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# Comparative study of Pre-cooked and Post-cooked *Tungrymbai:* A fermented soya product of Meghalaya

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**Abstract**
*Tungrymbai* is a local fermented soybean food of the ethnic tribes of Meghalaya. Fermentation of soybean enhances the nutritional value by increasing the amount of proteins and vitamins. Lactic acid bacteria have been found to play a vital role in the preservation and production of nutritious fermented foods. In this study comparative analysis between pre-cooked and post-cooked *Tungrymbai* was carried out. Two types of *Tungrymbai* was prepared, one in traditional way (control) and another using *Lactobacillus fermentum* and *Lactobacillus plantarum* culture combination in a ratio of 1:1. Shelf-life of pre-cooked sample was found to be 4 days. The microbial load was found to be more in product prepared with *Lactobacillus* strains in both pre- and post-cooked samples. Two types of post-cooked sample were prepared, sample-I and sample-II. *Lactobacillus* strains was absent in sample-I and present only in sample-II. Sensory analysis of post-cooked samples revealed that product prepared with *Lactobacillus* strains was more acceptable than traditional *Tungrymbai*. *Tungrymbai* can be prepared using *Lactobacillus* strains and different cooking procedure can be adapted without harming the *Lactobacillus* strains.

**Keywords:** *Tungrymbai*, pre-cooked, post-cooked, *Lactobacillus fermentum*, *Lactobacillus plantarum*, sensory analysis, beneficial microbes

## Introduction
Fermentation is one of the oldest and cost-effective methods for producing and preserving foods. In addition to preservation, fermentation also increases texture, aroma, food digestibility, prolonged shelf-life of food, improved nutritional as well as pharmacological values. Fermentation involves the action of promising microorganisms, or their enzymes, on food substrates leading to biochemical changes which results in significant changes to the food [1]. *Tungrymbai* is a naturally fermented food of *Khasi* and *Jaintia* tribes of Meghalaya [2]. Preparation of *Tungrymbai* was solely practice by people using local techniques [3]. Fermented foods are produced world-wide using a variety of traditional methods incorporating different natural resources and starter culture. The North-Eastern state of India is known for the preparation and consumption of many varieties of indigenous fermented food. The fermented soybean foods of northeastern states share a common feature of being sticky, slightly alkaline and having ammoniacal odour, even though the preparation methods is different [2-3]. Soybean fermentation is a natural process and involves mixed cultures of bacteria, fungi and yeasts. Lactic acid bacteria that are involved in the fermentation, is associated with substrate utilization, flavour promotion, food preservation and probiotic properties [4-5]. Lactic Acid Bacteria (LAB) are the most commonly used microorganisms in fermented foods which are associated mainly with their physiological features such as substrate utilization, metabolic capabilities and probiotic properties. Lactic acid bacteria has drawn maximum attention in food and nutrition science due to their nutraceutical potential producing certain biologically active peptides, along with other functional and probiotic attributes and most importantly having GRAS status [6]. Some of the health benefits of fermented foods observed are prevention of cardiovascular diseases, synthesis of important nutrients, prevention of cancer such as breast cancer, gastrointestinal disorders, allergic reactions and diabetes [7]. The traditional methods of ethnic fermented foods and their mode of consumption can be useful to understand and unexplored the knowledge of food production [8].
The present paper aims to compare the pre-cooked and post-cooked *Tungrymbai* sample by assessing the microbial composition and sensory analysis of the final product.

## Materials and Methods
### Soybean Used
Small, smooth, yellow colour seeds, local variety of soybean (*Glycine max* (L.) Merill) were purchased from local market of Meghalaya.

### Starter Culture
In this study the LAB strain *Lactobacillus fermentum* and *Lactobacillus plantarum (NCBI GenBank Accession No.KU644575 and MF155569.1)* were used which was isolated from fermented foods of Meghalaya by the department of RDAP, NEHU, Tura Campus.

