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Review article

Naturally fermented ethnic soybean foods of India

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ABSTRACT

Kinema, *hawaijar*, *tungrymbai*, *bekang*, *aakhone*, and *peruyaan* are naturally fermented ethnic soybean foods of India; they are popular among the Mongolian-origin races in the Eastern Himalayas. *Bacillus subtilis* is the dominant functional bacterium in all naturally fermented soybean foods of these regions. Although there is a good demand for ethnic fermented soybean foods among local consumers in north-east India, the production is limited to household level. A ready-to-use pulverized starter culture for *kinema* production can be introduced to *kinema*-makers or similar sticky fermented soybean foods of north-east India and adapted to local conditions for additional income generation. Ethnic fermented soybeans are one of the major food resources in the Eastern Himalayas; they supplement inexpensive, high-digested plant protein in the local diet with low fat/cholesterol content and high nutritive value as well as antioxidant and other health-promoting properties.

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1. Introduction

In the Eastern Himalayas, soybean [*Glycine max* (L.) Merrill, family Leguminosae, sub-family Papilionaceae] is grown under rain-fed conditions in upland terraces as a sole crop as well as a mixed crop with rice and maize up to an elevation of 1500 m (Fig. 1). Soybean, locally known as *bhatmas* in Nepali language, is traditionally used to prepare various fermented and nonfermented recipes in the Eastern Himalayan regions of Nepal, India, and Bhutan [1]. Soybean was probably introduced into India from China through the Himalayas several centuries ago and some believe that soybeans were also brought via Myanmar by traders from Indonesia [2]. Two indigenous varieties of soybeans "yellow cultivar" and "dark brown cultivar" (Fig. 1) are grown between May and June and harvested in November. Locally grown soybeans are harvested, and the dry seeds of soybeans are naturally fermented into a flavorsome and sticky product in eastern parts of Nepal, Darjeeling hills, Sikkim, north-eastern regions of India, and southern parts of Bhutan close to the Mongolian races. Some of the common ethnic nonsalted sticky fermented soybean foods of the Eastern Himalayas are *kinema* (Nepal, Darjeeling hills, Sikkim, and south Bhutan), *hawaijar* (Manipur), *tungrymbai* (Meghalaya), *bekang* (Mizoram), *aakhone* (Nagaland), and *peruyaan* (Arunachal Pradesh) (Table 1). This article will examine the characteristics, microbiology, and health benefits of *kinema* as well as *hawaijar*, *tungrymbai*, *bekang*, *aakone*, and *peruyaan*.

2. *Kinema* production

Kinema is an ethnic fermented soybean food of the Nepali community in the Eastern Himalayas; it is a sticky, slightly alkaline product with a slight ammoniacal flavor that is produced by natural fermentation. It is a whole-soybean fermented food with a sticky texture, gray tan color, and is flavorsome [8]. During traditional production of *kinema*, the small-sized (~6 mm) "yellow cultivar" soybean dry seeds are selected, washed, and soaked overnight (8–10 h) in water. Soaked soybean seeds are taken out and put into a container with fresh water, and boiled for 2–3 hours until they are soft. Excess water is drained off and the cooked soybean seeds are placed into a wooden mortar (locally called "okhli") and are cracked lightly using a wooden pestle (locally called "muslo") to split the cotyledons. This practice of cracking the cooked seeds of soybeans is observed only during *kinema* production, unlike other similar fermented soybean foods of Asia and north-east India, probably to increase the surface area for speed fermentation by aerobic spore-forming *Bacillus* spp. Approximately 1% of firewood ash is added directly to the cooked soybeans and mixed thoroughly to maintain the alkaline condition of the product. Soybean grits are placed in a bamboo basket lined with locally grown fresh fern called (*Glaphylopteriolopsis erubescens*). The basket is covered with

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Fig. 1. Soybean cultivation in Sikkim, India.

a jute bag and left to ferment naturally at ambient temperatures (25–40°C) for 1–3 days above an earthen kitchen oven (Fig. 2). During summer, the fermentation time may require 1–2 days whereas in winter it may require 2–3 days. In eastern Nepal, local consumers prepare dark brown local varieties of soybean seeds rather than yellow-colored seeds for making *kinema* [9]. Similarly, they commonly use *Ficus* (fig plant) and banana leaves as wrapping materials instead of fern fronds. Other methods remain the same. Completion of fermentation is indicated by the appearance of a white viscous mass on the soybean seeds and the typical *kinema* flavor with a slight odor of ammonia.

The shelf-life of freshly prepared *kinema* (Fig. 3) is 2–3 days in summer and a maximum of 1 week in winter without refrigeration. It may be prolonged by drying in the sun for 2–3 days. Dried *kinema* is stored for several months at room temperature. Preparation of *kinema* varies from place to place and is still restricted at the household level. It is interesting to note that mountain women use their indigenous knowledge of food production to prepare *kinema*. This unique knowledge of *kinema*-making has been protected as an hereditary right and passed from mother to daughter, mostly among the Limboo.

Kinema is eaten as curry with steamed rice. The delicacy of *kinema* can be perceived by its appealing flavor and sticky texture. Fresh *kinema* is fried in vegetable oil, with chopped onions, tomatoes, and turmeric powder. Salt and sliced green chillies are added and fried for 3–5 minutes. A little water is added to make

Table 1
Naturally Fermented Soybean Foods of India.

