

Original Research Article

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## Standardization of Technology for the Preparation of “*Tungrymbai*” using Selected *Lactobacillus* Strain

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### ABSTRACT

*Tungrymbai* is a traditional fermented dish of the *Khasi* and *Jaintia* tribes of Meghalaya. In this study an attempt was made to standardize the preparation of “*Tungrymbai*” using *Lactobacillus fermentum* and *Lactobacillus plantarum* culture obtained from fermented foods of Meghalaya. Three samples were prepared and the bacterial strain was added aseptically to the cooked soybeans in a combination of 1:1 ratio at 1, 2 and 3% culture combination respectively, a traditional sample was used as control. The samples were fermented at 37°C for 3-4 days. Sensory analysis of pre-cooked sample was done for 1, 2, 3 and 4 days by keeping at 6°C and 33°C. Pre-cooked sample prepared with 1% cell biomass was more preferred among other samples by the panellist and was carried out for post-cooked preparation. Two types of sample was prepared in post-cooked, sample I were sample was cooked along with ingredients and sample II were sample was mixed separately after ingredients was cooked. Sample II was found to be more preferable by the panellist in terms of aroma, taste, texture, colour and general acceptability. Laboratory prepared *tungrymbai* sample with *Lactobacillus* strain may provide as an alternative to traditional *tungrymbai* which can impart health benefits to consumers providing better quality, hygienic product and to enhance the nutritional and medicinal value of the product.

#### Keywords

*Tungrymbai*,  
*Lactobacillus*,  
Traditional, Pre-  
cooked, Post-cooked

#### Article Info

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### Introduction

*Tungrymbai* is a soybean-based fermented product prepared by using soybean seeds (*Glycine max* L. Meri) by the ethnic *Khasi* and *Jaintia* tribes of Meghalaya (Sohliya *et al.*, 2009). It serves as a cheap source of plant protein in local diet (Chettri and Tamang, 2016). *Tungrymbai* is similar to other fermented soybean products such as *akhone* of Nagaland, *hawaijar* of Manipur, *bekang* of Mizoram and *kinema* of Sikkim (Tamang

1996). In the traditional method of preparation, the soybean seeds are soaked overnight in double the quantity of water, they are then boiled well till the seeds became soft, and place in a bamboo basket aligned with leaves of *Clinogyne dichotoma* locally known as ‘*slamet*’ and covered with the same leaves at the top, it is then packed tightly and wrapped with a jute bag and kept near the fire place (25- 40°C) for 3-4 days. Fermentation of soybean is done only at the household and village level, and this skill is passing on from

one generation to another, this art is slowly dying out, as the process is tedious and because the fermented product can simply get from the market (Agrahar-Murugkar and Subbulakshmi, 2006).

There has been an increasing interest in probiotic products that contain Lactic acid bacteria (LAB) of intestinal origin in terms of functional food. Probiotic microorganisms can provide a number of health benefits, which include antimicrobial effects against pathogens, anti-tumour effects, anti-cholesterol, immuno-modulation, anti-diabetic and the treatment of diarrhea and lactose intolerance (Nagpal *et al.*, 2007, 2010, and 2012). In addition, probiotics also play a beneficial role in several medical conditions, including lactose intolerance, cancer, allergies, hepatic disease, urinary tract infections, hyperlipidaemia and assimilation of cholesterol (Ejtahed *et al.*, 2011).

Probiotic organisms used in foods have the potential to resist gastric juices, exposure to bile, and have the ability to proliferate and colonize the digestive tract (Saad *et al.*, 2013). Lactic acid bacteria (LAB) are considered as a major group of probiotic bacteria which are commonly used in both humans and animals (Nousiainen and Setala, 1998). The most commonly used LAB in humans is *Lactobacillus* and *Bifidobacterium* (Soccol *et al.*, 2010). Lactic acid bacteria (LAB) perform an essential role in the preservation and production of wholesome fermented foods (Steinkraus 1983).

Probiotic foods enhance health after consumption and contain microorganisms which are viable, specific and effective on main systems of nutritional physiology (Vaughan and Mollet, 1999; Soccoll *et al.*, 2010). Lactic acid bacteria, involved in the fermentation are associated with substrate utilization, flavour promotion, food preservation and probiotic properties. The

proteolytic activity of Lactic acid bacteria also affects the product texture, aroma and flavour (Thokchom and Joshi, 2013). Functional or technological properties of LAB isolated from fermented foods are important criteria for selection of starter cultures to be used in the manufacture of functional foods (Durlu-Ozkaya, 2001).

Detailed studies on the nutritional and therapeutic value of laboratory scale prepared *tungrymbai* using *Lactobacillus plantarum* and *Lactobacillus fermentum* can provide valuable information and bring benefits in the use of this product on a wider scale. Fermentation of soybean with lactic acid bacteria considerably increases its health value (Božanić *et al.*, 2011). Incorporation of probiotic organisms in *tungrymbai* can provide a potential as healthy food to improve its quality and the health status of consumers (Fukushima *et al.*, 2001).

## **Materials and Methods**

### **Soybean sample**

Small, smooth, yellow seed “local variety” of soybean [*Glycine max* (L.) Merrill] were purchased from the local market of Meghalaya.