### Starter culture (s) preparation
Starter culture preparations were followed as described by [9] with few modifications. A loopful culture of *Lactobacillus plantarum* and *Lactobacillus fermentum* was inoculated in 10 ml MRS broth (M255, HiMedia) respectively and incubated overnight at 37˚C. One ml of each culture was centrifuge at 10,000 RPM for 15 minutes, the supernatant was discarded

and one ml of sterile saline was added to the pellet, cells were resuspended and again centrifuged at 10,000 RPM for 10 minutes, the supernatant was discarded and one ml of sterile distilled water was added. Through this procedure the desired inoculum was achieved, and their growth activity was evaluated in skim milk medium [10].

**Preparation of pre-cooked traditional *Tungrymbai* (Control)**

About 50 grams of soybean was cleaned, washed and soaked in 100ml Reverse osmosis (RO) water and kept overnight at room temperature. Soaked soybeans was cleaned and boiled in pressure cooker for 15 minutes at 100˚C till it softens. The cooked soybeans were transferred into a sterile bamboo basket aligned with fresh leaves of *Clinogyne dichotoma* locally known as "*slamet*". The bamboo basket was wrapped with sterile muslin cloth and kept for fermentation for 3-4 days at 37˚C[11].

**Preparation of laboratory scale pre-cooked *Tungrymbai* using *Lactobacillus* strains**

About 50 grams of soybean was cleaned, washed and soaked in 100ml Reverse osmosis (RO) water and kept overnight at room temperature. Soaked soybeans was cleaned and autoclaved for 20minutes at 121˚C till it softens. The cooked soybean is allowed to cool till it reaches 30˚C. It is then transferred into a sterile bamboo basket aligned with fresh leaves of *Clinogyne dichotoma*; inoculate with the cell biomass of *Lactobacillus fermentum* and *Lactobacillus plantarum* in 1: 1 ratio. *Clinogyne dichotoma* leaves are covered on top of the soybean. The whole basket was wrapped with sterile muslin cloth and kept for fermentation in an incubator at 37˚C for 3-4 days [11].

**Preparation of post-cooked *Tungrymbai***

The sample was divided into two parts Sample I and Sample II.

- **Sample I:** Mustard oil was heated in a pan at 100˚C; followed by garlic paste and fried until golden brown. Next, pre-cooked *tungrymbai* sample was fried until brownish in colour and grounded chillies, black sesames seeds and salt was added, 50ml of RO water was poured for mixing the ingredients properly. The product was cooked for 5-10 minutes till all the water evaporates.
- **Sample II:** Mustard oil was heated in a pan at 100˚C; and garlic paste was fried until golden brown, next spices like grounded chillies, black sesames seeds and salt were added. 50ml of RO water was poured for mixing the ingredients properly. The mix was cooked for 5-10 minutes. The spices was allowed to cool down till 25-30˚C and pre-cooked *tungrymbai* sample was mixed with it.

**Microbial Analysis**

10 grams of soybean sample was taken and mixed in a sterile mortar and pestle. 90 mL of distilled water was added to homogenise. 1ml of liquid sample was taken and mixed thoroughly with 4ml of sterile 0.1% peptone water and serial dilutions was performed. The sample was diluted from $10^{-1}$ - $10^{-4}$ dilutions. 200µl of the sample was taken and plated on de Mann, Rogosa and Sharpe (MRS) agar (M255, HiMedia, India), for Lactic acid bacteria count. The MRS plates were incubated at 37˚C for 24hours. This was carried out for 1, 2, 3 and 4 days of the sample [11].

The calculated results were expressed as colony forming units (cfu) per ml. CFU/ml= No. of colonies x Dilution factor / volume of inoculum.

**Consumer preference trial**

Sensory analysis of post-cooked *Tungrymbai* was judged by 5 panellists (consumers familiar with the taste of traditional *Tungrymbai*) it was evaluated in terms of aroma, taste, colour, mouth feel, texture, overall acceptability using a nine-point hedonic scale [12].

**Statistical Analysis**

The experimental results were expressed as mean ± standard deviation (SD) of three replicates and the data were analyzed by using one way analysis of variance with a significance level of 0.05.

