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Storage study of Pre-cooked and Post-cooked *Tungrymbai* (Fermented Soy Food of Khasi Hills, Meghalaya)

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ABSTRACT

Tungrymbai is a traditional fermented food of *Khasi* and *Jaintia* tribes of Meghalaya. In this study *Tungrymbai* was analyzed for physico-chemical and microbial quality of pre-cooked and post-cooked samples prepared by using *Lactobacillus fermentum* and *Lactobacillus plantarum* culture combination in 1:1 ratio at 1, 2 and 3% culture combination. Shelf-life of the product was carried out by storing at 6°C and 33°C for 4 days. pH was found to decrease while titratable acidity increases as the number of storage days increases. Protein content was highest in *Tungrymbai* with 3% cell biomass. pH and titratable acidity was more in pre-cooked samples, whereas protein content was more in post-cooked samples. *Lactobacillus* count was found in all the samples, yeast and mold was observed only in the 4th day of storage and coliforms was absent in pre-cooked samples. However, microbes were absent in post-cooked sample I and only *Lactobacillus* strain were detected in post-cooked samples II which indicates that the cooking procedure and heat intensity while cooking affect the *Lactobacillus* strains. Therefore, awareness can be created for the preparation method of *Tungrymbai* without cooking the product, to get the beneficial properties of *Tungrymbai* prepared with *Lactobacillus* strains.

Keywords: *Tungrymbai*, *Lactobacillus*, Shelf-life, pH, Total titratable acidity

Soybean is a major leguminous crop in the world, and its application as food nutrients are generally confined to Asia (Shurtleff *et al*. 2010). *Tungrymbai* is a naturally fermented soybean food prepared by *Khasi* and *Jaintia* tribes of Meghalaya in India (Tamang *et al*. 2009). *Tungrymbai* is prepared by fermenting the soybean seeds for 3-4 days. It is primarily consumed as a side dish throughout winter season. The North-Eastern region of India is well known for the preparation and consumption of many diversities of native fermented food. These fermented foods provide essential components beneficial for the health with nutritious values, flavour, palatability and texture (Sohliya *et al*. 2009; Tamang *et al*. 2009. Traditional fermentation is a method of food processing done by using microorganisms, especially lactic acid bacteria (LAB), and yeast (Guarner *et al*. 2008). Unlike their unfermented counterparts, fermented foods are preferred by consumers because of their typical taste, texture, and colour (Nout *et al*. 1997). The function of LAB in increasing the shelf life and nutritional quality of fermented foods and beverages, controlling diarrhea, in addition to their antimicrobial properties have been established (Thokchom *et al*. 2012). These microorganisms are harmless and have enzymes such as proteases, amylases and lipases that hydrolyze food complexes into simple non-toxic products (Alokail *et al*. 2013; Steinkraus 1997). LAB, involved in the fermentation, is associated with substrate utilization, flavour promotion, food preservation and probiotic properties (Leroy and De Vuyst, 2004, Chao *et al*. 2009; Tu *et al*. 2010; Liu *et al*. 2011). The present paper aim to analyse the physico-chemical and microbial quality of pre-cooked and post-cooked

Tungrymbai, to study the shelf-life and the effect of cooking processes in the preparation of post-cooked *Tungrymbai*.

MATERIALS AND METHODS

Collection of soybean sample and other materials

Local variety of soybean (*Glycine max* (L.) Merill), ginger, garlic, black sesame seeds, mustard oil, salt and chillies were obtained from the local market of Meghalaya.

Microorganisms and Media

The strains used in this study were obtained from fermented foods of Meghalaya by the department of RDAP, NEHU Tura Campus. Pure bacterial strain *Lactobacillus fermentum* and *Lactobacillus plantarum* was transfer in de Mann, Rogosa and Sharpe (MRS broth) (M255, HiMedia, India) and incubate at 37˚C for 24 hours. The activated culture was inoculated in 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 (Gajbhiye *et al*. 2012).

Starter culture(s) preparation

A loopful culture of *Lactobacillus fermentum* and *Lactobacillus plantarum* 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 (Hati *et al*. 2014).

