

Ethnic Fermented Food Products of Nagaland, India

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Received: December 15, 2019

Accepted: February 05, 2020

Published: February 08, 2020

Citation: Deb CR, Bendangnaro J. 2020. Ethnic Fermented Food Products of Nagaland, India. *J Food Chem Nanotechnol* 6(1): 18-27.

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Published by United Scientific Group

Abstract

Ethnic food fermentation process forms one of the oldest methods of food preparation and preservation which not only increases the shelf life of the food but have benefits of improving the physiochemical characteristics and nutritional quality. The major objective of this study was to document indigenous knowledge of ethnic people of Nagaland on production of some of the popularly consumed fermented food products, analyse the nutritional value and to isolate, characterise and identify the dominant microorganisms of five fermented food products *viz*, *axone/akhuni*, *anishi*, *hungrii*, *rhujuk/bastanga* and *tsutuocie*. A total of 25 samples of *axone/akhuni*, *anishi*, *hungrii*, *rhujuk/bastanga* and *tsutuocie* were analyzed for the microbial population. On the basis of a combination of phenotypic and genotypic characterization, *Bacillus* species was found to be the most dominant microorganism in most of the foods. *Axone/akhuni* and *tsutuocie* were found to be alkaline in nature with a pH of 8.0 and 8.2. *Anishi* and *hungrii* had lower pH of 5.8 and 5.2, whereas *Rhujuk/bastanga* was acidic (4.7). The protein content in *axone*, *anishi*, *hungrii* and *rhujuk* were in the range of 42-38.9g/100g, whereas the protein content in *tsutuocie* was found to be as low as 3.2g/100g. The crude fibre content in *anishi* was found to be higher compared to the other fermented products. Of the five fermented food products it was observed that *rhujuk* had the highest level of phenolic content, followed by *hungrii*, *anishi* and *axone*, while lowest was in *tsutuocie*. The total flavonoid content in *anishi* was highest, followed by *hungrii*, *axone* and *rhujuk* whereas lowest was in *tsutuocie*. Maximum antioxidant activity was recorded in *anishi*, followed by *hungrii*, *rhujuk* and *axone*. Lowest antioxidant activity was recorded in *tsutuocie*.

Keywords

Anishi, *Axone*, Ethnic fermented foods, *Hungrii*, *Rhujuk*, *Tsutuocie*

Introduction

Ethnic foods are defined as foods originated from a particular ethnic group having their own unique heritage and culture. They are generally divided into fermented foods (including beverages) and non-fermented foods [1]. However, there is loss of ethnic food culture of many indigenous people due to various factors like change in climate, global economy, rapid urbanization and increasing availability of fast foods in the market [1].

Indigenous fermented foods are widely consumed as an important part of the diet throughout the world. Foods that are invented centuries ago and even predate written historical records can be prepared by household or cottage

industry using relatively simple techniques and equipment are called indigenous fermented foods [2] and associated with good health and longevity and contribute in reducing hunger by adding nutritional value to food and increase the bioavailability of nutrients.

Nagaland has rich diversity of indigenous fermented foods, which are least explored; therefore, these food items serve as rich reserve of unexplored microorganisms. The type of bacterial flora developed in each fermented food depends on the water activity, pH, salt, concentration, temperature and the composition of the food matrix [3]. Fermentation is spontaneous and uncontrolled process, thus resulting in a product of variable quality. Spontaneous fermentation typically results from the competitive activities of different microorganisms whereby strains best adapted and with the highest growth rate will dominate during particular stages of the process. This study explored the dominant microorganisms present in the final fermented product and to analyse the nutritional value of five major fermented food products viz., *axone*, *anishi*, *hungrii*, *rhujuk* and *tsutuocie*. Survey was conducted in different regions in Nagaland, based on personal interaction with the local fermented food producer and the information data collected is documented. Fermented food products produced by the ethnic people using their native knowledge of preservation of perishable raw materials without using starter culture and chemicals, was found both as low-cost ethnic foods and also beneficial for socio-cultural upliftment of the people. Most of the fermented food and beverages in Nagaland are associated with a particular tribe and in a way give a cultural identification value for communities through its food. The different types of traditional fermented foods and beverages of this state are unique from the other states and the people of this state have preserved the taste for fermentation products and processes for the production of fermented foods from generations on. The fermented foods and beverages documented in this communication are *Zutho*, *Axone/Akhuni*, *Anishi*, *Jang kap*, *Hungrii*, *Rhujuk/Bastanga*, *Jangpangngatsu*, *Tsutuocie*, fermented pork fat and fermented fruit beverages. These fermented food products form an important component of the staple diet of the people in Nagaland.

Materials and Methods

Documentation

Survey was conducted in different parts of Nagaland. Since documentation of the traditional knowledge associated with preparation of indigenous fermented foods was region and tribe specific, the following major regions were surveyed for collection of information; Kohima, Tseminyu, Wokha, Mokokchung and Zunheboto. Information on step-wise method for production of various traditional fermented products was collected through personal discussion with the local people. Fermented foods are categorized as follows:

1. Cereal and legume based
2. Vegetable based
3. Bamboo shoot based

4. Meat based

5. Fruit based fermented beverages

Cereal and Legume Based Fermented Product

(i) Zutho

'Zutho' (rice beer) is a traditional alcoholic beverage prepared from rice (*Oryza sativa* L.), named according to the *Angami Naga* dialect. It is prepared in two parts.