### **Collection of Starter Culture**

The LAB strain *Lactobacillus fermentum* and *Lactobacillus plantarum* used in this study was isolated from fermented foods of Meghalaya by the department of RDAP, NEHU, Tura Campus in a ratio of 1:1.

### **Development of Inoculum**

*Lactobacillus fermentum* and *Lactobacillus plantarum* bacterial strains was transfer in MRS broth (M255, HiMedia, India) and incubate at 37°C for 24 hours. Each activated culture was inoculated into MRS broth and

incubated at 37°C for 16 hours. These working cultures were then transferred into skim milk medium to check their activity in this medium thereby evaluating the growth of these cultures in skim milk (Hati *et al.*, 2014)

### **Starter culture (s) preparation**

A loopful culture of selected *Lactobacillus* species was inoculated in 10 ml MRS broth (M255, HiMedia) 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 (Chettri and Tamang, 2016)

### **Preparation of Tungrymbai in traditional way**

Local variety of soybean was used and about 50g of soybean was cleaned, washed and soaked in 100ml RO water and kept overnight at room temperature. Soaked soybeans was cleaned and without dehulling it was boiled in pressure cooker for 15 minutes at 100°C till it softens.

The cooked soybeans were transferred into a pre-sterile bamboo basket aligned with fresh leaves of *Clinogyne dichotoma* or *Phrynium pubinerve* locally known as “slamet” on the base and on the inner sides of the basket. *Slamet* leaves are then covered on top of the soybean. The whole basket was wrapped with pre-sterile muslin cloth and kept for fermentation in an incubator at 37° C for 3-4 days (Thokchom and Joshi, 2012)

### **Laboratory scale preparation of tungrymbai using different culture percentages**

Local variety of soybean was used and about 50g of soybean was cleaned, washed and soaked in 100ml RO water and kept overnight at room temperature. Soaked soybeans was cleaned and without dehulling it was boiled in pressure cooker for 15 minutes at 100°C till it softens. The cooked soybean is allowed to cool till it reaches 30°C. It is then transferred into a pre-sterile bamboo basket aligned with fresh leaves of *Clinogyne dichotoma* or *Phrynium pubinerve* on the base and on the inner sides of the basket, inoculate with the cell biomass of *Lactobacillus fermentum* and *Lactobacillus plantarum* in 1: 1 ratio in different percentages of 1, 2, and 3%. *Slamet* leaves are then covered on top of the soybean. The whole basket was wrapped with pre-sterile muslin cloth and kept for fermentation in an incubator at 37° C for 3-4 days (Thokchom and Joshi, 2012)

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

The sample was divided into two parts Sample I and Sample II, and was prepared as under:

#### **Sample I**

Firstly, Mustard oil was heated in a pan at 100°C; garlic paste was added and fried until golden brown. Next, pre-cooked *Tungrymbai* sample was added and fried until brownish in colour followed by grounded chillies, black sesames seeds and salt, 50ml of RO water was poured for mixing the ingredients properly. The product was cooked for 5-10 minutes and ginger was added for garnishing.

#### **Sample II**

Mustard oil was heated in a pan at 100°C; garlic paste was added and fried until golden brown followed by grounded chillies and black sesames seeds and salt were added and 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, ginger was added for garnishing.

### **Consumer preference trial**

Sensory analysis of the pre-cooked *tungrymbai* samples was judged by 5 panellists (consumers who are familiar with traditional *tungrymbai*) it was evaluated in terms of aroma, taste, colour, mouth feel, texture, overall acceptability using a nine-point hedonic scale (Peryam and Girardot, 1952). The sensory analysis and shelf-life studies for pre-cooked *Tungrymbai* samples were carried out for 1, 2, 3 and 4<sup>th</sup> day of storage keeping at 6°C and 33°C.

### **Statistical Analysis**

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

### **Results and Discussion**

#### **Sensory analysis of pre-cooked *tungrymbai* sample stored at 6°C**

Results presented in Table 7 showed that *Tungrymbai* sample prepared with 1% cell biomass has evaluated under 9 point hedonic rating scale and found highest score for overall acceptability with  $7.82 \pm 0.607$  and when it was compared with 2% and 3% culture product the score was  $5.88 \pm 0.717$  and  $5.48 \pm 0.223$ . Results also revealed that the score of sensory analysis decreases from  $7.82 \pm 0.607$ ,  $7.71 \pm 0.447$ ,  $5.88 \pm 0.717$  and  $5.48 \pm 0.223$  as on first day to  $5.48 \pm 0.665$ ,  $5.08 \pm 0.552$ ,  $4.52 \pm 0.223$  and  $3.89 \pm 0.414$  as on 4<sup>th</sup>

day for 1%, traditional, 2% and 3% *tungrymbai* samples respectively. Our results were in agreement with (Naz, 2012) who have worked on other products. *Tungrymbai* prepared in traditional way (control) was preferred 2<sup>nd</sup> best as it has better taste, consistency and aroma compared to *tungrymbai* with 2% and 3% cell biomass. Moreover, 2% and 3% cell biomass was found to be bitter in taste, dry texture, pale yellowish color and strong ammonia smell and it was absent in 1% cell biomass.

The analysis of variance was observed to be highly significant ( $p < 0.05$ ) in taste, mouth feel and overall acceptability. Whereas, there was no significant ( $p < 0.05$ ) difference for aroma, colour and texture.