**Results**

**Microbial Analysis**

The microbial population showed differences between the pre-cooked and post-cooked *Tungrymbai*. In pre-cooked *Tungrymbai* the analysis for *Lactobacillus* count was kept for 4 days to study the shelf-life of the product. It was found that *Lactobacillus* count increases in 1st and 2nd day i.e., from 6.212 ± 0.015 to 6.892 ± 0.100 (log cfu/ml) for traditional prepared *Tungrymbai* and from 7.119 ± 0.022 to 7.357 ± 0.130 (log cfu/ml) for *Tungrymbai* prepared with *Lactobacillus fermentum* and *Lactobacillus plantarum* culture combination in 1:1 ratio. *Lactobacillus* count was found to decline on 3rd and 4th i.e., 6.756 ± 0.106 to 6.512 ± 0.063 (log cfu/ml) for traditional prepared *Tungrymbai*, and from 7.183 ± 0.150 to 7.012 ± 0.015 (log cfu/ml) for *Tungrymbai* prepared with *Lactobacillus* strains. The *Lactobacillus* count was found to be more in *Tungrymbai* prepared with *Lactobacillus* strains than the traditionally prepared *Tungrymbai* with 7.119 ± 0.022 log cfu/ml. The analysis of variance of pre-cooked *Tungrymbai* showed that there was significant difference ($p < 0.05$) in *Lactobacillus* count of the samples.

In post-cooked sample the *Lactobacillus* count was absent in Sample-I. This can be due to the heat produced and the cooking temperature at 100ºC, in which the *Lactobacillus* species cannot survive[11]. In Sample-II the *Lactobacillus* count was found to be 6.65 ± 0.150 and 7.91 ± 0.112 (log CFU/ml) for traditionally prepared *Tungrymbai* and *Tungrymbai* prepared with *Lactobacillus* strains respectively. Further, it was found that the *Lactobacillus* count was more in pre-cooked *Tungrymbai* than in post-cooked *Tungrymbai*. Moreover, in post-cooked sample-I it was found that the *Lactobacillus* species does not survived because of the cooking method and the cooking temperature.

**Table 1:** Microbial analysis of pre-cooked *Tungrymbai* sample

| Microbial load (log CFU/ml) | Storage day | Storage temp. ($^0$C) | Traditional *Tungrymbai* | *Tungrymbai* with **Lactobacillus* strains |
|---|---|---|---|---|
| *Lactobacillus* viable cell count | 1 | 6 | 6.212 ± 0.015 | 7.119 ± 0.022 |
| | 2 | 6 | 6.892 ± 0.100 | 7.357 ± 0.130 |
| | 3 | 6 | 6.756 ± 0.106 | 7.183 ± 0.150 |
| | 4 | 6 | 6.512 ± 0.063 | 7.012 ± 0.015 |

Values are mean ± standard deviation of triplicate determinations (n=3), **Lactobacillus* strains: *L. plantarum* and *L. fermentum* culture combination in 1:1 ratio.

**Table 2:** Microbial analysis of post-cooked *Tungrymbai* sample

| Microbial load (log CFU/ml) | Traditional *Tungrymbai* (Sample I) | *Tungrymbai* with **Lactobacillus* strains (Sample I) | Traditional *Tungrymbai* (Sample II) | *Tungrymbai* with **Lactobacillus* strains (Sample II) |
|---|---|---|---|---|
| *Lactobacillus* viable cell count | Absent in 1ml | Absent in 1ml | 6.65 ± 0.150 | 7.91 ± 0.112 |

Values are mean ± standard deviation of triplicate determinations (n=3), Sample I- Product prepared by cooking along with spices, Sample II- Product prepared without cooking along with spices. **Lactobacillus* strains: *L. plantarum* and *L. fermentum* culture combination in 1:1 ratio.

**Sensory Analysis**

The organoleptic evaluation was performed only on post-cooked sample as the product was never consumed mainly at pre-cooked level. Sensory analysis revealed that sample-II prepared product was more acceptable than sample-I in terms of aroma, taste, colour, mouth feel, texture and overall acceptability. Sample-II *Tungrymbai* prepared with *Lactobacillus* strains (*L. plantarum* and *L. fermentum* culture combination) was found out to be superior and more preferable by the panellist (consumers acquainted with taste of *Tungrymbai*), among traditional and *Tungrymbai* sample prepared with *Lactobacillus* strains, with 7.4 ± 0.894 overall acceptability.