A. Preparation of malt: Malt is prepared by soaking the unhulled rice grains in water for about 2-3 h and allowed to germinate. The germinated grains are then spread on bamboo mats and left to dry in the sun followed by pounding it into powder.

B. Preparation of Zutho: Polished rice grains are first washed and soaked in water for 30 min, after which the excess water is drained off. It is then spread over bamboo mats and allowed to air dry. It is pounded into powder and hot boiling water is added to the rice powder bit by bit and kept side for some time to allow it to cool down. The powder of malt and polished rice grain powder are mixed together in the ratio of 3:7. After proper mixing, it is kept at room temperature and allowed to ferment for about 4-5 days (Figure 1a). The first stock in its pure form is called 'Thutshè' and after it is diluted with some amount of water it is called 'Zutho'. It is consumed as a popular alcoholic beverage in Nagaland. These are generally made during cultural festivals and marriages.

(ii) Axone/Akhone

'Axone' is a fermented soybean (*Glycine max* L.) product, named according to the *Sema Naga* dialect. Soybean seeds are washed and cooked till it becomes soft. Cooked beans are allowed to cool and packed in bamboo basket with the base lined with leaves of *Ficus* species which is then covered with the same on top. The bamboo basket is then kept above the fire place to ferment naturally for about 3-4 days. Usually at this point of the step the final product of most of the other fermented soybean product are produced but during *akhuni/axone* preparation it is further made into a paste and then wrapped in banana leaves or *Phrynium pubinerve* leaves and kept above the fire place for about 3-4 days to undergo further fermentation (Figure 1b). Most people go for longer fermentation to reduce the strong smell of the fermented

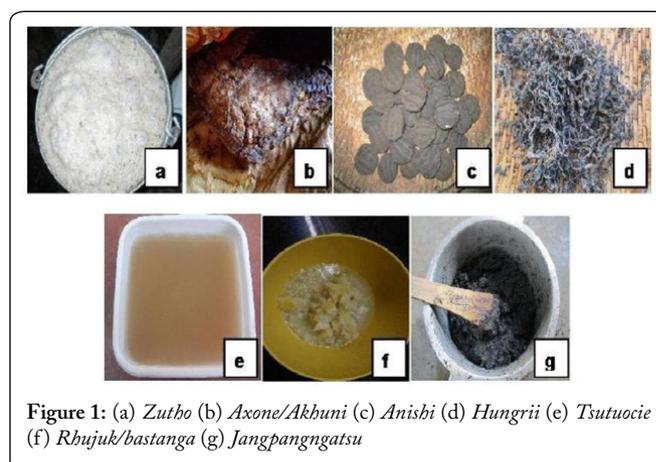


Figure 1: (a) Zutho (b) Axone/Akhuni (c) Anishi (d) Hungrii (e) Tsutuocie (f) Rhujuk/bastanga (g) Jangpangngatsu

product and to increase the shelf life. It is prepared mostly by the womenfolk in the household level and consumed as a popular condiment by almost all the tribes in Nagaland. It is sold in the local market for ~20 INR per packet and serves as a major source of income for some people.

Vegetable Based Fermented Food

(i) *Anishi*

'*Anishi*' is a fermented cake made from *Colocasia* leaves (*Colocasia esculenta* L.). It is exclusively prepared by the *Ao Naga* tribe. Its preparation involves the packing of the *Colocasia* leaves in gunny bags or wrapped in banana leaves for about 3-4 days till the leaves becomes yellow. It is then, pounded into pastes which are made into cakes. These cakes are then wrapped in banana leaves and kept under the hot ash near the fire place or exposed to the sunlight till it is completely dried and becomes hard (Figure 1c). It is consumed as a popular condiment and can be kept for long period of time. It is produced at a small scale household unit in *Sungratsü* and sold for ~300 INR per kg.

(ii) *Hungrii*

'*Hungrii*' is a fermented product prepared from brassica leaves (*Brassica juncea* L.) commonly prepared by the *Rengma Naga* tribe. Pit-fermentation method is followed during its preparation; where a pit of about 2-3 feet is dug on the ground and lined with banana leaves. The leaves are sun dried and allowed to wither. It is then pressed tightly into the pit and covered or plastered with mud on top. It is allowed to ferment naturally for about 15-18 days, after which they are again sun dried to get the final product (Figure 1d). It can be kept for 2-3 years and are consumed as a condiment. They are prepared in bulk during the peak season when brassica leaves are plenty and consumed during the long winter season. They are sold for ~400 INR per kg.

(iii) *Tsutuocie*

'*Tsutuocie*' is a cucumber based fermented product popularly prepared by the *Angami Naga* tribe. For the preparation, matured and ripened cucumber is first peeled and the seeds are removed. They are then cut into pieces and put into jars or earthen pots along with water and allowed to ferment for about 3-4 months (Figure 1e). It is used as a condiment during cooking. *Tsutuocie* can be kept for over 5-6 years.

