

Biochemical Characterization of Three Vegetable Based Fermented Food Products (*Hungrii*, *Rhujuk* and *Tsutuocie*) of Nagaland, India

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Abstract

Indigenous fermented foods are important constituent of staple diet of the Naga tribes of India. In Nagaland, there are variety of fermented foods and beverages with traditional and cultural value. Agriculture being the main occupation, preservation technique of perishable crops has been passed down from generation to generation. Here we present the biochemical characterization of some vegetable based fermented food products of Nagaland *i.e.*, *Hungrii* (*Brassica* leaves), *Rhujuk/Bastanga* (Bamboo shoot) and *Tsutuocie* (Cucumber). The comparative account of nutritive values like moisture content, pH, protein content, reducing sugars, crude fibre, total phenol content, flavonoid content and antioxidant activity of fermented foods and its constituent raw materials was done. Results indicated high amounts of protein in *Hungrii* (34.07 g/100g). Most of the fermented foods had low moisture content rendering it to have longer shelf life. *Rhujuk/Bastanga* was found to have significantly higher levels of phenolic content (1.44 mg GAE/g and 2.44 mg GAE/g), thus having high antioxidant activity in comparison to the other fermented products. This present study thus puts some light on the proximate composition as well as the antioxidant content of some major vegetable based fermented food products of Nagaland so as to popularize these products as nutritional support to the region for health improvement.

Keywords

Bastanga Rhujuk, *Hungrii*, Nagaland, *Tsutuocie*, Vegetable Based Fermented Foods

1. Introduction

Traditional fermentation processes have been practiced since ancient time and it forms an integral part of the world diet. It is one of the oldest methods of food preservation and has benefits of providing nutrients, enhancing the flavour, aroma and increases the shelf life of the food product [1]. Although advances in food science and technology have given rise to a wide variety of new food technologies, fermentation has remained an important technology throughout the history of mankind [2]. The traditional processes and technologies involved in the production of fermented food products have been inherited from generation to generation. Traditional fermented foods are indigenous to a particular area and have been developed through age-old practices using locally available raw materials. In the Indian sub-continent, preparation of these fermented food products using local food crops and other biological resources is very common [3]. The North Eastern Region of India inhabited by a large number of indigenous tribes has developed their own fermentation techniques for transformation of different raw material of plant and animal origin [4].

In Nagaland agriculture being the main occupation, preservation of perishable crops has been practiced since time immemorial. It not only contributes to the dietary intake but also improves safety, quality and availability of food and generates income to the rural people. Sun-drying and fermentation are the two important traditional techniques undertaken for the processing of vegetables. Vegetable fermentation normally involves minor changes in both nutrients and other physiochemical properties of vegetables. Changes in vegetable nutrients include increasing free amino acids, improvement in protein digestibility and development of desirable flavor and colors [5]. Today, industrial vegetable fermentation are carried out at a large scale however, it is still prepared at the household level in Nagaland. Present study aimed at documentation of different vegetable based fermented foods traditionally prepared by the ethnic tribes of Nagaland and to study the nutritional values of the fermented food products.

2. Materials and Method

Chemicals used: All the chemicals used in this study were of analytical grade and procured from Hi-Media (Hi-Media, India).

Documentation and collection of the fermented products: The traditional process of preparation was documented by interviewing with the local people. The samples were collected and brought to the laboratory and stored at 4°C till used.

Sample preparation: For the assessment of nutritional value, all the samples were first oven dried at 60°C and were grinded to a fine powder. The finely powdered samples were kept separately in an airtight container at 4°C until the time of use.

2.1. Biochemical Analysis

Estimation of protein: Total soluble protein was estimated using the Fo-

lin-Ciocalteau method [6] [7] and Bovine Serum Albumin (BSA) was used as standard.