The significant ( $p < 0.05$ ) difference in taste, mouth feel and overall acceptability maybe due to the resemblance of samples inoculated with cell biomass with the traditional *tungrymbai* sample which were superior in terms of health benefits and probiotic attributes than the traditional sample.

#### **Sensory analysis of pre-cooked *tungrymbai* sample stored at 33°C**

Results showed that sample prepared with 1% cell biomass was more preferred by the panellist with  $7.66 \pm 0.707$  overall acceptability, whereas other samples i.e. 2% and 3% cell biomass scored  $5.18 \pm 0.436$  and  $4.81 \pm 0.836$  overall acceptability respectively. Except aroma, the sensory evaluation and shelf life study for other parameters was done only for the 1<sup>st</sup> and 2<sup>nd</sup> day as the quality of the product began to deteriorate, the aroma and flavour becomes unpleasant, taste becomes bitter and product began to spoil. *Tungrymbai* prepared in traditional way (control) was preferred 2<sup>nd</sup> best followed by sample prepared with 2% cell biomass.

**Table.1** Sensory analysis of Pre-cooked *Tungrymbai* samples stored at 6°C

Parameters	Storage days	TT	T-1%	T-2%	T-3%
Aroma	1	6.82 ± 1.140	7.41 ± 0.894	6.64 ± 0.836	6.40 ± 0.547
	2	6.21 ± 0.894	6.66 ± 0.467	6.15 ± 0.536	6.05 ± 0.707
	3	5.66 ± 0.547	6.44 ± 0.547	5.66 ± 0.547	5.46 ± 0.566
	4	5.20 ± 0.448	5.46 ± 0.466	5.46 ± 0.666	5.01 ± 0.356
Taste	1	7.00 ± 0.707	7.18 ± 0.836	5.84 ± 0.836	5.21 ± 0.836
	2	6.00 ± 0.110	6.45 ± 0.547	5.21 ± 0.707	4.23 ± 0.221
	3	5.66 ± 0.547	6.18 ± 0.547	5.01 ± 0.113	5.21 ± 0.447
	4	5.00 ± 0.346	5.46 ± 0.566	4.31 ± 0.112	4.10 ± 0.247
Colour	1	7.61 ± 0.836	7.66 ± 0.547	6.55 ± 0.547	6.42 ± 0.547
	2	6.71 ± 0.547	7.14 ± 0.547	5.56 ± 0.547	5.81 ± 0.447
	3	5.81 ± 0.447	6.68 ± 0.516	4.62 ± 0.547	3.44 ± 0.894
	4	5.40 ± 0.547	5.51 ± 0.447	4.02 ± 0.112	3.11 ± 0.552
Mouth feel	1	7.00 ± 0.707	7.81 ± 0.836	5.80 ± 0.836	5.00 ± 0.224
	2	6.82 ± 0.447	6.66 ± 0.547	5.14 ± 0.447	4.44 ± 0.547
	3	5.56 ± 0.110	6.55 ± 0.547	4.42 ± 0.547	4.16 ± 0.456
	4	5.14 ± 0.447	5.70 ± 0.836	3.95 ± 0.224	3.20 ± 0.412
Texture	1	7.72 ± 0.447	7.88 ± 0.520	6.21 ± 0.336	6.11 ± 0.800
	2	6.78 ± 0.246	7.36 ± 0.894	6.15 ± 0.707	6.05 ± 0.707
	3	5.77 ± 0.112	6.59 ± 0.215	5.67 ± 0.147	5.77 ± 0.354
	4	5.22 ± 0.217	5.77 ± 0.337	4.58 ± 0.220	4.42 ± 0.102
Overall acceptability	1	7.71 ± 0.447	7.82 ± 0.607	5.88 ± 0.717	5.48 ± 0.223
	2	6.56 ± 0.235	7.41 ± 0.894	5.00 ± 0.707	4.56 ± 0.737
	3	5.17 ± 0.447	6.88 ± 0.707	4.62 ± 0.345	4.04 ± 0.542
	4	5.08 ± 0.552	5.48 ± 0.665	4.52 ± 0.223	3.89 ± 0.414

Values are mean ± standard deviation of triplicate determinations (n=5). (TT- Traditional *tungrymbai*, T-*Tungrymbai*)