Product	Sensory features and nature	Microorganisms	States in India	References
Aakhone	Alkaline, sticky, paste	*B. subtilis, Proteus mirabilis*	Nagaland	Singh *et al.* (2014) [3]
Bekang	Alkaline, sticky, paste, curry	*B. subtilis, B. brevis, B. circulans, B. coagulans, B. licheniformis, B. pumilus, B. sphaericus, Lysinibacillus fusiformis*	Mizoram	Chettri and Tamang (2015) [4]
Hawaijar	Alkaline, sticky	*B. subtilis, B. licheniformis, B. amyloliquefaciens, B. cereus, Staph. aureus, Staph. sciuri, Alkaligenes* sp., *Providencia rettgers, Proteus mirabilis*	Manipur	Jeyaram *et al.* (2008) [5], Singh *et al.* (2014) [3]
Kinema	Alkaline, sticky; curry	*B. subtilis, B. licheniformis, B. cereus, B. circulans, B. thuringiensis, B. sphaericus, Ent. faecium, Cand. parapsilosis, Geotrichum candidum*	Sikkim, Darjeeling hills, Assam	Sarkar *et al.* (1994) [6], Tamang (2003) [7]
Peruyaan	Alkaline, sticky, side dish	*B. subtilis, B. amyloliquefaciens, Vagococcus lutrae, Ped. acidilactici, Ent. faecalis*	Arunachal Pradesh	Singh *et al.* (2014) [3]
Tungrymbai	Alkaline, sticky, curry, soup	*B. subtilis, B. licheniformis, B. pumilus*	Meghalaya	Chettri and Tamang (2015) [4]

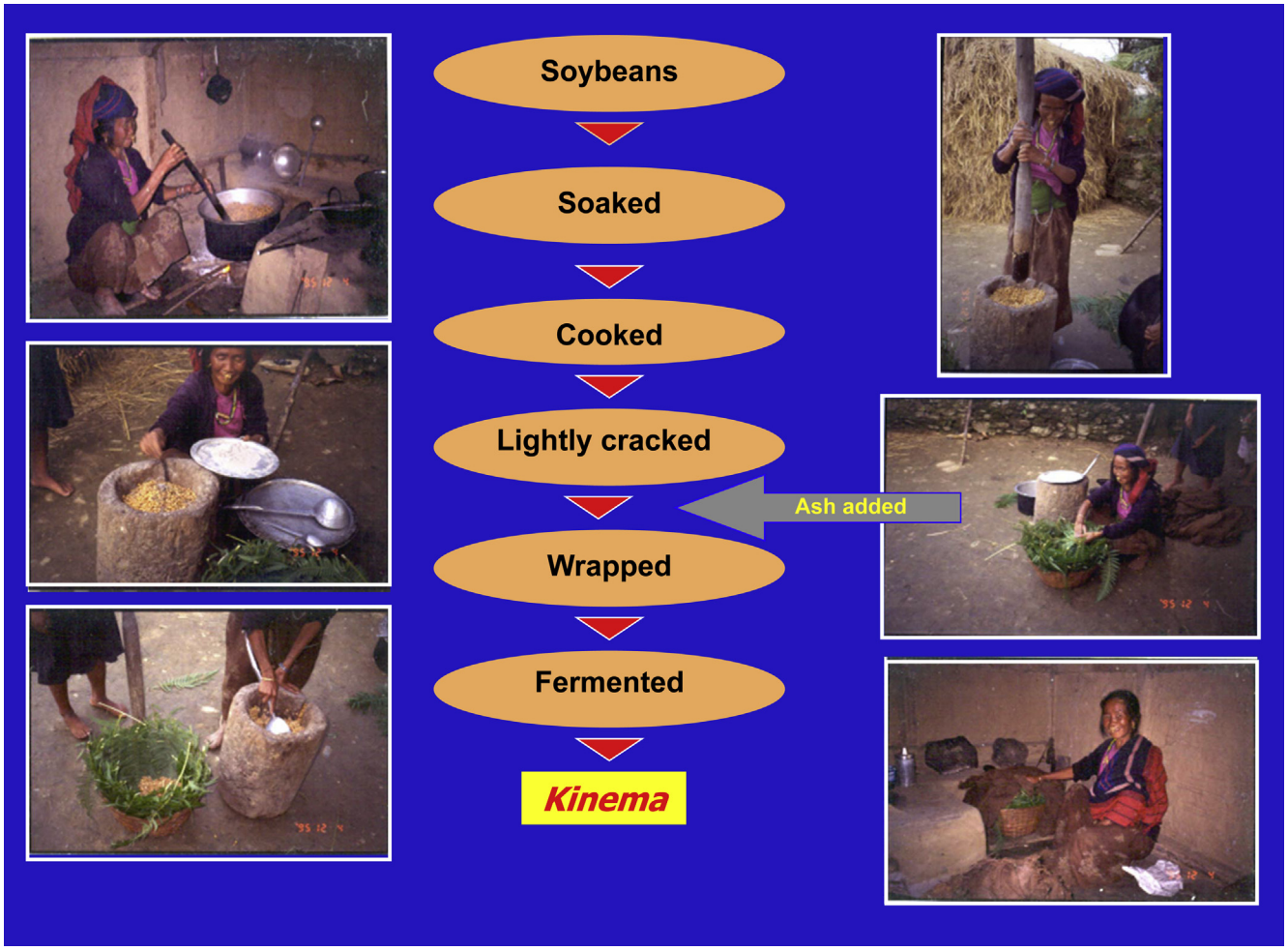

Fig. 2. Flow diagram of the traditional method of *kinema* production in Sikkim village, India.

thick gravy, and cooked for 5–7 minutes then the *kinema* curry (Fig. 3) is ready for serving with steamed rice. Dried *kinema* is sometimes mixed with leafy vegetables to make a mixed curry. Consumers like *kinema* mostly because of its typical flavor and sticky texture, and some people dislike the product due to its strong *umami*-type flavor [10] and mucilaginous texture.

