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Organoleptic Evaluation of *Tungrymbai* and *Bekang*, Naturally Fermented Soybean Foods, Produced by using Selected Species of *Bacillus*

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Tungrymbai and *bekang* are naturally fermented soybean foods of Meghalaya and Mizoram, respectively. Three strains of *Bacillus* viz. *Bacillus subtilis*, *B. licheniformis* and *B. pumilus* previously isolated from naturally fermented products *tungrymbai* and *bekang*, and were used singly and/or in mixture as starter culture(s) for the production of *tungrymbai* and *bekang* under optimized laboratory conditions. None of the strains of *Bacillus* used singly as starter could produce organoleptically acceptable *tungrymbai* and *bekang*. The sensory evaluation result showed that *tungrymbai* and *bekang* prepared in the laboratory by cell suspension mixture of pure culture of *Bacillus* spp. was more acceptable than conventionally prepared products.

Keywords: *Tungrymbai*, *bekang*, sensory evaluation, starter culture(s).

Introduction

Soybeans are mostly fermented naturally and made into different recipes by many ethnic people of Asia[1]. Soybean is a major leguminous crop in the world, and its utilization as foods are mostly confined to Asia. Some of the common ethnic non-salted sticky fermented soybean foods are *natto* (Japan), *kinema* (India, Nepal and Bhutan), *tungrymbai, bekang*, *hawaijar*, *aakhuni* and *peruyaan* (North Eastern India), *thua nao* (Thailand), *chungkokjang* (Korea), *pepok* (Myanmar) and *sieng* (Cambodia and Laos)[2].*Tungrymbai* is a naturally fermented soybean food of *Khasi* of Meghalaya[3]. It serves as a cheap source of high protein food in local diet. Species of *Bacillus*, lactic acid bacteria and yeast have been reported to be present in *tungrymbai*[4]. *Bekang* is also a naturally fermented soybean food consumed by Mizo ethnic people of Mizoram. Sun-dried *bekang* is called as '*bekang-ro*'. It is mostly consumed during the winter seasons and serves as a cheap source of high protein food. It is considered as an appetizer and is consumed as a side dish or pickle with cooked rice. It serves as a cheap source of high protein food[3]. On the basis of a combination of phenotypic and molecular characterization, species of *Bacillus* isolated from *tungrymbai* were identified as *Bacillus licheniformis* (25.5%), *B. pumilus* (19.5%) and *B. subtilis* (55%), and species of *Bacillus* from *bekang* were *B. brevis* (2%), *B. circulans* (7.5%), *B. coagulans* (6.5%), *B. licheniformis* (16.5%), *B. pumilus* (9.1%), *B. sphaericus* (4.6%), *B. subtilis* (51.8%), and *Lysinibacillus fusiformis* (2%)[5]. The most dominant species of *Bacillus* in these naturally fermented soybean foods on the basis of occurrence was *B. subtilis*[5].The aim of present paper is to select *Bacillus* species, previously isolated from naturally fermented products *tungrymbai* and *bekang*, for production of *tungrymbai* and *bekang* in laboratory scale.

Materials and methods

Soybean used

Small, smooth yellow seed coat and dark brown hilum 'local yellow' variety of soybean [*Glycine max* (L.) Merrill] was purchased from Police bazaar in Shillong (Meghalaya) and Bara bazaar in Aizawl (Mizoram).

Starter cultures

For *tungrymbai* preparation, three species of *Bacillus*, previously isolated from naturally fermented *tungrymbai* samples[5] via: *Bacillus subtilis* TS2:B24, *B. licheniformis* TSB:B13, and *B. pumilus* TSA:B15 were selected as starter culture preparation based on their occurrence percentage in the samples. Similarly, for *bekang* preparation, species of *B. subtilis* BT:B9, *B. licheniformis* BK1:B13, and *B. pumilus* BK2:B6, previously isolated from naturally fermented *bekang* samples[5] were selected as starter culture preparation based on their occurrence percentage in the samples.

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Starter culture(s) preparation

Preparation of starter cultures and laboratory-scale of fermented soybean were followed as described by Tamang (1999)[6].

A loopful culture of selected *Bacillus* spp. was inoculated in 5 ml nutrient broth (M002, HiMedia) and incubated overnight at 37 °C. One ml of each culture was centrifuged (Biofuge pico, Heraeus, Germany) at 8,000 *g* for 5 min, the supernatant was discarded, 1 ml of sterile distilled water was added to the pellet, cells were resuspended and again centrifuged at 8,000 *g* for 5 min. Cells were again suspended in 1 ml sterile distilled water. This procedure achieved an inoculum size containing 10^8 cfu ml^{-1}, and was checked as viable count in nutrient agar (MM012, HiMedia) plates.