Bamboo Shoot Based Fermented Food

(i) *Rhujuk/Bastanga*

Bastanga is made from succulent bamboo shoots (*Bambusatulda* Roxb., *Dendrocalamus hamiltonii* Nees et Arn. ex Munro). It is prepared mostly by the *Lotha Naga* tribe, named *Rhujuk* in *Lotha* dialect. Young shoots are taken and their sheaths are removed till only the soft white part of the shoot remains. The shoot is then pounded slightly and pressed tightly into bamboo baskets lined with banana leaves. A hole is made in the middle so as to let the juice drain out. The preparation is kept in that manner for about 2-3 weeks till the bamboo shoot is completely drained out of its juice. The fermented bamboo shoot is then dried. Different grades of

dried bamboo shoots are obtained depending on the way they are cut (Figure 1f). It is consumed as a popular condiment. The fermented bamboo shoot juice can also be stored for years. The thick paste of bamboo shoots is sold for 100-200 INR per container and the dried bamboo shoots are sold for 300-400 INR per kg.

Meat Based Fermented Food

(i) *Jangpangngatsu*

'*Japangangngatsu*' is a fermented food product made from crab (*Scylla* species), named according to the *Ao Naga* dialect. Crabs are first washed thoroughly and shredded into pieces leaving the hard coverings. The shredded crabs are then made into paste. Black 'til' (*Sesamum orientale* L.) are slightly simmered and grounded into powder. The mixture of the two are then wrapped in banana leaves or *Phrynium pubinerve* leaf or put into a pot and kept near the fire place for about 3-4 days for the fermentation to be complete (Figure 1g). It is used for preparation of chutneys. It is sold for 150-200 INR per box.

(ii) *Jang Kap*

'*Jang kap*' is made from buffalo skin, named according to the *Ao Naga* dialect. The skin is separated from the flesh completely and stacked in a tin or pot with tight covering. It is kept for about 1 week to allow the fermentation process. After the hairs are completely scrapped off it is either dried in the sun or kept above the fire place. The product is usually pressure cooked and consume as it becomes hard after it is dried. It is a lesser known fermented product consumed by only a few populations.

(iii) *Fermented pork fat*

Pork fat is fermented and taken as a condiment during preparation of vegetables and curries by almost all the *Naga* tribes. Pork fat is cut into small pieces and boiled. It is then put into bamboo containers and the mouth of which is sealed with banana leaves. The fermentation process gets completed in about 1 week time. It is also a lesser known fermented food product in Nagaland.

Fruit Based Fermented Beverage

The different *Naga* tribes usually prepare various kinds of fruit beverages from fruits like *Naga* apple (*Docyna indica*), passion fruit (*Passiflora edulis*), plum (*Prunus* spp.) and gooseberry (*Phyllanthus emblica*). During the preparation of fruit beverages using *Naga* apple and gooseberry, fruits are collected. Skin and seeds are removed, boiled in water and allowed to cool slightly. Sugar is then added and it is kept for 1-2 week for fermentation. The pulp of passion fruit and plum are directly soaked in sugar syrup. The fermented products are taken as a beverage. It is sold for 150-200 INR per litter in the local market.

Microbiology

Five samples each of the five different fermented foods viz., *axone*, *anishi*, *hungrii*, *rhujuk* and *tsutuocie* were taken from household products collected randomly from in and around Nagaland. Samples were kept in a refrigerator at 4 °C until processed.

Estimation of pH

Five grams of sample was blended with 10 ml of distilled water in a homogeniser and the pH of the slurry was determined directly using a digital pH meter.

Preparation of serial dilutions

Samplings were performed on stored finished fermented products to examine for its microbial groups. Ten grams of sample were taken aseptically and homogenized in sterile physiological saline (peptone, 0.1% w/v; NaCl, 0.85% w/v,) for 1 min. Then serial dilutions were prepared by transferring one ml from first dilution (10^1) to 9 ml peptone water and serially diluted further up to 10^{-10} dilutions with saline water. Then plate counts were carried out using the following media, temperature and incubation periods to enumerate different microbial group.

Total viable bacteria count

To determine the total bacterial count 0.1 ml of serially diluted 0.1% (w/v) sample was inoculated plate count agar (PCA) and incubated at 30–32 °C for 48h. Colony forming units (CFU) were counted using a colony counter and the results were presented as cfu ml⁻¹.

Enumeration of coliform bacteria

Appropriate decimal dilutions (0.1 ml) of the homogenate was spread on Nutrient Agar and TSA and incubated at 37 °C for 24h. Members of Enterobacteriaceae were enumerated using Violet red bile glucose agar and incubated at 30 °C for 48 h.

Enumeration of Lactic Acid Bacteria

From appropriate dilutions, 0.1 ml aliquots were spread plated in triplicates on pre-dried surfaces of MRS agar plates supplemented with 1% (w/v) calcium carbonate. The plates were incubated anaerobically in an Anaerobic Gas-Bag system at 30–32 °C for 48 h.

Enumeration of *Staphylococci*

Selective enumeration was carried out by spread plates on Baird-Parker agar media. The plates were incubated at 37 °C for 48 h.