Kinema production is a source of marginal income generation for many families in the Eastern Himalayas. *Kinema* is sold by rural women in all local periodical markets, locally called "haats", in eastern Nepal, Darjeeling hills, Sikkim, and southern parts of Bhutan. Usually, it is sold by volume taken in a small silver mug containing 150–200 g of *kinema*, and packed in the leaves of a fig plant (*Ficus hookeriana*) locally called "nevara", and then tied loosely by straw. Poly-bags are not used to pack *kinema.* One kilogram of *kinema* costs about Rs. 100. An average of 5 kg of *kinema* is sold by each seller in a local market and about 60% of expenses are incurred on the purchasing of raw soybeans, fuel for cooking, transportation from village to market, etc., and 40% profit is made [1]. This profit is spent on childrens' education, procuring essential commodities not locally available, and other domestic expenses. Although there is good demand for *kinema* in the local markets, the production of *kinema* is still confined to home production; there is no organized processing unit or factory for *kinema* production. *Kinema*-making technology has not been recognized as a small-scale industry for receiving financial support or a loan from any public sector bank or financial institution; neither has it been incorporated in the rural development programs of the government in Nepal, India, and Bhutan.

3. Microbiology of *kinema*

Several species of *Bacillus* have been isolated from *kinema* including *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus circulans*, *Bacillus thuringiensis*, and *Bacillus sphaericus* at an average load of 10^8 colony-forming units (CFU)/g [6,8,11]. However, *B. subtilis* is the dominant functional bacterium in *kinema* [12,13]. Besides *Bacillus*, *Enterococcus faecium* (10^7 CFU/g) and the yeasts *Candida parapsilosis* and *Geotrichum candidum* (10^4 CFU/g) have also been reported in *kinema* [6,7].

Rich microbial diversity is observed in various sources of soybean. The equipment and leaves used as wrapping materials harness microbiota for natural fermentation of *kinema* [7]. With the decline in protein nitrogen content, the nonprotein and soluble nitrogen contents increase during *kinema* fermentation [14] and a significant increase in the relative viscosity of *kinema* is found during maturation at 5°C and 10°C [15]. Keeping freshly prepared *kinema* below 10°C for 1 day stabilizes the quality of the product by preventing further biological activity of microorganisms and shows better stickiness, which is a very important sensory property of *kinema* [15]. Organoleptically, the monoculture fermentation of soybean by *B. subtilis* MTCC 2756 produces the best *kinema* because of a pleasant nutty flavor and highly sticky texture, and also

Fig. 3. Fresh *kinema*, sun-dried *kinema*, and *kinema* curry.

minimizes the conventional fermentation time, maintains better hygienic conditions and consistency, and increases the levels of soluble protein [16].

Inexpensive soybean extract broth after adjusting pH to 7 as a medium is prepared for enrichment of *B. subtilis* spores instead of discarding the soybean extract after autoclaving soybeans [16]. Moreover, nutrient broth, conventionally used for enrichment of *B. subtilis* spores, is composed of expensive beef extract, which is not acceptable to the majority of the Hindu population in the Himalayas. *Kinema* prepared using the *B. subtilis* KK2:B10 strain that is harvested in soybean extract broth is dried in an oven at 70°C for 10 hours and ground aseptically to make pulverized starter. The 1% of pulverized starter instead of *B. subtilis* is added aseptically to autoclaved soybeans and fermented to obtain *kinema*. Consumer preference trials show that *kinema* prepared using pulverized starter under optimized conditions is more acceptable than market *kinema* [16]. Water-soluble nitrogen and formol nitrogen contents are higher in *kinema* prepared using pulverized starter than market *kinema* [16]. Increased water-soluble nitrogen in *kinema* helps with digestibility and the high amount of formol nitrogen, which contains free amino acids supplements, imparts a better taste to *kinema* [17]. Application of ready-to-use pulverized starter may appear appropriate in *kinema* production for marginal *kinema* producers in the Himalayas because it is cost-effective and easy to handle [1]. In 2012, we obtained an Indian Patent on "*A process for production of kinema, fermented soybean food, using a pure starter culture*" (Patent No: 25346).

Although *E. faecium* does not add any sensory quality to the *Bacillus* fermentation of soybeans, it is always encountered in naturally fermented *kinema* [1]. The presence and growth of yeast during *kinema* preparation are associated with the development of rancidity in the products. In fact, *B. subtilis* is the sole fermenting organism in *kinema* preparation.

4. Health benefits of *Kinema*

Kinema has many health-promoting benefits including antioxidant, digested protein, essential amino acids, vitamin B complex, low-cholesterol content, etc. [1]; it is therefore considered a functional food. *Kinema* is the cheapest source of plant protein compared to milk and animal products on the basis of protein cost per kilogram. It contains all essential amino acids [18], and is rich in linoleic acid, an essential fatty acid [19]. Total amino acids, free amino acids, and mineral contents are increased during *kinema* fermentation [14,15,17]. Phytosterols (cholesterol-lowering effect) are increased during *kinema* fermentation [19]. Riboflavin and niacin increases in *kinema* during fermentation [20]. *Kinema* has antioxidant activities [21]. Due to the presence of a large amount of Group B saponins, *kinema* claims to have health-promoting benefits [22].