**Table.2** Sensory analysis of pre-cooked *Tungrymbai* samples stored at 33°C

Parameters	Storage days	TT	T-1%	T-2%	T-3%
Aroma	1	6.41 ± 0.707	7.22 ± 0.136	6.51 ± 0.547	6.20 ± 0.556
	2	6.11 ± 0.836	6.61 ± 0.547	5.01 ± 0.707	5.64 ± 0.547
	3	5.00 ± 0.346	5.46 ± 0.581	5.41 ± 0.512	4.05 ± 0.547
Taste	1	6.77 ± 0.836	6.85 ± 0.836	5.42 ± 0.140	4.83 ± 0.836
	2	5.81 ± 0.447	6.22 ± 0.447	5.43 ± 0.547	4.86 ± 0.447
Colour	1	6.01 ± 0.836	6.19 ± 0.836	5.66 ± 0.547	5.05 ± 0.547
	2	6.10 ± 0.547	6.11 ± 0.447	5.40 ± 0.547	5.02 ± 0.101
Mouth feel	1	6.28 ± 0.836	6.67 ± 0.547	5.20 ± 0.836	4.41 ± 0.547
	2	6.15 ± 0.547	6.38 ± 0.447	5.08 ± 0.447	4.12 ± 0.447
Texture	1	7.62 ± 0.547	7.22 ± 0.136	6.09 ± 0.707	6.08 ± 0.636
	2	6.54 ± 0.836	6.41 ± 0.881	6.00 ± 0.253	5.85 ± 0.447
Overall acceptability	1	7.55 ± 0.217	7.66 ± 0.707	5.18 ± 0.436	4.81 ± 0.836
	2	6.81 ± 0.836	7.48 ± 0.691	4.95 ± 0.673	4.15 ± 0.447

Values are mean ± standard deviation of triplicate determinations (n=5). (TT- Traditional *tungrymbai*, T-*Tungrymbai*) (\*- samples are rejected by the panellist on 3<sup>rd</sup> and 4<sup>th</sup> day as the sample began to deteriorate and spoiled)

**Table.3** Sensory evaluation of post-cooked *Tungrymbai* samples

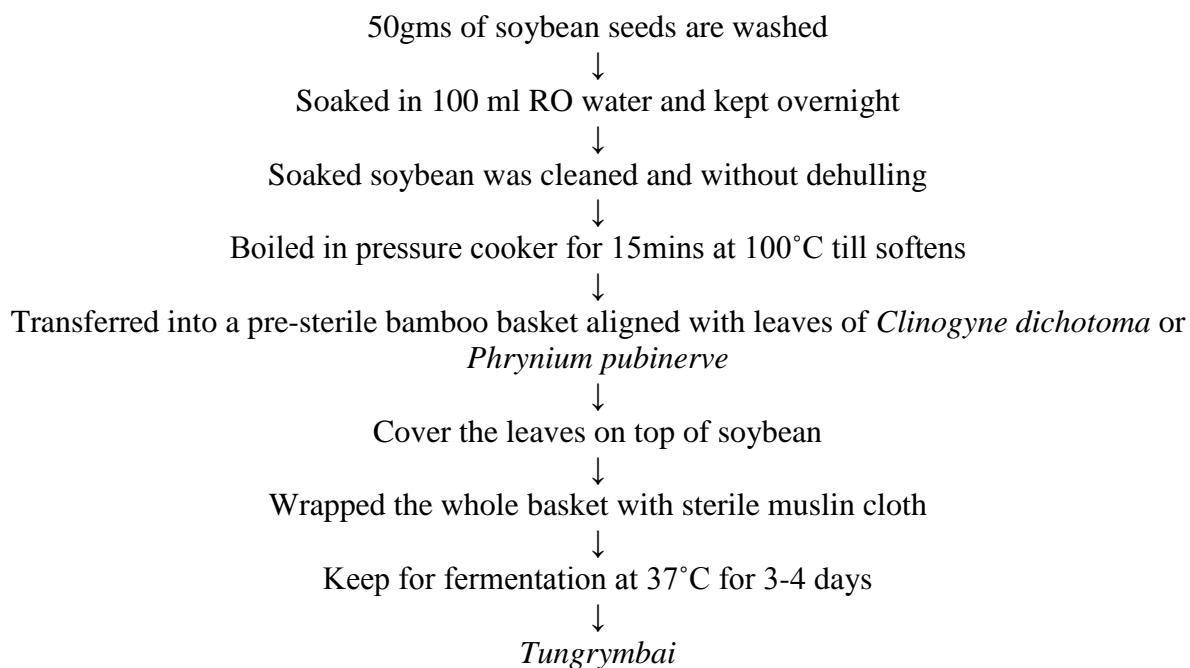
Parameters	TT (Sample I)	T-1% (Sample I)	TT (Sample II)	T-1% (Sample II)
Aroma	5.8 ± 1.303	5.8 ± 0.836	6.2 ± 1.923	6.8 ± 0.836
Taste	6 ± 0.707	5.6 ± 1.949	6.6 ± 0.894	7.6 ± 1.140
Colour	6 ± 1.22	6 ± 1.224	6.6 ± 0.547	7 ± 1.581
Mouth feel	6.4 ± 1.516	6 ± 0.707	6.2 ± 1.643	7 ± 1.224
Texture	6.2 ± 1.643	6 ± 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=5). (TT- Traditional *tungrymbai*, T-*Tungrymbai*)