Laboratory-scale preparation of *tungrymbai* and *bekang*

About 500 g of soybean was cleaned, washed and soaked in tap water overnight at room temperature. Soaked soybeans were autoclaved at 121 °C for 30 min and distributed into four parts containing 100-125 g each. For *tungrymbai* preparation, each of the four parts were inoculated with 1 ml cell suspension of *Bacillus subtilis* TS2:B24, *B. licheniformis* TSB:B13, *B. pumilus* TSA:B15, and the mixture of cell suspensions of all three strains of *Bacillus,* respectively. Similarly, for *bekang* preparation each of the four parts were inoculated with 1 ml cell suspension of *B. subtilis* BT:B9, *B. licheniformis* BK1:B13, *B. pumilus* BK2:B6, and the mixture of cell suspensions of all three strains of *Bacillus,* respectively. Strains of *Bacillus* were harvested in nutrient broth (M002, HiMedia). Inoculations were done while the temperatures of soybeans was above 80 °C. Inoculated soybeans were put into pre-sterile petri-dish (outer lid is replaced by perforated polythene film), and incubated at 40 °C for 48 h under 85 % relative humidity.

Sensory evaluation

Sensory evaluation of *tungrymbai* and *bekang* was prepared by selected starter culture(s) and later evaluated in terms of aroma, taste, texture, colour and general acceptability as described by Meilgaard *et al*. (1990)[7]. Sensory evaluation of laboratory prepared *tungrymbai* and *bekang* was done organoleptically by 10 judges (consumers who are familiar with traditional *tungrymbai* and *bekang* and also with *kinema*, similar fermented soybean food of Sikkim), with score rate of 1 (bad) to 5 (excellent), considering market *tungrymbai* and *bekang* as control with score rate of 3, moderate (Table 1).

Statistical analysis

The data were analysed by determining standard deviation (SD), standard error of measurement (SEM) and analysis of variance (ANOVA)[8].

Results and discussion

For *tungrymbai* preparation, three species of *Bacillus*, previously isolated from naturally fermented *tungrymbai* samples[5] via: *Bacillus subtilis* TS2:B24, *B. licheniformis* TSB:B13, and *B. pumilus* TSA:B15 were selected as starter culture preparation based on their occurrence percentage in the samples. Similarly, for *bekang* preparation, species of *B. subtilis* BT:B9, *B. licheniformis* BK1:B13, and *B. pumilus* BK2:B6, previously isolated from naturally fermented *bekang* samples[5] were selected as starter culture preparation based on their occurrence percentage in the samples.Starter cultures of *Bacillus* isolated from native *tungrymbai* and *bekang* were tested singly or in combination for their ability to ferment soybeans to produce the similar products. Species of *Bacillus* based on its superior technological properties such as acidifying ability, proteolytic activity[9] were selected. Different starter cultures used for *tungrymbai* preparation were: starter A-cells of *B. subtilis* TS2:B24; starter B-cells of *B. licheniformis* TSB:B13; starter C-cells of *B. pumilus* TSA:B15; starter D- cells of all *Bacillus* strains (*B. subtilis* TS2:B24, *B. licheniformis* TSB:B13, *B. pumilus* TSA:B15). Similarly, starter cultures used for *bekang* preparation were: starter E- cells of *B. subtilis* BT:B9; starter F- cells

Table 1—Format for sensory evaluation of *tungrymbai* and *bekang*

Please use market tungrymbai/bekang as a control with scoring rate of 3 (moderate)

Sample code: …… Name: ……

Attribute	Score					Comment
Aroma	Bad				Strong	
	1	2	3	4	5	
Taste:	Weak				Strong	
Mild acidic	1	2	3	4	5	
Texture:	Hard				Soft	
	1	2	3	4	5	
Colour:	Bad				Good	
	1	2	3	4	5	
General	Bad				Good	
Acceptability:	1	2	3	4	5	