5. Other fermented soybean foods of north-east India

Many *kinema*-like sticky naturally fermented soybeans are consumed by different ethnic communities living in the north-eastern part of India bordering with Bhutan, China, and Myanmar (Fig. 4)—these include *hawaijar* in Manipur, *bekang* in Mizoram,

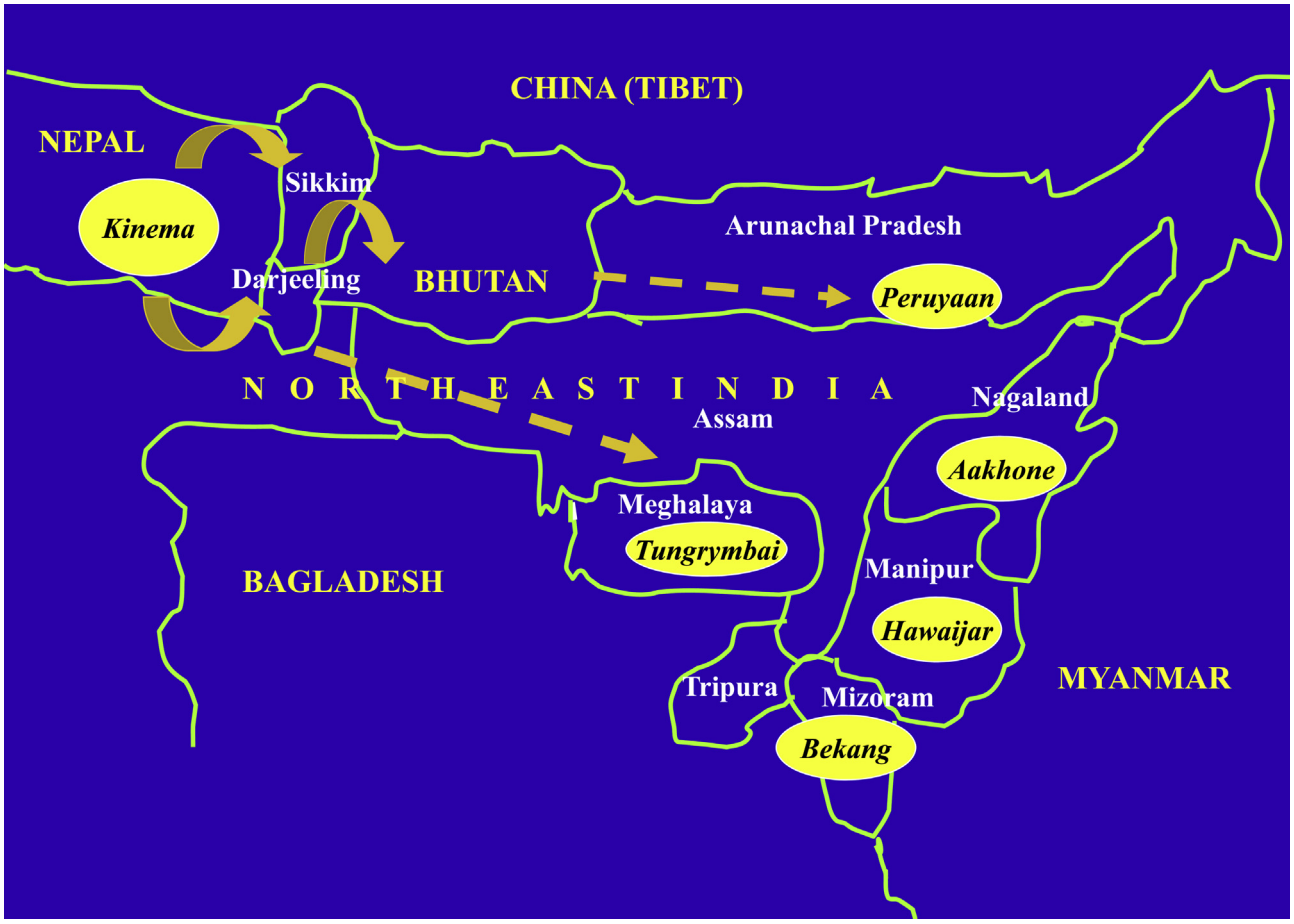

Fig. 4. Diversity of naturally fermented ethnic soybeans foods in the Eastern Himalayas.

peruyaan in Arunachal Pradesh, *aakhone* in Nagaland, and *tungrymbai* in Meghalaya (Fig. 5).

5.1. Hawaijar

Hawaijar is a traditional fermented soybean alkaline food of Manipur. It is prepared from the local variety of small-seeded soybean grown in the hilly terraces of Manipur [23]. It is similar to *kinema*. Small-sized soybean seeds are selected, washed, and boiled in an open cooker for 2–3 hours. Excess water is drained off, cooled to ~40°C, and then the whole soybean seeds are packed in a small bamboo basket that has a lid. The basket is lined with fresh leaves of the fig plant (*Ficus hispida*), locally called "assee heibong" in the Meitei language, or banana leaves. After placing cooled soybean seeds inside the basket, the lid is closed loosely and the basket is kept near the kitchen or a warm place for natural fermentation for 3–5 days (Fig. 6). The emission of the typical ammonia odor and appearance of sticky texture on the cooked soybean seeds are determined as good quality *hawaijar* by the Meitei. The shelf-life of *hawaijar* is a maximum of 7 days without refrigeration. Sometimes, it is sun-dried for 2–3 days and stored for several weeks for future consumption. Unlike *kinema*, the practice of cracking and the addition of ash is not adopted by the Meitei women in *hawaijar* production. *Hawaijar* is produced by the Meitei women, and the men support the process.