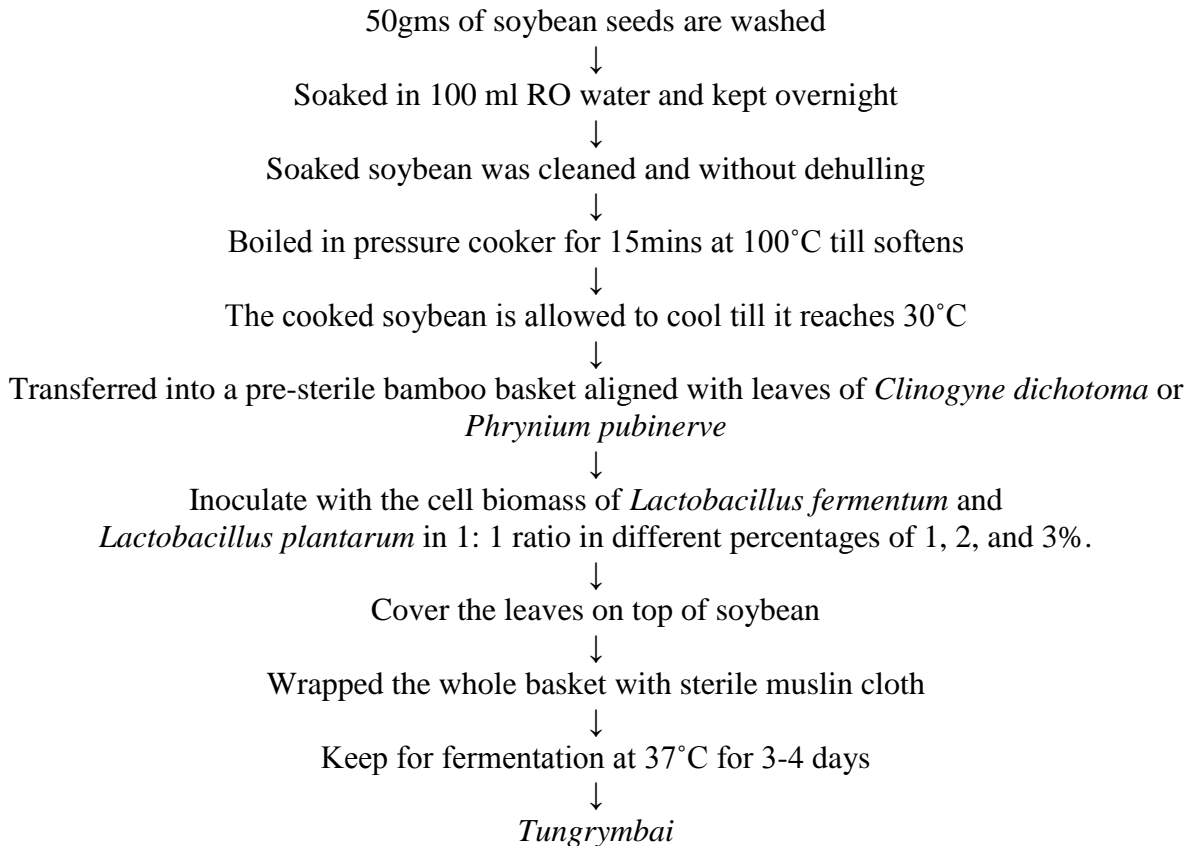
**Fig.1** Stepwise preparation of *tungrymbai*



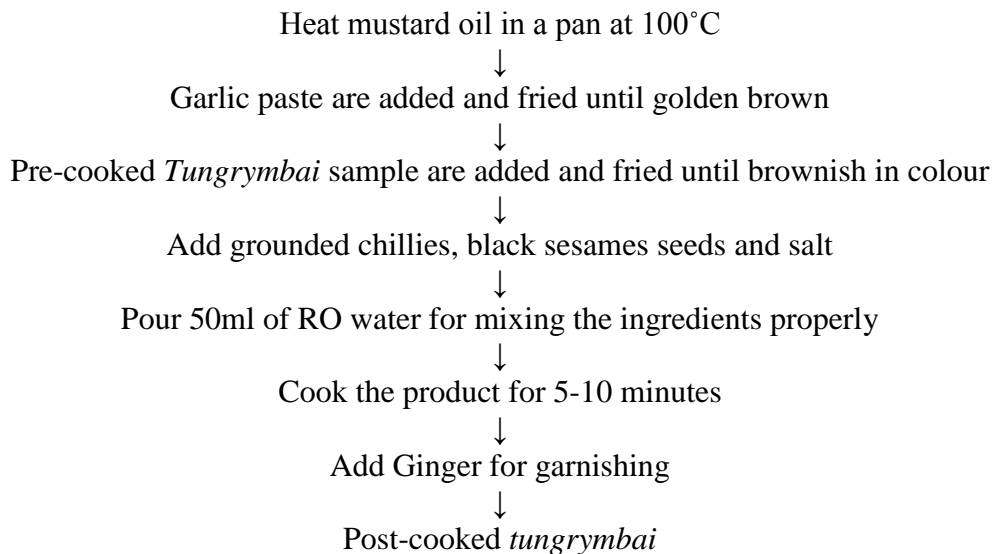
**Fig.2** Flow diagram of traditional method of *Tungrymbai* preparation



**Fig.3** Flow diagram of laboratory scale preparation of *Tungrymbai*



**Fig.4** Flow diagram of post-cooked preparation of *tungrymbai* (Sample I)



**Fig.5** Flow diagram of post-cooked preparation of *tungrymbai* (Sample II)

Heat mustard oil in a pan at 100°C  
 ↓  
 Garlic paste, grounded chillies, black sesames seeds and salt are added and fried until golden brown  
 ↓  
 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  
 ↓  
 Pre-cooked *Tungrymbai* sample was mixed with it  
 ↓  
 Ginger was added for garnishing.  
 ↓  
 Post-cooked tungrymbai