Preparation of *Tungrymbai* in traditional way (control)

Local variety of soybean was used and about 50g of soybean was cleaned, washed and soaked in 100ml Reverse Osmosis (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* locally known as "*slamet*", and covered on top by the same leaves. The bamboo basket was wrapped with sterile muslin cloth and kept for fermentation for 3-4 days at 37˚C (Thokchom and Joshi 2012).

Laboratory scale preparation of *Tungrymbai* using different culture combinations

About 50g of soybean was cleaned, washed and soaked in 100ml Reverse Osmosis (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 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 in different percentages of 1, 2, and 3%. *Slame*t leaves are then 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 (Thokchom and Joshi 2012).

Preparation of post-cooked *Tungrymbai*: The sample was divided into two parts Sample I and Sample II.

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 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, ginger was added for garnishing.

Physico-chemical analysis: The samples were analyzed for their pH, total titratable acidity and total protein content according to AOAC, 1990.

Determination of pH

This experiment was carried out with the help of digital pH meter (Hanna instruments, Model HI96107) after fermenting soybean for 3-4 days of incubation at 37 °C. The readings for all the samples were taken accordingly for 1, 2, 3 and 4 days.

Determination of Total titratable acidity

Total titratable acidity was determined by using 0.1N Sodium Hydroxide solution. 5ml of liquid soybean sample was taken in a beaker (previously obtained by homogenizing 10gm of soybean sample with 90ml of sterile distilled water) and mixed with 10ml distilled water and two drops of phenolphthalein indicator was added. Each sample was titrated with 0.1N NaOH solution until the sample turn light pink in colour. The reading was taken accordingly and recorded for each sample. The percent acidity was expressed in terms of % lactic acid. The titratable acidity was calculated as percent lactic acid as follows:

% of acidity = [mls of NaOH used] × [0.1 N NaOH] × [0.09g (milliequivalent factor)] × [100]/ grams of sample used.

Estimation of protein

This experiment was performed by using Biuret method. Freshly prepared solution of standard bovine serum albumin (0.1g of BSA is diluted in 5 ml sterile distilled water) is used and freshly prepared biuret reagent is taken. A standard solution of BSA with a concentrations range of 0, 2, 4, 6, 8 and 10 mg/ml was taken in test-tubes by keeping the volume of BSA solution 1ml in each test-tube), 1ml of unknown sample is taken in each test tubes and 5ml of Biuret reagent is added into all the test tubes and was properly mixed. The samples were kept in the dark at room temperature for 20 minutes and absorbance was recorded using spectrophotometer at 550nm within 10-15 minutes. A graph is plotted, with protein concentration on X axes and absorbance at Y axes and a standard graph was prepared. The values of protein in unknown samples were calculated by using slope value of the graph.

Microbial analysis

10 grams of soybean sample was mixed well in a sterile mortar and pestle and homogenised with 90 mL of sterile distilled water. From this solution, 1ml of liquid sample was taken and was 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), Sabouraud Chloramphenicol Agar (SM 023, SRL, India) and EMB Agar (M317, HiMedia, India) for Lactic acid bacteria count, Yeast and mould count and Coliform count respectively. The MRS and EMB plates were incubated at 37°C for 24hours whereas SCA plates were incubated at 32°C for 72 hours. This was carried out for 1, 2, 3 and 4 days of the sample (Thokchom and Joshi, 2012). The calculated results were expressed as colony forming units (cfu) per ml. CFU/ml= No. of colonies x Dilution factor / volume of inoculum

Statistical Analysis: The experimental results were expressed as mean ± standard deviation (SD) of three replicates.

RESULTS AND DISCUSSION

Physico-chemical analysis of pre-cooked *Tungrymbai* samples

Determination of pH and Total titratable acidity of pre-cooked Tungrymbai samples

The results showed that the pH decreased over a shelf life of 4 days in all the *Tungrymbai* samples i.e., traditional, 1%, 2% and 3% samples. The pH was found to decrease from 7.18 ± 0.088 to 7.01 ± 0.018, 7.34 ± 0.092 to 7.12 ± 0.0121, 7.44 ± 0.119 to 7.20 ± 0.0141