A special curry called "chagempomba" is commonly prepared by the Meitei in Manipur and is eaten with steamed rice. *Hawaijar* is eaten directly or used as a condiment or mixed with vegetables to make curry in the Manipuri cuisine. *Hawaijar* is commonly sold in local markets throughout Manipur by the Meitei women. Despite its popularity, there is no organized food sector for mass-scale production of *hawaijar* in Manipur. The product is still prepared at home and many women are dependent upon the product for their livelihood.

5.2. Tungrymbai

Tungrymbai is an ethnic fermented soybean food of Khasi and Garo in Meghalaya. It is similar to *kinema*. Soybean seeds are collected, cleaned, washed, and soaked in water for approximately 4–6 hours [24]. The seed coat of the soybean is normally removed before cooking by rubbing the soaked seeds gently. The soaked soybeans are cooked for about 1–2 hours until all the water is absorbed. Cooked beans are allowed to cool, and then they are packed with fresh leaves of *Clinogyne dichotoma* (locally called "lamet"), placed inside a bamboo basket, and covered with a thick cloth. The covered basket is kept over the fireplace and fermented naturally for 3–5 days to obtain *tungrymbai* (Fig. 7). *Tungrymbai* is mashed and put into a container with water and boiled until the water evaporates with continuous stirring. It is mixed with fried onion, garlic, ginger, chilli, grinded black sesame (locally called "til"), and salt. A thick curry is made and is served as a side dish with steamed rice by Khasi in Meghalaya. Pickle is also made from *tungrymbai*. Khasi women are commonly seen selling *tungrymbai* packed in fresh leaves of "lamet" or banana at the vegetable markets of Shillong.

Fig. 5. Naturally fermented ethnic soybean foods of India.

5.3. Functionality

Bacillus subtilis TS1:B25 (*tungrymbai*) and *B. subtilis* BT:B9 (*bekang*) accounted for the highest production of Poly-γ-glutamic acid (PGA) (2.8 mg/mL each) amongst the other strains tested [25]. Although lactic acid bacteria (LAB) showed antimicrobial activities, none of them produced bacteriocin and biogenic amines under the applied conditions. *Enterococcus faecium* TM2:L6 (*tungrymbai*) and BAV:E2 (*bekang*) showed the highest degree of hydrophobicity at 72.7% and 71.6%, respectively. LAB strains were able to degrade phytic acid and oligosaccharides, showing their ability to degrade antinutritive factors. *Tungrymbai* and *bekang* possess antioxidant and free radical (1,1-diphenyl-2-picryl hydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS)) scavenging activity [25].

Soybean
↓
Wash; boiled for 2-3 h
↓
Loosely pack in a bamboo basket lined with leaves
↓
Fermented (25-40^0 C, 2-3 d)
↓
HAWAIJAR

Fig. 6. Traditional method of preparation of *hawaijar* in Manipur. Microorganisms: *Bacillus subtilis* (dominant functional bacterium), *Bacillus licheniformis*, *Bacillus cereus*, and other nonbacilli bacteria, e.g., *Staphylococcus aureus*, *Staphylococcus sciuri*, and *Alkaligenes* spp [2].

5.4. Bekang

Bekang is an ethnic fermented soybean food commonly consumed by Mizo in Mizoram. It is also similar to *kinema*. During

Soybean
↓
Soaked for 4-6 h
↓
Seed coat of soybean removed
↓
Cooked and dewatered
↓
Cool down to room temperature
↓
Packed loosely in bamboo basket lined with leaves
↓
Basket is loosely covered with leaves and thick cloth
↓
Fermented (25-40^0 C, 3-5 d)
↓
TUNGRYMBAI

Fig. 7. Traditional method of *tungrymbai* preparation in Meghalaya. Microorganisms: on the basis of a combination of phenotypic and molecular characterization using ARDRA, ITS-PCR, and RAPD-PCR techniques, species of *Bacillus* isolated from *tungrymbai* were identified as *Bacillus licheniformis* (25.5%), *Bacillus pumilus* (19.5%), and *Bacillus subtilis* (55%) (dominant bacterium) [4]. ARDRA, The amplified amplification of polymorphic DNA; ITS-PCR, The amplification of 16S–23S rDNA intergenic transcribed spacer-Polymerase Chain Reaction; RAPD-PCR, Ribosomal DNA restriction analysis-polymerase chain reaction.