Yeast and Mold Enumeration

From suitable dilution of sample, 0.1 ml was transferred onto solidified PDA and YMA, supplemented with 12 µg ml⁻¹ Streptomycin to inhibit bacterial growth. Plates were then incubated at 27 °C for 48 h.

Phenotypic Characterization

Morphologically different colonies were isolated and purified cultures were grown on slants of the same medium and stored at 4 °C. Purified isolates were checked for gram stain and for catalase production.

Molecular Identification

DNA isolation and polymerase chain reaction (PCR)

Extraction of genomic DNA was done using CTAB protocol described by Moore et al. [4] with suitable modification. The extracted genomic DNA was tested qualitatively on 1% (w/v) agarose gel electrophoresis and

quantified using Nanodrop Spectrophotometer.

The 16S rDNA gene sequences were amplified using universal primers 9F and 1492R [5] and 27F and 1492R [6]. About 25 µL of PCR mixture was amplified in a PCR programmed with the following temperatures: 94 °C for 5 min then 35 cycles at 94 °C 1 min, 60 °C for 1 min and 72 °C for 30 sec. The final extension was at 72 °C for 5 min and stopped at 4 °C.

16S rDNA Sequencing

Amplified products were separated by electrophoresis in 1.2%, w/v agarose gel and were purified using a commercial purification kit. Sequencing was done at 1st Base, Singapore. To determine the closest known relatives of the partial DNA sequences obtained, searches for homologous nucleic acid sequences was performed using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/>). Percent similarity values of the most closely related identities were determined by a comparison with the sequences available in the database using BLAST software.

Nutritional Analysis

Comparative study was conducted among the selected fermented foods and their constituent raw materials to understand the degree of nutritional value addition during fermented process. Fermented food samples were collected from different households and stored at 4 °C till use. Raw materials were collected during specific seasons when these materials were abundantly available. For assessment of nutritional value, all the samples were first oven dried at 60 °C and were grinded to a fine powder and stored in an airtight container at 4 °C until use.

Proximate Analysis

Moisture content was done by oven drying method and represented in percentage. Protein estimation was done using the colorimetric method of Lowry et al. [7]. Reducing sugar was estimated using 3,5-dinitrosalicylic acid (DNSA) reagent [8].

Estimation of crude fibre

Crude fibre was determined following Maynard [9] with modification and is expressed as g/100 g.

Estimation of total phenolic content (TPC)

Total phenol content was determined following Folin-Ciocalteu method of Singleton and Rossi [10]. Gallic acid was used for making the standard graph and expressed as mg Gallic acid equivalents (GAE)/g of extract.

Estimation of total flavonoid content (TFC)

Total flavonoid content was determined following technique of Sahreen et al., [11] with slight modification. The absorbance was measured at 510 nm and standard curve was prepared using Quercetin and expressed as mg Quercetin equivalents (QE)/g of extract.

Antioxidant Activity

DPPH radical scavenging assay

The scavenging activity of stable 2, 2-Diphenyl-1-

picrylhydazyl (DPPH) free radical was determined following Aoshima et al. [12] with modification. Standard curve was calculated using Trolox and inhibition percentage was calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}} \times 100$$

Statistical Analysis

The experiments were done in triplicate (n = 3) and expressed as mean ± standard deviation. The results were processed using statistical software Microsoft Excel and Origin-Pro 8.

Results and Discussion

A total of 25 samples of *axone*, *anishi*, *hungrii*, *rhujuk* and *tsutuocie* were analyzed for their microbial population. In *axone* and *rhujuk* total viable microbial load was in the range of 10^7 cfu ml⁻¹. However, in *anishi*, *hungrii* and *tsutuocie* total viable microbial load was low in the range of 10^4 cfu ml⁻¹, which may be due to the pre or post fermentation treatment of drying and addition of water creating an environment suitable for the growth of only particular microorganisms. No yeast or moulds were detected in any of the samples. On the basis of a combination of phenotypic and genotypic characterization, *Bacillus* species was found to be the most dominant microorganism present in almost all the fermented food products.

Preparation and consumption of sticky, non-salty, flavoursome fermented soybean foods are the traditional wisdom of the people from several South-East Asian countries, which have fostered a distinct food culture of the people [13]. Sarkar et al. [14] and Jeyaram et al. [15] reported *Bacillus subtilis* to be the most dominant microorganisms involved in the production of *kinema* and *Hawaijar*. In most of the fermented foods from Nagaland *Bacillus* species was found to be the most dominant microorganism (*Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus cereus*) (Table 1). Although the method of production and culinary practices vary from product to product, all bacilli-fermented Asian soybean foods have a characteristic stickiness and typical flavour. Chetri and Tamang [16] reported *Bacillus subtilis* as dominant bacteria in production of *tungrymbai* and *bekang*. Furthermore, reports also show that the Gram-positive spore-forming *Bacillus subtilis* is responsible for the fermentation of *thuanao* and *chungkokjang* [17]. *Bacillus cereus* a Gram-positive, rod-shaped, spore forming food borne pathogen was reported from *hawaijar* and *Staphylococcus* species from *tungrymbai* and *hawaijar* which normally find their way into fermented products from the raw material, personnel, animal skins and the environment [15]. The prevalence of *Bacillus* species in the fermented products may be due to the alkaline condition (pH 8) that occurs during the fermentation process leading to favourable condition for some bacteria to grow, but also causing unfavourable condition for other microbes to grow.

