoic acidC13:0	16.757	74	6080868	99	15.04%
5	Tetradecanoic acidC14:0	18.994	74	9977306	99	24.67%
6	Pentadecanoic acidC15:0	20.38	74	20544973	98	50.80%
7	Stearic acid C18:0	9.374	74	116454	55	0.29%
8	Octanoic acidC8:0	17.73	73	138932	75	0.34%
9	Dotriacontanoic acidC32:0	15.987	57	243784	70	0.60%
10	4-Octadecenoic acidC18:1n-14(MUFA)	19.93	104	1477278	78	3.65%
	Total fatty acid=83.45%					
1	3-Ethyl-3-Methylheptane	2.817	71	213666	82	2.66%
2	Oxirane, [(Dodecyloxy)Methyl]-	3.755	83	35795	80	0.45%
3	Ritalin	5.977	91	1693396	86	21.12%
4	Benzene	7.6	175	487660	92	6.08%
5	Dl-Valine	7.761	72	209359	87	2.61%
6	Tridecane	8.7	57	178410	87	2.22%
7	Dichloroacetic Acid	9.087	69	126828	91	1.58%
8	3-Octadecene-1,2-Diol	9.768	73	61123	75	0.76%
9	Dl-Leucine	9.861	86	258833	83	3.23%
10	1-Hexadecene	11.066	55	167442	97	2.09%
11	Tetradecane	11.266	57	487326	95	6.08%
12	Octadecane, 1-Chloro-	14.773	104	628407	87	7.84%
13	1-Hexadecene	15.987	57	243784	96	3.04%
14	2-Propenoic Acid	18.308	55	2368545	97	29.53%
15	Oxirane, [(Hexadecyloxy)Methyl]-	18.433	57	858889	80	10.71%

Other volatile compounds=16.55%

Table 3: GC-MS:Fatty acid and other volatile compound profile.

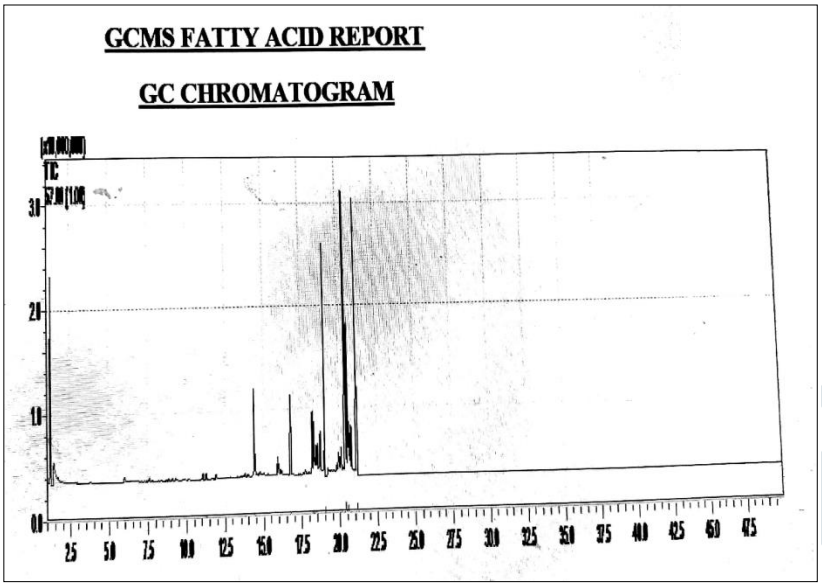

Figure4: GC-chromatogram of fatty acid composition of napham

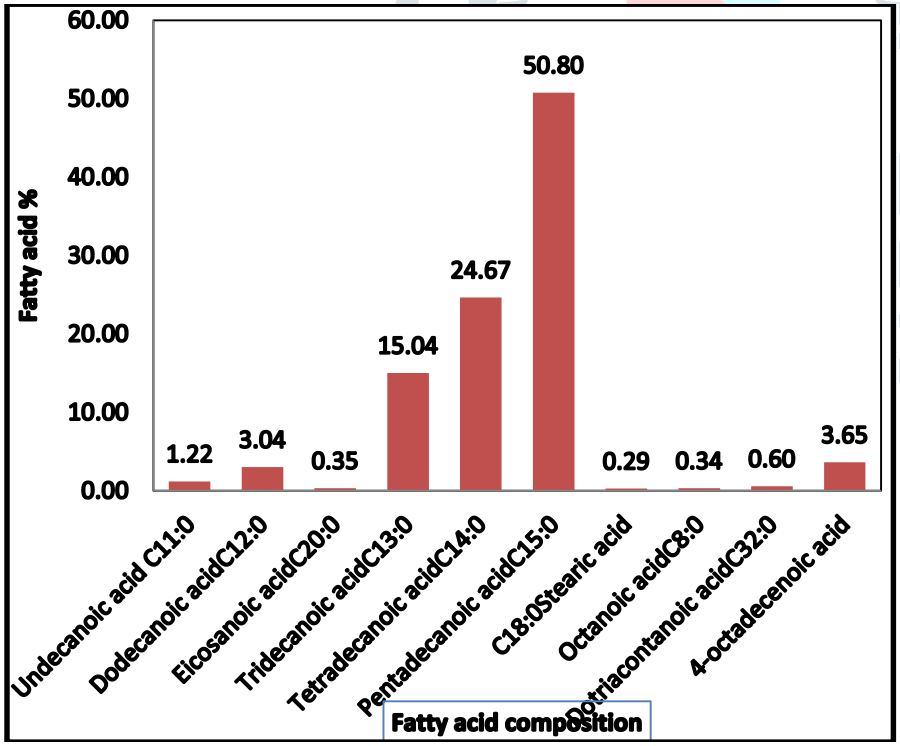

Figure5: Graphical representation of fatty acid composition of *Napham*

4. Conclusion:

From the present study it can be concluded that *napham* is good source of several minerals and essential amino acids. Fatty acid profile shows PUFA and MUFA are less as compared to the saturated fatty acid. This may be due to the fermentation process and activity of microbes that are responsible for biotransformation of fatty acid into several intermediate products. These intermediate products as shown in table 3 are ethers, acids, esters, hydrocarbons, pyrazines, phenols, benzenes which gives unique characteristic, especially in terms of aroma, flavor, and texture to *napham*.

This product is livelihood generating product and several Bodo women are making this product and selling it in markets for small economic gain. It is also a traditionally important cuisine of Bodo ethnic group. But due to lesser amount of available raw materials there is reduction in its ease of use as common household condiments. The factors may be the change in food habit, cause related to modernization like habitat destruction, global warming, indiscriminate fishing in wetlands to fulfill the demands of ever increasing human population etc.. In market products the hygiene and microbial load is a matter of concern amongst the consumers. A little intervention in processing of this product may help in producing better hygenic product with no harmful microbial load and perhaps with good sensory characters that will be liked by many. This can be a very good future prospect of this study that will benefit the indigenous Bodo tribal society.

5. Acknowledgments: I would like to acknowledge Department of Bio-technolgy and Botany, Bodoland University for allowing the laboratory facilities. My sincere gratitude to SAIF, NEHU, Meghalaya and Sandor life sciences, Hydrabad for instrumentation facilities.

6. Declaration of interest statement: The authors that they do not have any conflicts of **interest** to **declare.**

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