Table 1: pH of pre-cooked *Tungrymbai* samples

Parameters	Storage days	Storage temp. (°C)	TT	T-1%	T-2%	T-3%
pH	1	6	7.18 ± 0.088	7.34 ± 0.092	7.44 ± 0.119	7.52±0.168
		33	7.28 ± 0.02	7.36 ± 0.037	7.47 ± 0.036	7.62 ± 0.0458
	2	6	7.17 ± 0.014	7.255 ± 0.007	7.39 ± 0.0141	7.49 ± 0.0212
		33	7.11 ± 0.028	7.31 ± 0.092	7.43 ± 0.036	7.50 ± 0.0212
	3	6	7.13 ± 0.028	7.29 ± 0.0141	7.38 ± 0.0424	7.42 ± 0.0494
		33	7.00 ± 0.018	7.18 ± 0.022	7.284± 0.033	7.31 ± 0.0168
	4	6	7.01 ± 0.018	7.12 ± 0.0121	7.20 ± 0.0141	7.29 ± 0.0458
		33	6.90 ± 0.007	7.04 ± 0.0636	7.07 ± 0.0989	7.17 ± 0.0353

Values are mean ± standard deviation of triplicate determinations (n=3). (TT- Traditional Tungrymbai, T-Tungrymbai).

Table 2: Titratable Acidity of pre-cooked *Tungrymbai* samples

Parameters	Storage day	Storage temp. (°C)	TT	T-1%	T-2%	T-3%
TA (%lactic acid)	1	6	0.051 ± 0.055	0.063 ± 0.011	0.077 ± 0.055	0.087 ± 0.055
		33	0.055 ± 0.021	0.068 ± 0.035	0.082 ± 0.035	0.092 ± 0.011
	2	6	0.061 ± 0.055	0.073 ± 0.011	0.087 ± 0.055	0.094 ± 0.055
		33	0.075 ± 0.022	0.078 ± 0.035	0.092 ± 0.022	0.099 ± 0.034
	3	6	0.073 ± 0.15	0.086 ± 0.15	0.091±0.15	0.118 ± 0.15
		33	0.095 ± 0.057	0.088 ± 0.152	0.098 ± 0.152	0.126 ± 0.057
	4	6	0.096 ± 0.115	0.113 ± 0.152	0.119 ± 0.115	0.156 ± 0.173
		33	0.146 ± 0.115	0.173 ± 0.152	0.206 ± 0.351	0.366 ± 0.057

Values are mean ± standard deviation of triplicate determinations (n=3). (TT- Traditional Tungrymbai, T-Tungrymbai).

and 7.52 ± 0.168 to 7.29 ± 0.0458 for traditional, 1%, 2% and 3% *Tungrymbai* samples respectively stored at 6°C. The pH was also found to decreased from 7.28 ± 0.02 to 6.90 ± 0.0070, 7.36 ± 0.037 to 7.04 ± 0.0636, 7.47 ± 0.036 to 7.07 ± 0.0989 and 7.62 ± 0.0458 to 7.17 ± 0.0353 for traditional, 1%, 2% and 3% *Tungrymbai* samples respectively stored at 33°C for a period of 4 days. The result of total titratable acidity shows that all *Tungrymbai* samples i.e., traditional, 1%, 2% and 3% samples increased over a period of 4 days of shelf-life analyses. It was found that the titratable acidity increased from 0.051 ± 0.055 to 0.096 ± 0.115, 0.063 ± 0.011 to 0.113 ± 0.152, 0.077 ± 0.055 to 0.119 ± 0.115 and 0.087 ± 0.055 to 0.156 ± 0.173 for traditional, 1%, 2% and 3% *Tungrymbai* samples respectively stored at 6°C. The titratable acidity also increases from 0.055 ± 0.021 to 0.146 ± 0.115, 0.068 ± 0.035 to 0.173 ± 0.152, 0.082 ± 0.035 to 0.206 ± 0.351, 0.092 ± 0.011 to 0.366 ± 0.057 for traditional, 1%, 2% and 3% *Tungrymbai* samples respectively stored at 33°C. From the study, it was observed that the titratable acidity increased with the decrease in pH, which may indicate as a good characteristic of *Tungrymbai*, it could help to prevent growth of pathogenic bacteria, increased shelf life of the product and prevent food spoilage, as has been reported by Ng'ong'ola- Manani, 2014 in the study of lactic acid bacteria fermentations in pastes of soybeans and soybean–maize blends. Other reports by Bristone *et al.* 2016 in the study of yoghurt fermentation, the decrease in pH and increase in titratable acidity was found to be a desired quality and characteristics of good yoghurt. The decrease in pH may be due to the production of organic acids. Similar results were observed by Maftei *et al*. 2012 in which soymilk was combined with different percentages of sea buckthorn syrup and fermented with a culture of *Bifidobacterium bifidus*.