Fermented food products prepared from leafy vegetables for bio-preservation to extend the storage life and enhance

Table 1: 16S rRNA sequence-based identification of microbes from different fermented foods and GenBank accession numbers.

Fermented Food	Isolates	Closest related microorganism	GenBank Accession No.
Axone/ akhuni	BJ-DEBCR-2	<i>Bacillus licheniformis</i>	KU301334
	BJ-DEBCR-33	<i>Bacillus licheniformis</i>	MF487831
	BJ-DEBCR-3	<i>Bacillus subtilis</i>	KU301335
	BJ-DEBCR-24	<i>Bacillus subtilis</i>	MF487822
	BJ-DEBCR-22	<i>Bacillus cereus</i>	KX364205
	BJ-DEBCR-29	<i>Bacillus cereus</i>	MF487826
	BJ-DEBCR-1	<i>Staphylococcus epidermis</i>	KU301333
	BJ-DEBCR-21	<i>Alcaligenes faecalis</i>	KX364204
Tsuuocie	BJ-DEBCR-7	<i>Bacillus subtilis</i>	KU301335
	BJ-DEBCR-8	<i>Bacillus subtilis</i>	KU854956
	BJ-DEBCR-25	<i>Bacillus subtilis</i>	MF487823
	BJ-DEBCR-10	<i>Bacillus licheniformis</i>	KU854958
	BJ-DEBCR-12	<i>Bacillus pumilis</i>	KU854960
	BJ-DEBCR-34	<i>Bacillus pumilis</i>	MF487832
Hungrii	BJ-DEBCR-11	<i>Bacillus pumilis</i>	KU301334
	BJ-DEBCR-19	<i>Bacillus licheniformis</i>	KX258615
	BJ-DEBCR-26	<i>Bacillus licheniformis</i>	MF487824
	BJ-DEBCR-36	<i>Bacillus licheniformis</i>	MF487834
	BJ-DEBCR-23	<i>Bacillus subtilis</i>	MF487821
	BJ-DEBCR-35	<i>B. amyloliquefaciens</i>	MF487833
Rhujuk/ Bastanga	BJ-DEBCR-9	<i>Bacillus subtilis</i>	KU854957
	BJ-DEBCR-14	<i>Bacillus subtilis</i>	KU854961
	BJ-DEBCR-30	<i>Bacillus subtilis</i>	MF487828
	BJ-DEBCR-31	<i>Bacillus subtilis</i>	MF487829
	BJ-DEBCR-37	<i>Bacillus subtilis</i>	MF487835
	BJ-DEBCR-39	<i>Bacillus subtilis</i>	MF487837
	BJ-DEBCR-5	<i>Bacillus licheniformis</i>	KU301337
	BJ-DEBCR-38	<i>Bacillus licheniformis</i>	MF487836
	BJ-DEBCR-32	<i>B. amyloliquefaciens</i>	MF487830
Anishi	BJ-DEBCR-6	<i>Bacillus subtilis</i>	KU854954
	BJ-DEBCR-40	<i>Bacillus subtilis</i>	MF487838
	BJ-DEBCR-4	<i>Bacillus licheniformis</i>	KU301336
	BJ-DEBCR-17	<i>Bacillus licheniformis</i>	KU854963
	BJ-DEBCR-18	<i>Bacillus licheniformis</i>	KU854964
	BJ-DEBCR-28	<i>Bacillus licheniformis</i>	MF487826
	BJ-DEBCR-41	<i>Bacillus licheniformis</i>	MF487839
	BJ-DEBCR-20	<i>Bacillus pumilis</i>	KX258616
	BJ-DEBCR-16	<i>Enterococcus faecalis</i>	KU854962

safety of foods using the natural microflora, is popularly practiced in Nagaland. These products are mostly non-salted and are either sun dried or baked at high temperature after the completion of the fermentation period. The pH of *anishi* and *hungrii* was recorded to be 5.8 and 5.2 respectively, which

renders it to be acidic (Table 2). Production of organic acids and lactic acids by *Bacillus* species have been reported by Yan et al. [18]. Studies have reported the presence of lactic acid bacteria in most of the vegetable based fermented food products [19, 20]. However, in the present study the most dominant microorganisms in *anishi* were reported to belong to the members of the *Bacillus* species, which were identified as *Bacillus subtilis* and *Bacillus licheniformis*, after comparing with 16S rRNA sequences from the NCBI genbank (Table 1). Another bacteria isolated was identified as *Enterococcus faecalis*. The presence of a bacterial species representing *E. faecalis* is responsible for sensory characteristics of the final product, as this species is often prevalent in foods [21]. In addition, some *Enterococci* strains, especially *E. faecalis* and *E. faecium* may produce bacteriocins that are active against a plethora of food borne pathogens, making them suitable candidates for controlling emerging pathogens during food fermentation [22]. Despite the safety and pleasant sensory attributes imparted by *E. faecalis* in foods, some strains of *E. faecalis* and *E. faecium* are associated with infection that pose challenges to food safety [21]. Similarly, in *hungrii* the most dominant microorganisms isolated belonged to *Bacillus* species, which were identified as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilis* and *Bacillus amyloliquefaciens*. The presence of *Bacillus* species in such foods can be linked to different factors such as post fermentation method of baking at high temperature or drying during the process, which select for heat resistant microorganisms, especially spore forming bacteria.