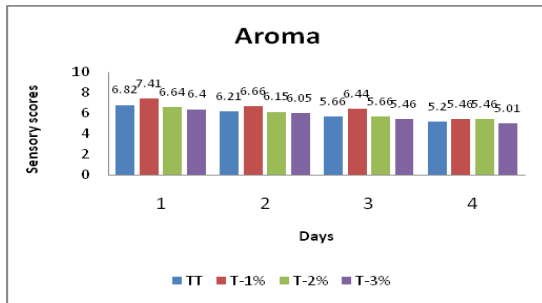


Fig. 8. Aroma of pre-cooked *tungrymbai* stored at 6°C

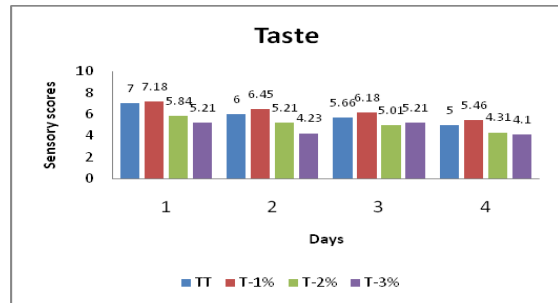


Fig. 9. Taste of pre-cooked *tungrymbai* stored at 6°C

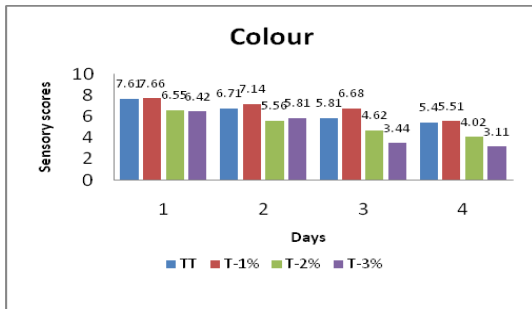


Fig. 10. Colour of pre-cooked *tungrymbai* stored at 6°C

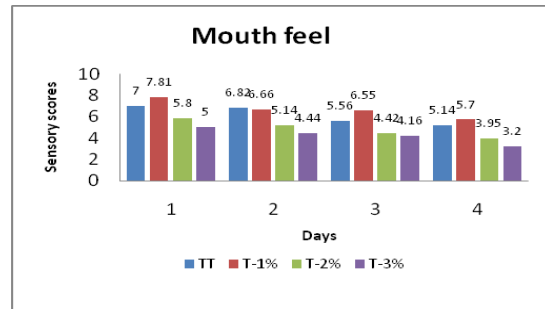


Fig. 11. Mouth feel of pre-cooked *tungrymbai* stored at 6°C

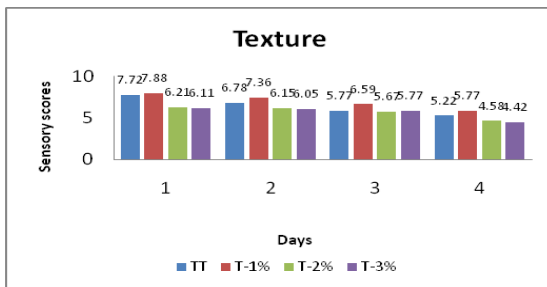


Fig. 12. Texture of pre-cooked *tungrymbai* stored at 6°C

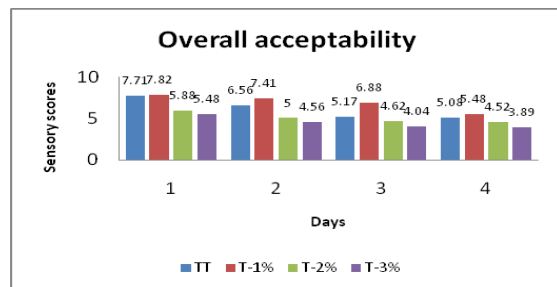


Fig. 13. Overall acceptability of pre-cooked *tungrymbai* stored at 6°C



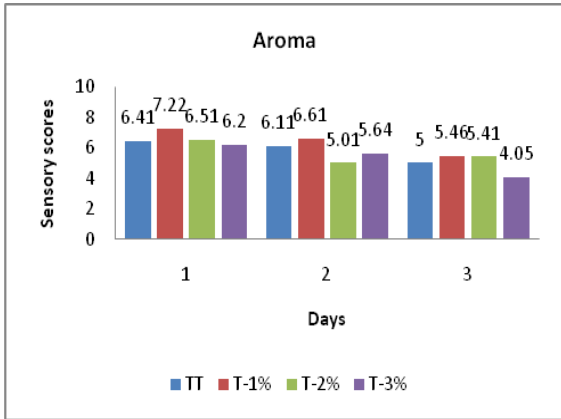


Fig.15. Aroma of pre-cooked *tungrymbai* stored at 33°C

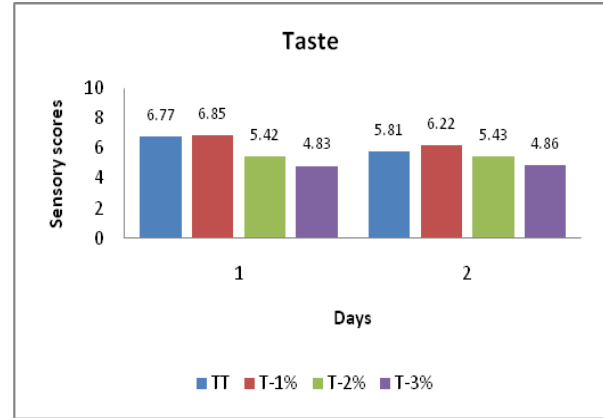


Fig.16. Taste of pre-cooked *tungrymbai* stored at 33°C

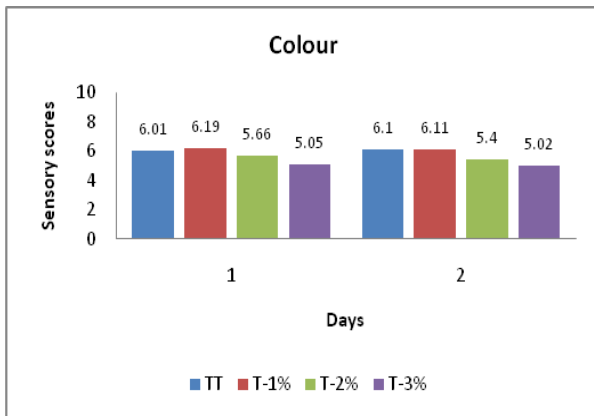


Fig.17. Colour of pre-cooked *tungrymbai* stored at 33°C

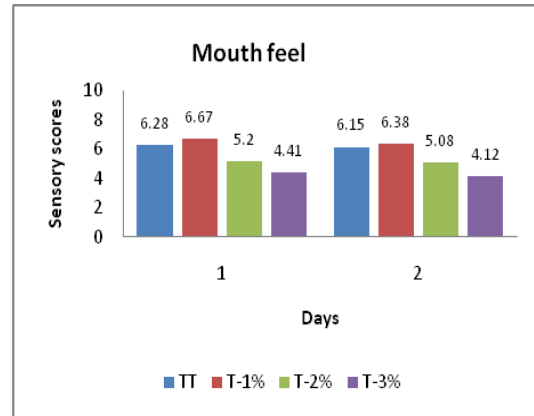


Fig.18. Mouth feel of pre-cooked *tungrymbai* stored at 33°C

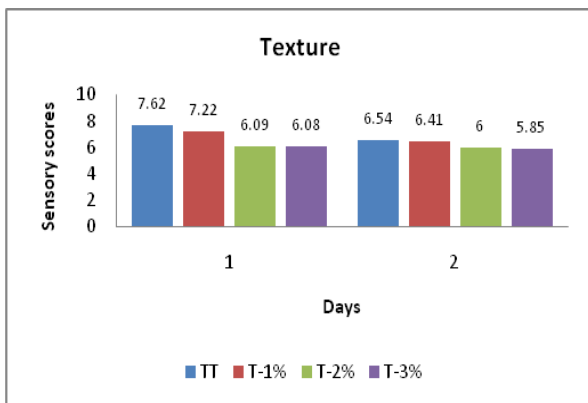


Fig.19. Texture of pre-cooked *tungrymbai* stored at 33°C

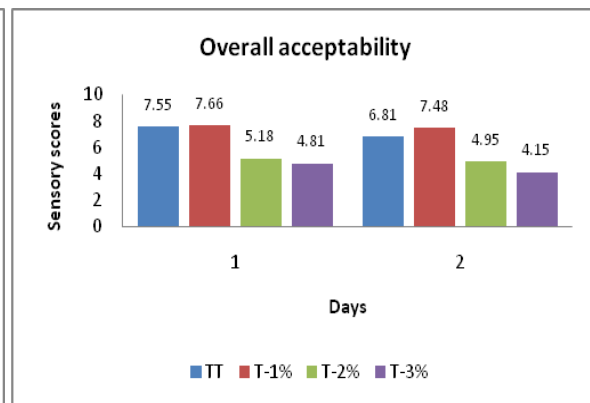


Fig.20. Overall acceptability of pre-cooked *tungrymbai* stored at 33°C

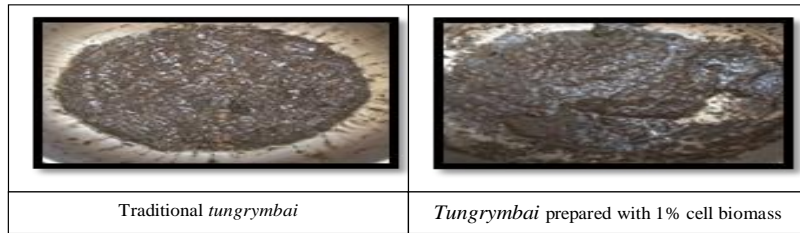


Fig.21. Sample I post-cooked *tungrymbai* samples

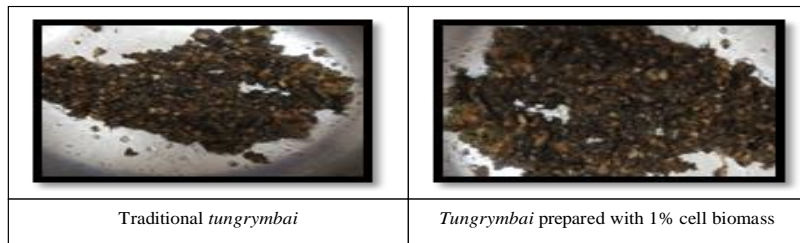
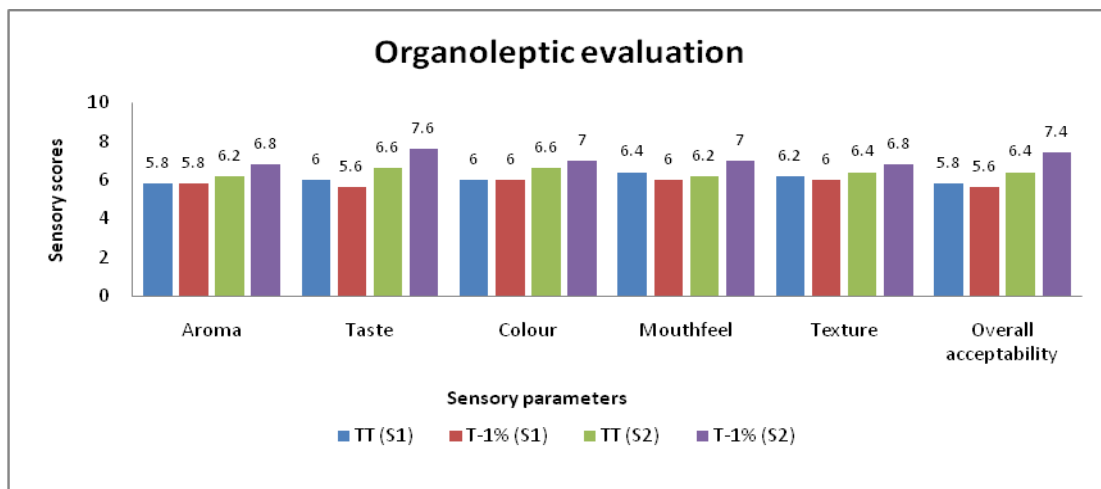


Fig.22. Sample II post cooked *tungrymbai* samples