Date: Signature of Judge

of *B. licheniformis* BK1:B13; starter G- cells of *B. pumilus* BK2:B6; starter H- cells of all *Bacillus* strains (*B. subtilis* BT:B9, *B. licheniformis* BK1:B13, *B. pumilus* BK2:B6).Table 2 and Table 3 show the sensory analysis of *tungrymbai* and *bekang* produced by selected strains of *Bacillus* (Starters A-H), respectively. There was no significant difference ($P<0.05$) in colour and taste attributes among the 4 samples of *tungrymbai*. However, a significant difference ($P<0.05$) in aroma, texture and general acceptability attributes was observed among some samples. Organoleptically, *tungrymbai* prepared by using starter D (mixture of *B. subtilis* TS2:B24, *B. licheniformis* TSB:B13 and *B. pumilus* TSA:B15) scored highest in taste, aroma, texture and general acceptability (Table 2). Similarly, there was no significant difference ($P<0.05$) in colour and taste attributes among the 4 samples of *bekang*. However, a significant difference ($P<0.05$) in aroma, texture and general acceptability attributes was observed in some samples. *Bekang* prepared by using starter H (mixture of *B. subtilis* BT:B9, *B. licheniformis* BK1:B13 and *B. pumilus* BK2:B6) scored highest in taste, aroma, texture and general acceptability (Table 3). None of the strains of *Bacillus* used singly as starters could produce organoleptically acceptable *tungrymbai* and *bekang*. The sensory evaluation result showed that *tungrymbai* and *bekang* prepared in the laboratory by cell suspension mixture of *Bacillus* spp. was more acceptable than conventionally prepared products. Application of starter cultures may appear appropriate in *tungrymbai* and *bekang* production at household level, since it is cost-effective and may contribute to small-scale fermentation[10]. *Tungrymbai* and *bekang* prepared by using a starter culture had thus advantages over the traditional method, which resulted in a shorter fermentation time that eliminates the chance of growth of contaminants, hygienic conditions, maintaining consistency with better quality and flavour. The final product is not always consistent in natural fermentation; the use of a mixed starter culture could provide more consistent fermentations and products of higher quality[11,12]. The quality, safety and acceptability of traditional fermented *tungrymbai* and *bekang* may be significantly improved through the use of starter culture(s) selected on the basis of multifunctional considerations. Due to possession of superior functional properties, some of the strains of bacilli can be used in mixed cell suspension as starter culture(s) for optimized production of fermented soybean products typical of the Meghalaya and Mizoram.

Table 2—Sensory evaluation of *tungrymbai** prepared using selected starter culture(s)

Starter culture	Attribute				
	Aroma	Taste	Texture	Colour	General acceptability
A	3.0 ± 1.0^{a}	3.0 ± 1.0^{a}	3.5 ± 0.5^{a}	3.6 ± 0.5^{a}	3.6 ± 0.5^{ac}
B	2.9 ± 1.0^{ac}	2.6 ± 0.5^{a}	3.8 ± 0.5^{b}	3.8 ± 0.5^{a}	3.8 ± 0.5^{bc}
C	2.4 ± 0.5^{bc}	1.9 ± 1.0^{a}	2.6 ± 0.5^{b}	2.6 ± 0.5^{a}	2.5 ± 0.5^{ac}
D	3.6 ± 0.5^{a}	3.6 ± 0.5^{a}	3.6 ± 0.5^{a}	3.5 ± 0.5^{a}	4.4 ± 0.5^{a}

* *Tungrymbai* was prepared using different starter(s) at 40° C for 48 h.
A, pure culture of *Bacillus subtilis* TS2:B24.
B, pure culture of *Bacillus licheniformis* TSB:B13.
C, pure culture of *Bacillus pumilus* TSA:B15.
D, mixed cultures of *B. subtilis* TS2:B24, *B. licheniformis* TSB:B13 and *B. pumilus* TSA:B15.
Data represents the means scores ± SD of three sets of experiments. Values bearing different superscripts in each column differ significantly ($P<0.05$).

Table 3—Sensory evaluation of *Bekang** prepared using selected starter culture(s)

Starter culture	Attribute				
	Aroma	Taste	Texture	Colour	General acceptability
E	2.5 ± 0.5^{a}	2.9 ± 1.0^{a}	3.5 ± 0.5^{a}	3.5 ± 0.5^{a}	3.5 ± 0.5^{ac}
F	2.5 ± 0.5^{ac}	2.0 ± 1.0^{a}	2.4 ± 0.5^{b}	2.6 ± 0.5^{a}	2.5 ± 0.5^{bc}
G	2.0 ± 0.0^{bc}	2.4 ± 0.5^{a}	2.0 ± 1.0^{b}	2.5 ± 0.5^{a}	2.6 ± 0.5^{ac}
H	3.8 ± 0.5^{a}	2.5 ± 0.5^{a}	3.6 ± 0.5^{a}	3.5 ± 0.5^{a}	3.6 ± 0.5^{a}

**Bekang* was prepared using different starter(s) at 40° C for 48 h.
E, pure culture of *Bacillus subtilis* BT:B9.
F, pure culture of *Bacillus licheniformis* BK1:B13.
G, pure culture of *Bacillus pumilus* BK2:B6.
H, mixed culture of *B. subtilis* BT:B9, *B. licheniformis* BK1:B13 and *B. pumilus* BK2:B6.
Data represents the means scores ± SD of three sets of experiments. Values bearing different superscripts in each column differ significantly ($P<0.05$).

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