microorganisms [23]. Mostly, the production of fermented bamboo shoots involves the natural fermentation process with various lactic acid bacteria playing dominant role in imparting flavour, taste and aroma to the product. However, in the present study *rhujuk* had microbial load in the range of 10^7 cfu ml⁻¹, which was mostly dominated by *Bacillus* species (Table 1). The different strains identified were *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus amyloliquefaciens*. Tamang et al. [24] reported presence of *Bacillus subtilis* and other *Bacillus* species like *Bacillus circulans*, *Bacillus firmus* and *Bacillus sphaericus* from *tuaitbur*, a fermented bamboo shoot product of Assam. Bioconversion ability of *Bacillus sp.* found in the metabolites of fermented succulent shoots of bamboo makes them ideal source of bioactive compounds like phytosterols (precursors of many pharmaceutically active steroids) [25]. Another bacteria isolated from *bastanga* was identified as *Staphylococcus* species. The presence of *Staphylococcus* species in food products is generally undesirable especially when the count is greater than 10^2 cfu g⁻¹. Low numbers of this organism is indicative of poor handling conditions whereas high counts are frequently associated with incidences of food poisoning.

Lactic acid fermentation usually plays a very important role in cucumber fermentation. Most commercial cucumber fermentations rely upon growth of the microorganisms that is naturally present on the surface of cucumbers [20]. Cucumbers are mostly fermented by adding salt or acetic acid to limit the growth of spoilage microorganisms. However, during the preparation of *tsutuocie* no salt is added, instead

Table 2: Proximate composition of the non-fermented and fermented products.

Parameters	Axone		Anishi		Hungrii		Rhujuk		Tsuuocie	
	Raw material (soybean)	Product	Raw material (Colocasia leaves)	Product	Raw material (Brassica leaves)	Product	Raw material (Bamboo shoot)	Product	Raw material (Cucumber)	Product
Moisture (%)	11.2 (0.02)	50.0 (0.01)	80.6 (0.1)	3.8 (0.06)	60.0 (0.04)	5.2 (0.03)	80.0 (0.1)	90.0 (0.03)	90.0 (0.04)	92.0 (0.04)
pH	6.8 (0.003)	8.0 (0.001)	6.2 (0.04)	5.8 (0.02)	6.2 (0.05)	5.2 (0.01)	6.2 (0.03)	4.7 (0.07)	6.1 (0.09)	8.2 (0.006)
Protein (g/100g)	41.8 (0.004)	42.1 (0.03)	20.64 (0.03)	34.19 (0.005)	23.34 (0.1)	34.07 (0.1)	33.07 (0.002)	30.89 (0.1)	6.7 (0.04)	3.2 (0.04)
Reducing sugars (%)	27.6 (0.013)	29.7 (0.01)	54.7 (0.1)	29.6 (0.04)	32.1 (0.004)	34.5 (0.04)	52.1 (0.05)	29.8 (0.03)	17.5 (0.007)	22.5 (0.06)
Crude fibre (g/100g)	1.04 (0.03)	1.61 (0.01)	10.52 (0.1)	12.26 (0.02)	2.88 (0.03)	1.019 (0.06)	0.17 (0.04)	0.27 (0.009)	0.128 (0.04)	0.05 (0.04)

Data represent mean of three sample analysis (n = 3) ± SD

Bamboo shoots constitute a major component of traditional cuisine in most of the Asian countries. It forms a rich ecological niche which harbours a plethora of

water was added. In *tsutuocie* microbial load was in the range of 10^4 cfu ml⁻¹, mostly *Bacillus* species (*Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilis*) (Table 1). *Tsutuocie* was

found to be alkaline in nature with a pH as high as 8.2. This high pH of the fermented product might have been the reason for the occurrence of *Bacillus* species in dominance over other microorganisms in the final product.

Shahcheraghi et al. [26] reviewed the various potential of *Bacillus subtilis* strains as a probiotic, producing antibiotics and enzymes that are important in both medical and industrial sciences. Studies have reported that *Bacillus* species possess wide range of inhibitory spectrum against pathogenic bacteria due to secretion of antimicrobial compounds viz. bacteriocins [27]. The absence of LAB in most of the fermented vegetables and fruit, probably owes to loss of survival during aging process. These results are also in agreement with the generally accepted concept that traditional fermentations are dominated by a few microbial species that are selected during the course of fermentation because of good adaptation to the food matrix [28]. LAB involved in vegetable fermentation were not detected, which may be due to dominance of Non-LAB over fastidious LAB and further it was also reported that fresh fruits and vegetables harbour only 0.1% of LAB and 99.9% Non-LAB [29] and hence may have gone undetected.