**Fig.23** Organoleptic evaluation of post-cooked *tungrymbai* sample



The least preferred sample was *tungrymbai* with 3% cell biomass which was found to be bitter in taste, dry in texture and less preferred by the panellist. Similar findings have been reported by Tamang (1999) and Tripathi *et al.*, (2015). There was no significant ( $p < 0.05$ ) difference for aroma, colour and texture. Whereas, the analysis of variance was observed to be highly significant ( $p < 0.05$ ) in taste, mouth feel and overall acceptability. No significant difference was found for aroma, colour and texture. This may be due to the fermentation process which was comparable with the study of Chettri and Tamang (2016).

On the basis of sensory analysis, *tungrymbai* sample prepared with 1% cell biomass was carried out for post-cooked preparation as it has scored the highest among other samples, with better quality and taste, typical soybean flavour and better overall acceptability.

#### **Sensory analysis of post-cooked *tungrymbai* sample**

The sensory analysis for post-cooked *Tungrymbai* samples was done on the same day of preparation; no shelf life study was carried out for the sample because this product is

generally cooked and consume on the same day in the study area. More research work is needed for the shelf life study of this product. From Table 21, it was observed that sample II of *tungrymbai* with 1% cell biomass was more preferred by the panelist/judges, as shown by the following parameters: aroma:  $6.8 \pm 0.836$ , taste:  $7.6 \pm 1.140$ , colour:  $7.00 \pm 1.581$ , mouth feel:  $7.00 \pm 1.224$ , texture:  $6.8 \pm 0.836$ , overall acceptability:  $7.4 \pm 0.894$ .