Estimation of reducing sugar: 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. One gram of dried samples was boiled with 200 ml of 0.25N sulphuric acid (H_2SO_4) for 30 min. It was then filtered with No. 1 Whatman filter paper. The filtrate was then boiled with 200 ml of 0.313N NaOH solution for 30 min followed by filtration and washed subsequently with 25 ml of boiling 1.25% H_2SO_4 and thrice with 50 ml distilled water and 25 ml of alcohol. The residue was removed and transferred to pre-weighed ashing dish (W_1 g). The filtrate was then dried for 2 h at $130^\circ C \pm 2^\circ C$ and then cooled. The ashing dish was cooled and weighed (W_2 g). It was ignited for 30 min at $600^\circ C$. After cooling in desiccators it was again reweighed (W_3 g). The crude fibre content was determined using the formula:

$$\text{Crude fibre (g/100g)} = \frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1)}{\text{Original weight of sample}} \times 100$$

Moisture content: Moisture content was estimated by taking 5 g of sample in a pre-weighed dish plate and placed in the oven for ~16 h at $70^\circ C \pm 1^\circ C$ till a constant weight was achieved. After drying, samples were weighed again and the moisture content was determined by using the formula:

$$\text{Moisture content (\%)} = \frac{\text{Loss of weight}}{\text{Weight of the sample}} \times 100$$

Estimation of pH: Five gram of sample was blended with 10 ml of distilled water in a homogenizer and the pH of the slurry was determined directly using a digital pH meter (Systronics, pH system).

Preparation of methanol extract: One gram of dried sample was ground and extracted in 10 ml of 80% (v/v) methanol by shaking for 24 h at room temperature. The extraction procedure was repeated until the extraction solvent became colorless. The extract was then filtered over with Whatman No. 4 filter paper. The filtered obtained was directly used for antioxidant analysis.

Estimation of total phenolic content (TPC): Total phenol content was determined following Folin-Ciocalteau method [10].

Estimation of total flavonoid content (TFC): Total flavonoid content was determined following technique of Sahreen *et al.* [11] with slight modification. To 0.3 ml of extract, 3.4 ml of 30% methanol, 0.15 ml of 0.5M sodium nitrite and 0.15 ml of 0.3M aluminum chloride were added. The mixture was then allowed to stand for 5 min and then added 1 ml of 1M NaOH. The absorbance was measured at 510 nm and standard curve was prepared using Quercetin and expressed as mg Quercetin equivalents (QE)/g of extract.

2.2. Antioxidant Activity

DPPH radical scavenging assay: Antioxidant scavenging activity of stable 2,

2-Diphenyl-1-picrylhydrazyl (DPPH) free radical was determined following Aoshima *et al.* [12] with modification. To 100 µL of methanol extract, 2.9 ml of DPPH reagent (0.1 mM in methanol) was added followed by vigorous shaking and incubated in dark and room temperature for 30 min before reading the absorbance at 517 nm in spectrophotometer (Multiskan Go, Thermo Scientific). Standard curve was calculated using Trolox and inhibition percentage was calculated using the formula:

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

where, OD is the optical density.

Statistical analysis: All the experiments were done in triplicate (n = 3) and expressed as mean ± standard deviation.

3. Results and Discussion

Proximate composition: The proximate composition of the fermented products in comparison with its raw material is given in **Table 1**. Moisture content of *Hungrii* (7%) was low due to post fermentation drying. However, *Rhujuk/Bastanga* (90%) and *Tsutuocie* (98%) had high moisture due to addition of water during its preparation [13]. *Hungrii* and *Rhujuk/Bastanga* had pH of 5.2 and 4.7, which may be due to acids produced by microorganisms during fermentation preventing the growth of contaminating microorganisms [14]. *Tsutuocie* was found to be alkaline in nature with a pH of 8.2, which may be due to release of ammonia and proteolysis [15].

Protein content: Protein content of *Hungrii* was 34.07 g/100g, which was found to increase as compared to its raw material *Brassica* leaves (23.34 g/100g) (**Table 1**). 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% respectively. Another product *goyang*, prepared from leaves of local *Brassica* species of Nepal was reported to contain 35.9% of protein [16]. However, protein content of *Rhujuk/Bastanga* and *Tsutuocie* were 30.89 g/100g and 3.2 g/100g, respectively which decreased in comparison to bamboo shoot (33.09 g/100g) and cucumber (6.7 g/100g). The reduction in

Table 1. Proximate compositions of the non-fermented and fermented products.

Parameters	Brassica leaves	<i>Hungrii</i>	Bamboo shoot	<i>Rhujuk/Bastanga</i>	Cucumber	<i>Tsutuocie</i>
Moisture (%)	60 (0.04)	7.0 (0.03)	80.0 (0.1)	90.0 (0.03)	90.0 (0.04)	98.0 (0.04)
pH	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)	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 (%)	32.1 (0.004)	34.5 (0.04)	52.1 (0.05)	29.6 (0.03)	59.1 (0.007)	22.5 (0.06)
Crude fibre (g/100g)	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 replicates (±Standard deviation).

protein content may be due to the denaturation of protein during fermentation [17]. A similar result was reported in *khori*, a bamboo shoot based fermented product of Assam, where the protein content was lower than its raw material (3.78% to 2.40%) [18].