Total Protein Content of pre-cooked *Tungrymbai* samples

Results showed that the protein content was highest in *Tungrymbai* with 3% cell biomass ranging from 108.808- 89.989 mg/ml stored at 6˚C and 122.818- 98.306 mg/ml at 33˚C followed by *Tungrymbai* with 2% cell biomass with protein content ranging from 105.175- 81.747 mg/ml at 6˚C and 112.022- 94.962 mg/ml at 33˚C, *Tungrymbai* with 1% cell biomass has protein content ranged from 102.644- 77.495 mg/ml stored at 6˚C and 109.602- 92.669 at 33˚C and the protein content was found to be least in traditional

Table 3: Protein estimation of pre-cooked *Tungrymbai* samples

Parameters	Storage day	Storage temp (^{0}C)	TT (mg/ml)	T-1% (mg/ml)	T-2% (mg/ml)	T-3% (mg/ml)
Protein content	1	6	98.520	102.644	105.175	108.808
		33	106.983	109.602	112.022	122.818
	2	6	82.645	86.523	93.247	94.218
		33	98.280	106.602	112.426	120.288
	3	6	81.530	83.282	87.758	90.565
		33	96.397	96.646	102.178	107.557
	4	6	80.200	77.495	81.747	89.989
		33	90.137	92.669	94.962	98.306

Values are mean ± standard deviation of triplicate determinations (n=3). (TT- Traditional Tungrymbai, T- Tungrymbai).

Table 4: Microbial Analysis of Pre-cooked *Tungrymbai* Samples

Microbial load (log CFU/ml)	Storage day	Storage temp. (˚C)	TT (control)	T-1%	T-2%	T-3%
Lactobacillus count	1	6	6.212 ± 0.015	7.119 ± 0.022	7.856 ± 0.161	8.199 ± 0.109
		33	6.568 ± 0.011	7.542 ± 0.109	8.232 ± 0.201	9.057 ± 0.200
	2	6	6.892 ± 0.100	7.357 ± 0.130	7.443 ± 0.441	8.668 ± 0.080
		33	7.114 ± 0.109	8.243 ± 0.101	9.068 ± 0.099	10.117 ± 0.221
	3	6	6.756 ± 0.106	7.183 ± 0.150	7.252 ± 0.109	8.386 ± 0.101
		33	7.018 ± 0.011	8.057 ± 0.330	8.843 ± 0.426	9.152 ± 0.200
	4	6	6.512 ± 0.063	7.012 ± 0.015	7.116 ± 0.011	8.158 ± 0.150
		33	6.791 ± 0.018	7.619 ± 0.022	8.168 ± 0.101	8.882 ± 0.208
Yeast and *Mold* count	1	6	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
		33	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
	2	6	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
		33	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
	3	6	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
		33	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
	4	6	2.416 ± 0.070	3.011 ± 0.019	3.140 ± 0.0205	3.473 ± 0.070
		33	2.736 ± 0.070	3.315 ± 0.089	3.680 ± 0.0205	3.890 ± 0.060
Coliforms count	1	6	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
		33	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
	2	6	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
		33	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
	3	6	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
		33	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
	4	6	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml
		33	Absent in 1ml	Absent in 1ml	Absent in 1ml	Absent in 1ml

Values are mean ± standard deviation of triplicate determinations (n=3). (TT- Traditional Tungrymbai, T-Tungrymbai).

Tungrymbai (control) with 98.52- 80.20 mg/ml at 6°C and 106.983 – 90.137 mg/ml at 33°C. The protein content of laboratory prepared *Tungrymbai* was found to be more than the traditional product. It can be assumed that fermentation with *Lactobacillus* cell biomass slightly increases the protein content of *Tungrymbai* as reported by Mohiedeen *et al*. 2010 there was a slight gain of protein content during microbial growth in maize fermentation. From the result, it was observed that there was a decrease of protein content as the number of storage days increases, this could be due to protein degradation by proteolytic activities of microorganisms. Similar reports were found by Khetarpaul and Chauhan *et al.* 1989 in the study of effect of fermentation on protein of pearl millet, and in the study of soy proteins during fermentation of *thua-nao* inoculated with *Bacillus subtilis* (Visessanguan *et al*. 2005).