Proximate Composition

The proximate composition of the fermented products in comparison to its raw material is given in table 3. Moisture content of *axone/akhuni* decreased considerable from 50% (raw material) to 11.2% (product), which is due to the addition of water during cooking and washing of the cotyledon. Moisture content of *anishi* (3.8%) and *hungrii* (5.2%) were low in comparison to its raw material, as they were baked or dried after fermentation (Table 2). While, moisture content of *rhujuk* (90%) and *tsutuocie* (92%) had high moisture content this may be due to addition of water during its preparation.

In *axone/akhuni* protein content was 42.1 g/100g, which was found to increase just slightly compared to constituent soybean (41.8 g/100g). Several studies indicated the increase in protein content of soybean based fermented products as compared to its raw material such as in *hawaijar* [30]; *tungrymbai* [31]; *kinema* [14, 24].

found to be 34.19g/100g and 34.07g/100g, which increased significantly compared to its raw materials having 20.64g/100g and 23.34g/100g respectively. The protein content in other leaf based fermented product like *gundruk*, prepared from mustard, *rayo-sag* (local brassica leaves) and cauliflower leaves was reported to be 37.4%. Another product *goyang*, prepared from leaves of local brassica species of Nepal was reported to contain 35.9% protein [13].

Bamboo shoots are known as 'wild or forest vegetable' and are consumed either in their fresh form or dried, fermented or pickled and canned. The protein content of *rhujuk*, prepared from young succulent bamboo shoots was found to contain 30.89g/100g, which decreased from its raw material having 33.09g/100g of protein. Decrease in protein content may be due to the denaturation of protein during fermentation. A similar result was reported in *kborisa*, a bamboo shoot based fermented product of Assam, where the protein content was lower than its raw material [32]. However, Agrahar-Murugkar and Subbulakshmi [31] found enhancement of protein content in *lungsiej*, a fermented bamboo shoot product of Meghalaya.

Tsutuocie prepared from ripened cucumber fruits, protein content was low compared to its raw material (from 6.7g/100g to 3.2g/100g).

The reducing sugar in *axone* increased after fermentation from 27.6 to 29.7%. This increase may be due to increase in the activity of native or microbial amylases which hydrolyses starch to sugars. Similar report was also seen in *hawaijar*, where reducing sugars increased as compared to soybean from 1.10 to 3.1% respectively [30]. Similarly, reducing sugars in *hungrii* and *tsutuocie* were also found to increase compared to their raw materials (Table 2). However, the reducing sugars in *anishi* and *rhujuk* decreased as compared to its counterpart. The reduction in the reducing sugar content of fermented product could be due to utilization of some of the sugars by fermenting organisms for growth and metabolic activities.

Besides above, crude fibre content in *axone* was found to be 1.605g/100g increased from 1.035g/100g in soybean seeds. Keishing and Banu [30] also reported the increase in crude

Table 3: Total phenol and total flavonoid content of non-fermented and fermented products.

Parameters	Axone		Anishi		Hungrii		Rhujuk		Tsutuocie	
	Raw material (soybean)	Product	Raw material (Colocasialeaves)	Product	Raw material (Brassica leaves)	Product	Raw material (Bamboo shoot)	Product	Raw material (Cucumber)	Product
TPC(mg GAE/g)	0.2 (0.003)	0.86 (0.024)	0.88 (0.05)	1.44 (0.09)	2.72 (0.1)	1.66 (0.03)	1.52 (0.1)	2.44 (0.21)	0.4 (0.016)	0.22 (0.002)
TFC (mgQE/g)	0.46 (0.03)	0.64 (0.02)	0.66 (0.06)	2.06 (0.07)	1.08 (0.004)	0.76 (0.03)	0.36 (0.18)	0.62 (0.05)	0.028 (0.007)	0.12 (0.056)

While, protein content in *anishi*, prepared from *Colocasia* leaves and *hungrii*, prepared from brassica leaves were

fiber in *hawaijar* as compared to soybean. However, Omadara and Olowomofe [33] reported decrease in the level of crude

fiber in soy-*iru* than in the unfermented soybean.

The crude fibre content in *rhujuk*, increased from its raw material i.e., from 0.172 to 0.265 g/100g. The crude fibre in other bamboo shoot based fermented products had wide variations in comparison with the results in the present study, which may be due to the different bamboo species used and also on their conditions of growth [34]. Crude fibre in *anishi* was found to be 12.26 g/100g and in *hungrii* it was 1.02 g/100g, which decreased from its raw material having 2.88 g/100g. The crude fibre content in cucumber was relatively low and it decreased further after fermentation in *tsutuocie*. The reduction of crude fibre content in diet might be due to enzymatic degradation of the fibrous material during fermentation [35]. The variation in the levels of proximate composition of foods after fermentation may be influenced by various factors like the different varieties of raw material used or the influence of environmental factors and also on the conditions involved during its processing.