This may be due to its high palatability, soft texture, better colour and aroma with its typical *tungrymbai* flavour. Moreover, it was found that in sample I, *Lactobacillus* and other beneficial microbes did not survive during the cooking process, similar findings has been reported by Thokchom and Joshi (2012) in which *Lactobacillus* species was not found in the post-cooked samples, because of the cooking procedures used for consumption of the *tungrymbai*. *Tungrymbai* prepared with *Lactobacillus fermentum* and *Lactobacillus plantarum* was more preferable than the traditionally prepared *tungrymbai* which have added advantages of health benefits, hygienic conditions with better quality and flavour. Tamang (1999) also reported that *kinema* prepared by using pulverised starter have more advantages over traditional method of fermentation. Other reported when soybeans were fermented by the LAB showed the potential for developing a healthy food supplement and dietary adjunct (Chonkeeree *et al.*, 2013).

The results indicate that sample II *tungrymbai* with 1% cell biomass was most preferred by the panellist/judges and could be used for the preparation of probiotic *tungrymbai* using *Lactobacillus fermentum* and *Lactobacillus plantarum* culture. Probiotic *tungrymbai* can be more beneficial than the traditional *tungrymbai* in terms of health benefit, hygienic quality and probiotic attributes of the product. Therefore, it is imperative to create an awareness of the beneficial aspects of *tungrymbai* without cooking and frying it as the beneficial microbes will not survive during cooking procedure.

Considering its health benefits, hygienic quality and probiotic attributes, this product can serve as a novel and fortified alternative to traditional *tungrymbai* and can be popularised within the local people of Meghalaya and globally as well.

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