Microbial analysis of pre-cooked *Tungrymbai* samples

The microbial analysis was determined through *Lactobacillus*, Coliforms, Yeast and mold viable cell count. Analysis was done for the study of shelf-life and to examine the presence of pathogenic organisms in the product. Samples were stored for 1, 2, 3 and 4th days at 6°C and 33°C. *Lactobacillus* counts were found to be highest in *Tungrymbai* prepared with 3% cell biomass ranging from 8.199 ± 0.109 to 8.158 ± 0.150 log cfu/ml at 6°C and 9.057 ± 0.200 to 8.882 ± 0.208 log cfu/ml at 33°C. Yeast and mold count was absent in 1, 2 and 3rd day of storage, but found only on 4th day. Coliforms were not observed in all four days of storage. From the results it can be concluded that the product was free from any contamination, while only on the 4th day of storage contamination was observed which may arise from the surrounding environment**.** Thus, we can assume that all the samples were safe and have the characteristics of good quality product. Similar results was observed by Thokchom and Joshi., 2013 in which *Enterobacteriaceae* and LAB count was highest in *Tungrymbai* sample, while coliforms, yeast and mold count was comparatively less than the bacterial counts. Other reports were observed that LAB population was more compared to microbial load of yeasts in log CFU/ml in the *Tungrymbai* sample (Chettri and Tamang., 2014).

Physico-chemical analysis of post-cooked *Tungrymbai* samples:

pH and Titratable acidity of post-cooked *Tungrymbai* samples

It was observed that the pH and titratable acidity was more in sample II than in sample I of *Tungrymbai*. In Sample I, the pH was found to be 7.0 ± 0.078 and 7.05 ± 0.141 for traditional sample and 1% cell biomass samples. The titratable acidity was observed to be 0.061 ± 0.141 and 0.060 ± 0.012 for traditional and 1% cell biomass sample. In Sample II, the pH was found to be 7.10 ± 0.124 and 7.12 ± 0.070 for traditional and 1% cell biomass sample. The titratable acidity was observed to be 0.054 ± 0.056 and 0.063 ± 0.070 for traditional and 1% cell biomass sample**.** The pH and titratable acidity was found to be lesser in post cooked sample than in pre-cooked sample. This could be due to the preparation method and ingredients used when cooking, which was similar with other studies done by Thokchom and Joshi., 2013 in which the pH and titratable acidity was lower in the post-cooked *Tungrymbai* sample.

Total protein content of post-cooked *Tungrymbai* samples

The total protein content was found to be more in sample II than in sample I. In sample I, the protein content was 116.094 and 121.309 mg/ml for traditional and 1% cell biomass. Whereas, in sample II, the protein content was 136.679 and 142.242 mg/ml for traditional and 1% cell biomass samples**.** During protein analysis it was found that *Tungrymbai* samples inoculated with *Lactobacillus* cell biomass has slightly more protein content than traditional *Tungrymbai* samples this may be due to the microbial growth during fermentation which was in agreement with Mohiedeen *et al*. 2010 in the study of maize fermentation there was a slight gain of protein content during microbial growth. Sample II *Tungrymbai* has more protein content than sample I, this could be due

Table 5: pH and Titratable Acidity of post-cooked *Tungrymbai* samples

Parameters	Post Cooked TT(Sample I)	Post Cooked T-1%(Sample I)	Post Cooked TT (Sample II)	Post Cooked T-1% (Sample II)
pH	7.0 ± 0.078	7.05 ± 0.141	7.10 ± 0.124	7.12 ± 0.070
TA (%lactic acid)	0.061 ± 0.141	0.060± 0.012	0.054 ± 0.056	0.063 ± 0.070

(TT- Traditional Tungrymbai, T-Tungrymbai).

Table 6: Protein estimation of post-cooked *Tungrymbai* samples

Parameters	TT (Sample I)	T-1% (Sample I)	TT (Sample II)	T-1% (Sample II)
Total Protein (mg/ml)	116.094	121.309	136.679	142.242

to the preparation method and heat intensity while cooking the samples. Similar reports were found by Sohliya *et al*. 2009 in the study of chemical changes during preparation of *Tungrymbai*.