**Table 3:** Sensory analysis of post-cooked *Tungrymbai* samples

| Parameters | Traditional *Tungrymbai* (Sample I) | *Tungrymbai* with **Lactobacillus* strains (Sample I) | Traditional *Tungrymbai* (Sample II) | *Tungrymbai* with **Lactobacillus* Strain (Sample II) |
|---|---|---|---|---|
| Aroma | 5.8 ± 1.303 | 5.8 ± 0.836 | 6.2 ± 1.923 | 6.8 ± 0.836 |
| Taste | 6.0 ± 0.707 | 5.6 ± 1.949 | 6.6 ± 0.894 | 7.6 ± 1.140 |
| Colour | 6.0 ± 1.22 | 6.0 ± 1.224 | 6.6 ± 0.547 | 7.0 ± 1.581 |
| Mouth feel | 6.4 ± 1.516 | 6.0 ± 0.707 | 6.2 ± 1.643 | 7.0 ± 1.224 |
| Texture | 6.2 ± 1.643 | 6.0 ± 1.732 | 6.4 ± 1.140 | 6.8 ± 0.836 |
| Overall acceptability | 5.8 ± 1.303 | 5.6 ± 1.949 | 6.4 ± 1.673 | 7.4 ± 0.894 |

Values are mean ± standard deviation of triplicate determinations (n=3), Sample I- Product prepared by cooking along with spices, Sample II- Product prepared without cooking along with spices. **Lactobacillus* strains: *L. plantarum* and *L. fermentum* culture combination in 1:1 ratio.

**Discussion**

The reduction of *Lactobacillus* count as the number of storage days increased can be because as the organisms reaches the death/declining phase and the nutrients absorption depleted as the number of storage days increased. It has also been noticed that the *Tungrymbai* prepared with bacterial strains has more *Lactobacillus* count than the traditionally prepared *Tungrymbai*; this is due to the growth of *L. fermentum* and *L. plantarum* in the product which increased the microbial count. The microbial growth was found to be more in pre-cooked *Tungrymbai* than in post-cooked sample, which may be due to the ingredients added such as garlic and ginger which are having anti-microbial properties that reduced the microbial load and other microbes in post-cooked *Tungrymbai* [13]. The loss of viability of *Lactobacillus* strains during heating at 65ºC and above was found to denature the protein and damage the cell wall which may be responsible for thermal death of *Lactobacillus* species [14]. Other findings were observed [15] were *Lactobacillus* species were absent in the post-cooked samples of *Tungrymbai* due to the cooking procedure. Hence, Sample-II shows better result compared to Sample-I.

In sensory analysis, the sample prepared with *Lactobacillus* strains was found to be more acceptable than the traditionally prepared sample. Similar findings have been studied [16] in which *Kinema* prepared with pulverised starter culture was more acceptable than market *Kinema*. Other studies have been done[17] in which the modified *Doenjang* (Korean fermented soybean) inoculated with *Bacillus* species have better sensory attributes than the traditionally prepared *Doenjang*. Hence, *Tungrymbai* prepared with *Lactobacillus* strains was found to be better than traditional *Tungrymbai.*

**Conclusion**

The result of this study showed that addition of *L. fermentum* and *L. plantarum* culture combination to *Tungrymbai* can improved its probiotic properties, flavour promotion and food preservation. The microbial load was found to be more in pre-cooked samples than in post-cooked *Tungrymbai* samples. Moreover, the cooking procedure of post-cooked sample-I has drastically affect the microbial load of beneficial microbes; this can be avoided by adopting new methods of cooking such as sample-II method of post-cooked *Tungrymbai*, in which the product was added separately to the cooked spices. Sensory analysis also revealed that preparation of post-cooked *Tungrymbai* by sample-II procedure was more acceptable by the consumers. Keeping in view of the above result, adoption

of new method for the production of post-cooked *Tungrymbai* can be more beneficial for the consumers, in which the probiotic microorganisms will also retain in the process which is highly recommended.

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