Reducing sugars: Reducing sugars like glucose plays a very important role during fermentation as the microorganisms utilize them to undergo fermentation [19]. The reducing sugars decreased considerably in *Rhujuk/Bastanga* (29.8%) as compared to its counterpart (52.1%), which may be due to the utilization of some of the sugars by fermenting organisms for growth and metabolic activities [20]. Singh *et al.* [11] reported a sharp consistent decrease in the level of reducing sugars in two varieties of *Soibum*, a fermented bamboo shoot product of Manipur, from 1.47 to 0.62 g/100g and 3.16 to 1.2 g/100g respectively. However, there was increase in the level of reducing sugars in *Hungrii* (34.5%) and *Tsutuocie* (22.5%) as compared to its raw material (32.1% and 17.5%) which may be due to increase in the activity of native or microbial amylases which hydrolyses starch to sugars [21].

Dietary fiber: Studies have indicated that components of plants such as dietary fibre have beneficial effects in lowering blood cholesterol levels aside from the decreased intake of saturated fat and cholesterol that occurs with high intakes of plant foods [22]. Crude fibre was low in *Tsutuocie* (0.05 g/100g) and *Hungrii* (1.019 g/100g) as compared to cucumber (0.128 g/100g) and *Brassica* leaves (2.88 g/100g), which may be due to enzymatic degradation of the fibrous material during fermentation [23]. There was however, increase in the level of crude fibre in *Rhujuk/Bastanga* (0.27 g/100g) in comparison to its raw material (10.5 g/100g and 0.17 g/100g). The crude fibre content in *soibum*, a fermented bamboo shoot product of Manipur as reported by Singh *et al.* [19] was in the range of 0.35 - 0.60 g/100g. Choudhury *et al.* [24] also reported the presence of crude fibre in processed bamboo shoots to be 1.8 g/100g. 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 [25].

Total phenolic and flavonoid: Comparison of total phenolic and flavonoid content of the fermented products and its raw materials is given in **Figure 1**. The amount of total phenolic and flavonoid content of *Rhujuk/Bastanga* (2.44 mg GAE/g and 0.62 mg QE/g) was found to be higher than in the raw bamboo shoot (1.52 mg GAE/g and 0.36 mg QE/g). Sonar *et al.* [26] reported the presence of total phenolic and flavonoid content in fermented bamboo shoots of North East, India and the highest phenolic content was observed in *eup* (920 µg/g), whereas, the lowest phenolic content (718.03 µg/g) was observed in *soidon*. The highest total flavonoid content was observed in *hiring* (308.72 µg/g) and lowest in *eup* (568.54 µg/g). Fermentation have been reported to increase the phenolic and flavonoid content by inducing structural breakdown of the substrate cell wall