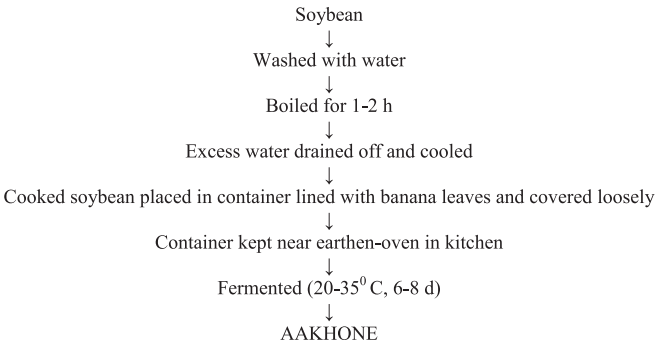

Fig. 8. Traditional method of preparation of *aakhone* in Nagaland. Microorganisms: *Bacillus subtilis*, and *Proteus mirabilis* [3].

the traditional method of preparation of *bekang*, small-sized, dry seeds of soybean are collected, cleaned, and soaked in water for 10–12 hours. Excess water is removed and beans are boiled for 2–3 hours in an open cooker until the beans become soft. Excess water is drained off and wrapped in fresh leaves of *Calliparpa aroria* (Family: Verbanaceae), locally called "nuhlhan", or in leaves of *Phrynium* sp. (Family: Merantaceae), locally known as "hnahthial". The wrapped beans are kept inside a small bamboo basket. The basket is then placed near the earthen oven or in a warm place and is allowed to ferment naturally for 3–4 days. Sticky soybean with an emission of ammonia odor is produced, which is liked by the local consumers. The product is called *bekang* in Mizoram (Fig. 8). *Bekang* is consumed as it is, or made into curry with the addition of salt, green chillies, and tomatoes. It is consumed as a side dish with steamed rice. *Bekang* is sold in the local markets by Mizo women, who earn their livelihood this way [26].

5.5. Aakhone

Aakhone, also called *axone*, is an ethnic fermented sticky soybean food of Sema Naga in Nagaland, similar to *kinema*. The preparation is the same as for other fermented soybean foods of northeast India. Soybean seeds are soaked, cooked, and the beans are wrapped in fresh leaves of banana or *Phrynium pubinerve* Blume (Family: Marantaceae) or *Macaranga indica* Wight (Family: Euphorbiaceae), and kept above the fireplace to ferment for 5–7 days [27]. The shelf-life of freshly fermented *aakhone* is a maximum of 1 week. Fresh *aakhone* is molded and made into cakes and dried above the earthen oven. Sometimes, each fermented bean is separated by hand and dried in the sun for 2–3 days. Dried *aakhone* is stored in containers for future consumption (Fig. 9). Pickle is made from freshly fermented *aakhone* by mixing with green chilli, tomato, and salt. The dried *aakhone* cakes are cooked with pork and are eaten as a side dish with steamed rice by Sema.

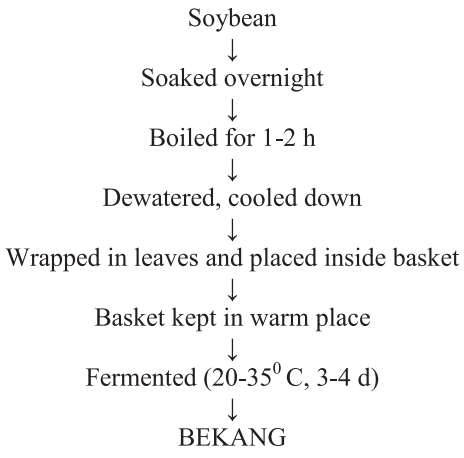

Fig. 9. Traditional method of preparation of *bekang* in Mizoram. Microorganisms: on the basis of a combination of phenotypic and molecular characterization using ARDRA, ITS-PCR, and RAPD-PCR techniques, the species of *Bacillus* isolated from *bekang* were identified as *Bacillus brevis* (2%), *Bacillus circulans* (7.5%), *Bacillus coagulans* (6.5%), *Bacillus licheniformis* (16.5%), *Bacillus pumilus* (9.1%), *Bacillus sphaericus* (4.6%), *Bacillus subtilis* (51.8%; dominant bacterium), and *Lysinibacillus fusiformis* (2%) [4]. ARDRA,; ITS-PCR, internal transcribed spacer-polymerase chain reaction; RAPD-PCR, random amplified polymorphic DNA-polymerase chain reaction.

5.6. Peruyyan

Peruyaan is an ethnic fermented soybean food of Apatani tribes in Arunachal Pradesh. The word *peruyaan* has been derived from the Apatani dialect—*perun* means beans and *yannii* means packing in leaves [26]. During the traditional preparation of *peruyaan*, soybean seeds are collected, washed, and cooked for 2–3 hours until the beans become soft. The excess water is drained off and is cooled for some time. The cooked soybeans are kept in a bamboo basket (vessel) lined with fresh ginger leaves, locally called "taki yannii". The basket is loosely covered with ginger leaves and is kept on the wooden rack above the fireplace for fermentation for 3–5 days. The stickiness of the product is checked, and if the product is considered sticky enough then it is ready for consumption (Fig. 10). *Peruyaan* is consumed mostly as a side dish with steamed rice by the Apatani tribes in Arunachal Pradesh. It is mixed with hot water, chillies locally called "tero", and salt, and directly consumed without frying or cooking, unlike *kinema* curry preparation.

6. The similarity between *kinema* and other Asian nonsalted *Bacillus*-fermented soybean foods

Kinema is similar to other Asian *Bacillus*-fermented sticky soybean foods such as *natto* of Japan [28], *chungkukjang* of Korea [29], and *thua nao* of northern Thailand [30] (Fig. 11), *pepok* of northern Myanmar [31], and *sieng* of Cambodia and Laos [32]. The preparation of *kinema* is very similar to that of *natto*. In *itohiki-natto* production, whole soybeans are used for fermentation; in *hikiwari-natto* production, dehulled soybeans are cracked into two to four pieces and used [33]. Some of the steps in *kinema* preparation do not resemble those in *natto* and *chungkukjang*, and thus make *kinema* a unique nonsalted soybean-fermented product. The cooked beans are lightly crushed to dehull most of the seeds.