Microbial Analysis of Post cooked *Tungrymbai* samples

The microbial analysis of post-cooked sample showed that *Lactobacillus* count was present only in Sample II and not in Sample I of *Tungrymbai*. In sample II the microbial load was found to be 6.65 ± 0.150 and 7.31 ± 0.112 (log CFU/ml) for traditional and *Tungrymbai* sample prepared with 1% cell biomass. This can be due to the heat generated from cooking temperature in which the *Lactobacillus* species cannot survive. Coliforms, Yeast and mold viable cell count were found to be absent in all the samples. Other findings have been reported by Thokchom and Joshi., 2012 were *Lactobacillus* species are absent in the post-cooked samples of *Tungrymbai*. Absence of coliform implies that the samples were contamination free, and has good quality standard. Other findings have been reported by Oyeniyi *et al*. 2014 in the study of soy-yoghurt sample. Hence, sample II shows better result compared to sample I.

CONCLUSION

Lactic acid bacteria have been widely used in food industries due to their probiotic attributes. In the present investigation, physico-chemical and microbial compositions of *Tungrymbai* were analyzed for both pre- and post-cooked samples. It was found that the pH and titratable acidity decrease in post-cooked *Tungrymbai* samples, whereas, the protein content was more in post-cooked samples which may be due to the preparation method and spices used while cooking the samples. Through microbial analysis, it was found that *Tungrymbai* prepared with *Lactobacillus fermentum* and *Lactobacillus plantarum* was more in *Lactobacillus* count as compared to the traditional sample. However, it was observed that the microbial load was absent in sample I post-cooked *Tungrymbai* and present only in sample II, this could be due to the heat generated while cooking, in which all the beneficial microbes did not survived. Shelf-life study revealed that the product is free from contamination and pre-cooked *Tungrymbai* could be kept for 4 days without getting spoiled. Further, *Tungrymbai* prepared with *Lactobacillus fermentum* and *Lactobacillus plantarum* was found to be more beneficial with probiotic attributes than the traditional *Tungrymbai* and could be used as an alternative for the preparation of traditional product. Moreover, awareness can be created for the preparation method of *Tungrymbai*, so that consumers can get the beneficial properties of *Tungrymbai* prepared with *Lactobacillus fermentum* and *Lactobacillus plantarum*.

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REFERENCES

AOAC.1990.Official methods of analysis of the Association of Official Chemists. Washington, D.C: Association of Official Chemists.

Alokail, M.S., S. Sabico, Y. Al-Saleh, N.M .Al- Daghri, K.M. Alkharfy and P.M. Vanhoutte. 2013. Effects of probiotics in patients with diabetes mellitus type 2: *Study protocol for randomized, double- blind, placebo controlled.* **14:** 1-8.

Bristone, C., E.C. Igwe, M.H. Badua, O. Boafo and V.G. Zira. 2016. Microbial ecology and effectiveness of different starter cultures in yoghurts produced from whole cow milk and soybeans. *African Journal of Food Science and Technology*, **7(5):** 107-117.

Chao, S.H., R.J. Wu, K. Watanabe and Y.C. Tsai. 2009. Diversity of lactic acid bacteria in suan-tsai and fu-tsai, traditional fermented mustard products of Taiwan. *International Journal of Food Microbiology*, **135(3):** 203–210.

Chettri, R. and J.P. Tamang. 2014. Functional properties of *Tungrymbai* and *Bekang*, naturally fermented soybean foods of North East India. *International Journal of Fermented foods,* **3(1):** 87-103.

Chettri, R. and J.P. Tamang. 2016. Organoleptic Evaluation of *Tungrymbai* and *Bekang,* Naturally Fermented Soybean Foods, Produced by Using Selected species of Bacillus. *Journal of Scientific and Industrial Research*, **75:** 416-419.

Gajbhiye, M., D. Prakash, S. Jagdale, S.N. Ahiwale, Patil and B. Kapadnis. 2012. Pomegranate borne fungicidal lactic acid bacteria and their biodiversity. *Proceedings of The National Academy Of Sciences India Section B-Biological Sciences*, **82(3):** 413–419.