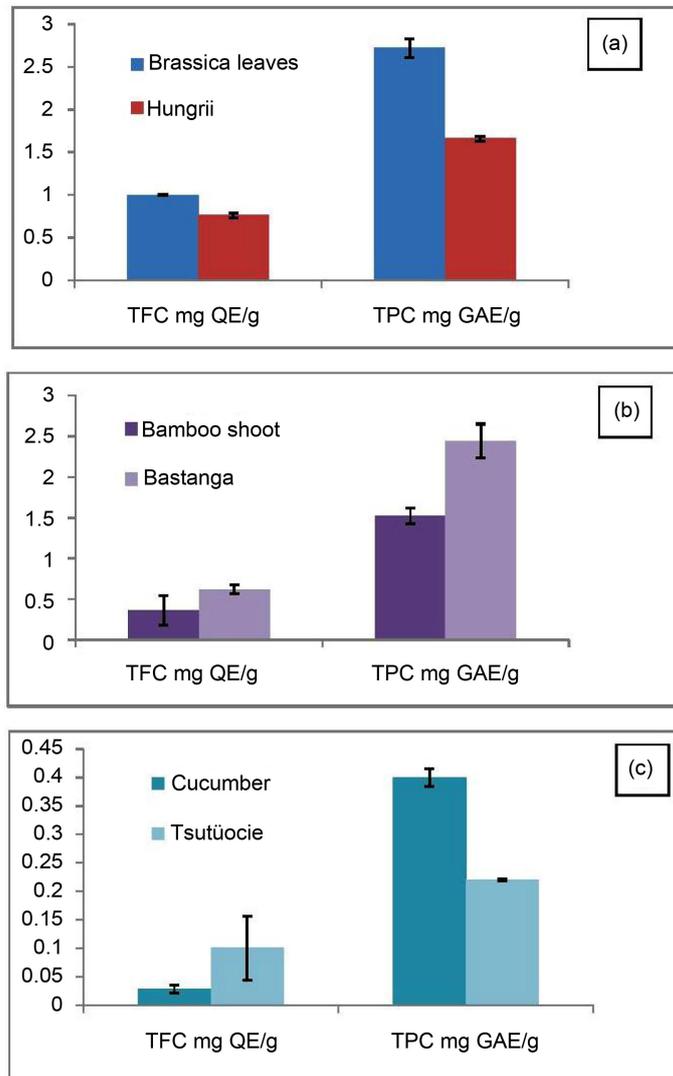


Figure 1. Total phenolic and flavonoid content of non-fermented and fermented products. (a) TPC and TFC of *Hungrii* in comparison with its raw material; (b) TPC and TFC of *Rhujukl Bastanga* in comparison with its raw material; (c) TPC and TFC of *Tsutuocie* in comparison with its raw material.

leading to release of bioactive in plant based functional foods [27]. However, *Hungrii* (1.66 mg GAE/g and 0.76 mg QE/g), had relatively low levels of phenolics and flavonoid as compared to *Brassica* leaves (2.72 mg GAE/g and 1.08 mg QE/g). In, *Tsutuocie* (0.22 mg GAE/g) the total phenolic content was lower but the total flavonoid content (0.12 mg QE/g) increased from that of its raw material (0.4 mg GAE/g and 0.028 mg QE/g).

It has been reported that fermentation as well as thermo mechanical processes such as extrusion cooking and alkaline hydrolysis, increases total free phenolic content and antioxidant activity [28]. Decrease in the level of bioactive compounds may be due to strengthening of plant cell walls into lignans and lignins by polymerization [29]. Ng *et al.* [30] also reported that plant parts have an

Table 2. IC50 value of DPPH scavenging capacity of non-fermented and fermented products.

Raw materials	DPPH IC50 (mg/ml)	Fermented product	DPPH IC50 (mg/ml)
Brassica leaves	1.09	<i>Hungrii</i>	1.22
Bamboo shoot	1.87	<i>Rhujukl Bastanga</i>	1.55
Cucumber	2.01	<i>Tsutuocie</i>	3.65
Trolox			0.204

increase in total phenols after fermentation, and that the observed antioxidant activity may be due to the increase in the total phenolic compounds.

Antioxidant activity: Antioxidants are substances which inhibits or delay oxidative processes which occur due to influence of atmospheric oxygen or reactive oxygen species. 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 [31].

Free radical scavenging activity for DPPH radical was expressed as IC50 value (the concentration required to scavenge 50% of DPPH) (Table 2). The free radical scavenging activity of *Rhujukl Bastanga* (1.55 mg/ml) was higher than in bamboo shoot (1.87 mg/ml). However, the free radical scavenging activity of *Hungrii* (1.22 mg/ml) and *Tsutuocie* (3.65 mg/ml) were lower compared to *Brassica* leaves (1.09 mg/ml) and cucumber (2.01 mg/ml). Oh *et al.* [32] reported that during *kimchi* manufacturing process the antioxidant components contained in leaf mustard were degraded, similarly in the present study *hungrii* had lower antioxidant activity than *Brassica*/mustard leaves.

The results showed a significant correlation, where it was seen that higher the concentration of phenolic compounds, the more the scavenging activity. It may not only depend on the concentration of phenolic compounds but also on the kind of phenolic compounds which varies with the degree of hydroxylation and polymerization [33]. 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 [34].

4. Conclusion

Fermented products are still prepared at household levels, increasing the risk of contamination affecting the quality of the food. In foods, it is necessary to know the nutritional composition to be labeled as a quality food product. Present study revealed that most of the vegetable fermented food products get nutritionally value added compared to its raw materials. Thus, 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. There is a need to intensively study

and develop the traditional fermented products for better quality so as to benefit and improve the health as well as to commercialize these products at a larger scale.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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