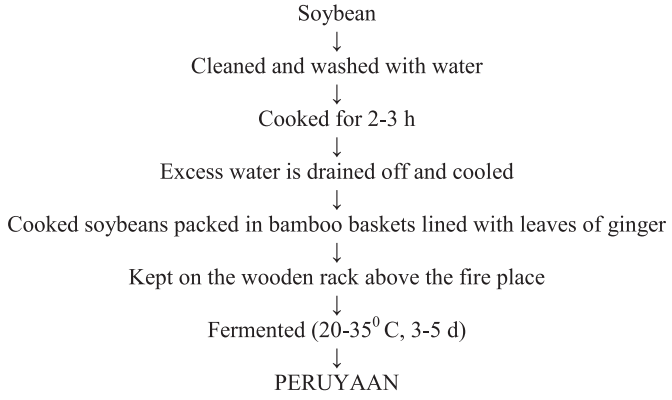

Fig. 10. Traditional method of preparation of *peruyaan* in Arunachal Pradesh. Microorganisms: *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Vagococcus lutrae*, *Pediococcus acidilactici*, and *Enterococcus faecalis* [3].

Fig. 11. *Bacillus*-fermented soybean foods of Asia.

However, fermentation is carried out with the kernels as well as the seed coats. Unlike *natto* and *chungkukjang*, *kinema* is always fried in oil and made into curry. The practice of frying *kinema* may have developed to drive out the unpleasant ammonia smell that masks the pleasant and persistent nutty flavor.

7. Phylogenetic similarity of *Bacillus* strains from Asian sticky fermented soybeans

The phylogenetic relationship among bacilli isolated from *kinema* (India), *chungkokjang* (Korea), and *natto* (Japan), similar fermented sticky soybean foods of Asia, on the basis of 16S rDNA sequences has been studied [34]. Strains of *Bacillus* isolated from *kinema* and *chungkokjang* show a central to paracentral position of spores with few strains showing negative nitrate reduction tests, whereas *B. subtilis* (*natto*) isolated from *natto* show a central position of spores and all reduce nitrate [34]. However, all strains of *B. subtilis* isolated from *kinema*, *chungkokjang*, and *natto* show stickiness on phytone agar and cooked soybean, which are characteristic properties of nonsalty fermented soybean foods of Asia [13]. However, the strain *B. subtilis* JCM 1465 does not produce any stickiness [34].

In order to investigate the phylogenetic relationship of isolates to other bacteria, the sequence of 16S rRNA gene polymerase chain reaction products was determined and it was found that strains *B. subtilis* KD:B1 and KG:B1 isolated from *kinema*, *B. subtilis* CA:B1 and CK:B2 isolated from *chungkokjang*, and *B. subtilis* JN-1 isolated from *natto* have identical sequences except that of JA-1 (*natto*), which has one ambiguous nucleotide [34]. The evolutionary distance between the four strains CK:B1, KD:B1, JN-1, and JA-1 and *B. subtilis* is 0.002 K_{nuc} as calculated by the ratio of nucleotide substitution per nucleotide site, indicating 99% homology with the *B. subtilis* type strain. However, the evolutionary distance between the strains CA:B1 and KG:B1 and *B. subtilis* is 0.005 K_{nuc}, showing approximately 99.5% homology with the type strain [34]. The phylogenetic analyses reveal that all six strains belonged to *B. subtilis*. This is the first report to describe the phylogeny of *B. subtilis* isolated from similar nonsalty fermented sticky soybean foods of Asia [34]. The plasmid of the *B. subtilis* (*natto*) strain resembles that of the *B. subtilis* strain in the partial nucleotide sequences [35–37]. The diversity of *Bacillus subtilis*-fermented soybean foods of Asia, including that of the Eastern Himalayas, needs to be studied in detail to determine the similarity. The probable source of a common stock of similar sticky fermented soybean foods will help food scientists to trace the antiquity of fermented soybean foods in Asia.

8. *Kinema-natto-thua nao* triangle hypothesis

Nonsalted fermented soybean foods are concentrated in a triangle with three vertices in Japan (*natto*), India and Nepal (*kinema*), and Indonesia (*tempe*). Nakao [38] named a "*natto* triangle" and