Guarner, F., A.G. Khan, J. Garisch, R. Eliakim, A. Gangal and A. Thomson. 2008. Probiotics and prebiotics. Milwaukee, USA: World Gastroenterology Org.p.22.

Hati, S., S .Vij, B.P. Singh and S .Mandal. 2014.β- Glucosidase activity and bioconversion of isoflavones during fermentation of soymilk. *Journal of the Science of Food and Agriculture,* **95:** 216-220.

Khetarpaul, N. and B.M. Chauhan. 1989. Effect of fermentation on protein, fat, minerals and thiamine content of pearl millet. *Plant Foods for Human Nutrition*, **39:** 169–177.

Leroy, F. and L. De Vuyst. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*. **15:** 67–78.

Liu, S.N., Y. Han and Z.J. Zhou. 2011. Lactic acid bacteria in traditional fermented Chinese foods. *Food Research International*, **44:** 643–651.

Maftei, N.M., R.Dinica and G. Bahrim. 2012. Functional characterization of fermented beverage based on soy milk and sea buckthorn syrup. *The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology*, **36(1):** 81-96.

Mohiedeen, I.E., A.H.E. Tinay, A.E.O. Elkhalifa, E.E. Babiker and L.O. Mallasiy. 2010. Effect of fermentation on in vitro protein digestibility, protein fractions and amino acids composition of maize (*Zea mays* Linnaeus) cultivars. *Electronic Journal of Environmental, Agricultural and Food Chemistry,* **9:** 838–847.

Ng'ong'ola- Manani, T.A., H. Marit Ostlie, A. Mbachi Mwangwela and T. Wicklund. 2014. Metabolite changes during natural and lactic acid bacteria fermentations in pastes of soybeans and soybean-maize blends. *Food science and Nutrition*, **2(6):** 768-785.

Nout, M.J.R and Y. Motarjemi. 1997. Assessment of fermentation as a household technology for improving food safety: A joint FAO/ WHO workshop. Food contr. **8**: 221-6.

Oyeniyi, A.O., A.C. Aworh and J.O. Olaniyan. 2014. Effect of Flavorings on Quality and Consumer Acceptability of Soy-Yoghurt. *Journal of Environmental Science, Toxicology and Food Technology*, **8(1):** 38-44.

Shurtleff, W and Aoyagi, A, 2010. History of soybeans and soyfoods in Southeast Asia (13th century to 2010): extensively annotated bibliography and sourcebook. SoyInfo Center Pulishers, Lafayette, USA. pp 8.

Sohliya, I., S.R. Joshi, R.K. Bhagobaty and R. Kumar. 2009. *Tungrymbai*- A traditional fermented Soybean food of the ethnic tribes of Meghalaya. *Indian Journal of Traditional Knowledge,* **8(4):** 559-561.

Steinkraus K.H. 1997. Classification of fermented foods: worldwide review of household fermentation techniques. Food Control. **8(5/6):** 311–317.

Tamang, J.P., R. Chettri and R.M. Sharma.2009. Indigenous knowledge of Northeast women on the production of ethnic fermented soybean foods. *Indian Journal of Traditional Knowledge*, **8(1):** 122–126.

Thokchom, S and S.R. Joshi. 2012. Microbial and chemical changes during preparation in the traditionally fermented soybean product *Tungrymbai* of ethnic tribes of Meghalaya. *Indian Journal of Traditional Knowledge*, **11(1):** 139-142.

Thokchom, S. and S.R. Joshi. 2013. Physicochemical Analysis of Ethnically Fermented Soybean Products of North-East India and Molecular Characterization of associated Lactic Acid Bacteria. *Proceeding of the National Academy of Sciences, India, Section B Biological Science.*

Tu, R.J., H.Y. Wu, Y.S. Lock and M.J. Chen. 2010. Evaluation of microbial dynamics during the ripening of a traditional Taiwanese naturally fermented ham. *Food Microbiology*, **27(4):** 460–467.

Visessanguan, W., S. Benjakul, W. Potachareon, A. Panya and S. Riebroy. 2005. Accelerated proteolysis of soy proteins during fermentation of *thua-nao* inoculated with *Bacillus subtilis*, *Journal of Food Biochemistry*, **29:** 349-366.