Total phenolic and flavonoid content

The total phenol content and flavonoid contents of different fermented products are presented in table 3. The TPC and TFC in *axone* was found to be 0.86 mg GAE/g and 0.64 mg QE/g respectively while, in *anishi* (1.44mg GAE/g and 2.06mg QE/g) and *rhujuk* (2.44mg GAE/g and 0.62mg QE/g) which were significantly higher than in the raw materials. Fermentation have been reported to increase the phenolic and flavonoid content by inducing structural breakdown of the substrate cell wall leading to release of bioactive in plant based functional foods [36]. However, the total phenolic and flavonoid content in *hungrii* (1.66 mg GAE/g and 0.76 mg QE/g), decreased from its raw material. The total phenolic and flavonoid content in *tsutuocie* (0.22mg GAE/g and 0.1mg QE/g) had relatively lower levels of phenolics as compared to its counterpart; the reason for the decrease in the level of bioactive compounds may be due to strengthening of plant cell walls into lignans and lignins by polymerisation [25].

Antioxidant activity

During fermentation, bacterial enzyme transforms organic substances into simpler compounds such as peptides, amino acids and other nitrogenous compounds which not only contribute to the flavour and aroma of the fermented products but some exhibit antioxidant capacity [37]. The free radical scavenging activity of *axone*, was found to be 98.79 µg/ml, which increased from its raw material soybean seeds having 186.75 µg/ml, similarly in *anishi* (60.2 µg/ml) and *rhujuk* (92.85 µg/ml) it was found to be higher as compared to *Colocasia* leaves (100.9 µg/ml) and young succulent bamboo shoots (112.3 µg/ml). However, the free radical scavenging activity of *tsutuocie* (219.3 µg/ml) was lower compared to cucumber fruit (120 µg/ml) (Table 4). Sonar et al. [38] reported the increase in antioxidant activity of fermented bamboo shoots with time in fermentation. Diverse fermentation processes of fermented bamboo shoots and their method of preparation could contribute for the higher antioxidant activity. Oh et al. [39] reported that during *kimchi* manufacturing process the antioxidant components contained in leaf mustard were

degraded, similarly in the present study *hungrii* (73.3 µg/ml) had lower antioxidant activity than brassica/mustard leaves (65.7 µg/ml).

Table 4: IC50 value of DPPH scavenging capacity of non-fermented and fermented products.

Raw materials	DPPHIC50 (µg/ml)	Fermented product	DPPHIC50 (µg/ml)
Soybean seeds	186.75 (0.81)	Axone/Akhuni	98.79 (1.09)
Colocasia leaves	100.9 (0.98)	Anishi	60.2 (0.62)
Brassica leaves	65.7 (0.54)	Hungrii	73.3 (0.56)
Bamboo shoot	112.3 (1.2)	Rhujuk/Bastanga	92.85 (0.76)
Cucumber	120.8 (1.32)	Tsutuocie	219.32 (1.23)
Trolox		240.5	

Data represent mean of three sample analysis (n = 3) ± SD.

Microorganisms during fermentation are exposed to oxidative stress making the cells evolve protective mechanisms involving enzymatic antioxidation, which may contribute to the antioxidative effect of fermentation. High antioxidant activity might be due to fermentation process and also biochemical changes that could promote binding of dietary fibre to polyphenols followed by decomposition into free phenolic compounds. Diverse fermentation processes and their method of preparation could alter the availability of antioxidant activity in the different fermented product.

Conclusion

In the present study documentation of some popular fermented food was done along with nutritional analysis and characterization of dominant microorganisms present in the sample. The findings of the present study will help in popularizing these ethnic foods at larger platform/market. Food contaminant like *Staphylococcus epidermis*, *Bacillus cereus* and *Alcaligenes* species were detected in some of the fermented products, as fermented foods in Nagaland are still prepared at the household level using traditional methods. Poor hygienic standards during the preparation of these fermented method also explains the presence of the contaminants in the food product. Thus, improvement of crude traditional methods by employing modern scientific technologies is the need of hour to upgrade the quality and production of fermented products at commercial scale while keeping intact their unique natural flavour, taste and aroma. Result from this study demonstrates that most of the fermented food products were rich in nutrients in comparison to its raw material and thus, their proper utilization, exploitation and conservation is of utmost importance. Fermented products *axone/akhuni*, *anishi*, *hungrii* and *rhujuk/bastanga* were found to be good source of protein; *axone/akhuni*, *anishi* and *hungrii* had good amount of crude fiber; *axone/akhuni*, *anishi* and *rhujuk/bastanga* were found to

be good source of total phenolic and flavonoid content, thus having high levels of antioxidant activity. Therefore, it can be concluded that fermentation helps in the improvement of the nutritional profile of these fermented products, which can contribute to the dietary status of consumers, leading to improvement in product acceptability. Further, more detailed investigation on the microorganisms and their role in the nutrition and health value of the food is needed. Also, starter cultures with desired microorganisms are required to accelerate the fermentation process as well as to improve the quality of the fermented product.

Acknowledgements

Authors are thankful to Department of Biotechnology, Ministry of Science & Technology, Govt. of India, New Delhi for financial assistance through Institutional Biotech Hub to Prof. C. R. Deb vide order no. BT/22/NE/2011. Facilities used from the UGC-SAP (DRS-III) and DST-FIST programmes are duly acknowledged.

Conflict of Interest

The authors declare that there is no conflict of interest.

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