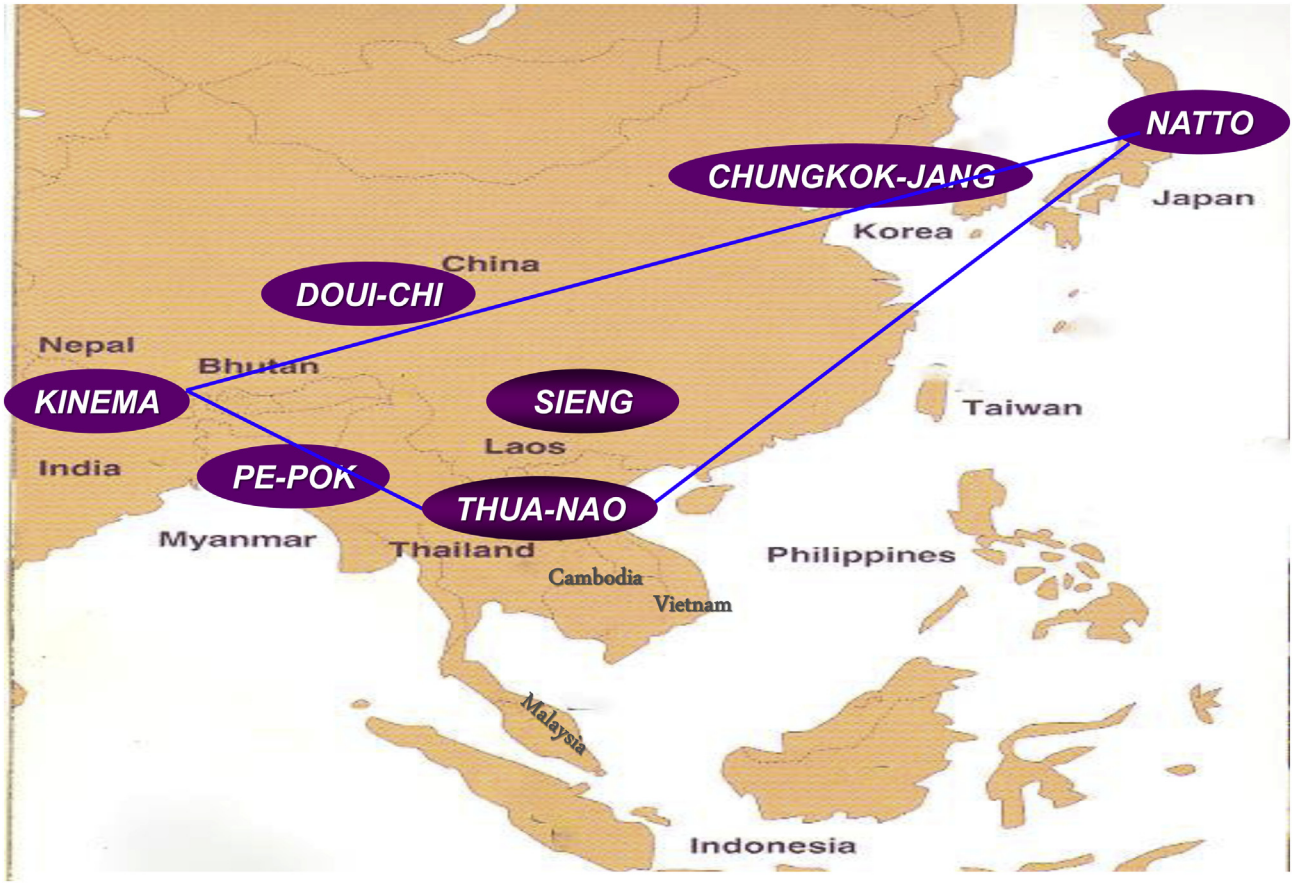

Fig. 12. The *kinema-natto-thua nao* (KNT) triangle representing only *Bacillus*-fermented soybean foods of Asia [1].

included both bacilli and mold-fermented soybean products, including *tempe,* and extended the triangle up to Indonesia. Tamang [1] renamed this hypothetical triangle the "*kinema-natto-thua nao* triangle" (KNT triangle) and included only nonsalty, *Bacillus*-fermented soybean foods with three vertices on India and Nepal (*kinema* and similar products), Thailand (*thua-nao*), and Japan (*natto*) (Fig. 12). Within the proposed triangle-bound countries, many fermented sticky nonsalty soybean foods are consumed by the different ethnic groups of people such as *kinema* (India, Nepal, and Bhutan), *natto* (Japan), *tungrymbai*, *bekang*, *hawaijar*, *aakhone*, and *peruyaan* (India), *thua nao* (Thailand), *chungkokjang* (Korea), *pepok* (Myanmar), and *sieng* (Cambodia and Laos). Beyond this hypothetical "KNT triangle", there is no report of *kinema*-like products with sticky and ammonia-flavored fermented soybean foods and the proposed "KNT triangle" does not include salted, nonsticky, and nonbacilli fermented soybean products such as *tempe*, *miso*, *sufu*, soy sauce, etc. [1]. Although the method of production and culinary practices vary from product to product, all bacilli-fermented Asian soybean foods have characteristic stickiness and a typical flavor. Hara *et al.* [35] reported that the plasmid of the *B. subtilis* (*natto*) strain resembles that of the *B. subtilis* isolated from *thua-nao* and *kinema.* The phylogenetic relationships among bacilli isolated from *kinema* (India), *chungkokjang* (Korea), and *natto* (Japan), similar fermented sticky soybean foods of Asia, reveal that all bacilli strains belonged to *B. subtilis* [34]. This suggests that *B. subtilis* strains responsible for fermentation of sticky soybean foods in Asia might have originated from the same stock. Another theory was proposed that nonsalted fermented soybean foods originated in the Yunnan province of China, which was the center of the hypothetical triangle [28].

9. Discussion

It has been observed that flavorsome mucilaginous fermented soybean foods *kinema*, *hawaijar*, *tungrymbai*, *bekang*, *aakhone*, and *peruyaan* are popular among the Mongolian-origin races in the Eastern Himalayas. The Mongolian people prefer the *umami*-flavored foods due to specific sensory development. *B. subtilis* is the dominant functional bacterium in all fermented soybean foods of these regions. Fermented soybean foods are consumed only in the Eastern Himalayas; no such product is consumed in other parts of the Himalayas. Although there is good demand for ethnic fermented soybean foods among the local consumers in north-east India, the production is limited to household level. The scientific findings have correlated the indigenous knowledge of the ethnic people of the Himalayas and acknowledged the innovative skills of mountain women. Fermented soybean foods supplement the local diet with inexpensive, high-digested plant protein content and they have health-promoting properties.

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