Kinema and similar products

Handbook of Indigenous Foods Involving Alkaline Fermentation

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continue to maintain and strengthen the cultural heritage and can compete successfully with imported products.

In conclusion, douchi is one of the traditional alkaline-fermented foods, which has been widely consumed as a relish by the Chinese people for more than 2000 years. Although there has been some research on douchi, further study needs to be taken to improve various aspects in the production of douchi and supply people with more scientific information. If innovations in processing technology, functionality, and storage are made, douchi may become more popular not only in China but also in the world.

2.1.1.3 Kinema and Similar Products

Prabir K. Sarkar and M. J. Robert Nout

Kinema is a unique flavorsome delicacy to many of the people of Nepal and the neighboring countries. This indigenous soybean-fermented condiment is a low-cost meat substitute and a source of income for many rural households.

2.1.1.3.1 Origin and Culture The word “kinema” is derived from “kinambaa” of the Yakthung or Limbu (a tribe indigenous to Limbuwan comprising nine districts within Mechi and Kosi zones of east Nepal) dialect where “ki” means fermented and “nambaa” means flavor. Although the history of origin of kinema is lost in antiquity, it is certain that among the Nepalese, the Limbu started its production and consumption. After unification of Limbuwan and Nepal in the 18th century, there was creation of a mixed society of multiethnic communities. This might have resulted in the spread of kinema culture to the allied Nepali communities, such as the Rai, Tamang, Gurung, and Mangar (Tamang, 2001). Because of trans-boundary movement of the people, kinema is also popular in the adjacent Darjeeling district of the State of West Bengal and the State of Sikkim in India, and also in Bhutan (Tamang et al., 1988). In northeast India, kinema is traditionally consumed by the non-Nepalis and called by various names (Table 2.3; Figure 2.12). On visiting a local periodic market (called “haat”), one might catch a glimpse of small, neatly packed, green-colored (wrapped in large-sized locally available leaves) bundles, tied loosely using a piece of straw. This is how these foods are commonly sold by women of the ethnic communities (Figure 2.13).

Table 2.3 Kinema Variants, Used by Non-Nepali Communities in Northeast India

PRODUCT NAME	LOCATION	USER TRIBE	MAIN PROCESSING STEPS	MODE OF CONSUMPTION
Aagya	Basar town in West Siang district of the State of Arunachal Pradesh	Galo	Whole beans are boiled and fermented for 12–14 days.	Mixed with boiled vegetables and chutney
Axone/akhone/aakhuni/kheuha/sabrocha/sadocha Bekang/bekanthu	State of Nagaland	Sema	Whole beans are boiled and fermented for 5–8 days.	Ground with tomato, chillies, and salt to make chutney
Bhari/bari	State of Mizoram	Mizo	Crushed whole beans are boiled and fermented for 3 days.	Mixed with herbs and boiled leafy vegetables
Chukchoro	State of Sikkim	Bhutia	Beans are soaked, boiled, crushed to grits, and fermented for 4–5 days.	Ground with vegetables and herbs to make chutney or cooked to curry
Hawaijar/hawaizaar	Shergaon area of West Kameng district of the State of Arunachal Pradesh	Sherdukpen	Beans are soaked, dehulled, boiled, and fermented for 10–12 days (15–30 days in winter).	Mixed with vegetables, or ground with vegetables and yak cheese to make chutney
Peron namsing	State of Manipur	Meitei	Whole beans are soaked, boiled, and fermented for 2–5 days.	Ground to make chutney, or cooked with rice, vegetables, spices, and prawn/fish to make curry
Satlyangser	East Siang district of the State of Arunachal Pradesh	Adi	Whole beans are boiled and fermented for 10–12 days (25–30 days in winter).	Mixed with boiled vegetables and herbs or ground to make chutney
Tungrymbai	State of Sikkim	Lepcha	Beans are soaked, boiled, crushed to grits, and fermented for 2–3 days.	Cooked with vegetables to make curry
Yanni perung/peruyyan	State of Meghalaya	Khasi	Whole beans are soaked, dehulled, boiled, and fermented for 3–5 days.	Mixed with herbs and spices to make curry or pickle
	Lower Subansiri district of the State of Arunachal Pradesh	Apatani	Whole beans are boiled and fermented for 3–4 days (10–12 days in winter).	Mixed with herbs to make chutney

Source: Tamang, J.P. et al., *Journal of the Science of Food and Agriculture*, 44, 375–385, 1988; Tamang, J.P. et al., *Indian Journal of Traditional Knowledge*, 8, 122–126, 2009; Singh, A. et al., *Indian Journal of Traditional Knowledge*, 6, 79–94, 2007; Premarani, T. and Chhetry, G.K.N., *Assam University Journal of Science & Technology: Biological and Environmental Sciences*, 6, 82–88, 2010.

Figure 2.12 Localities of use of kinema and its similar products in eastern part of Nepal, Bhutan, and northeast Indian States.

Figure 2.13 Packages of fresh kinema, as sold in a market of Kalimpong, India.

2.1.1.3.2 Indigenous Preparation of Kinema and Its Socio-Economic Impact The preparation of kinema (Figure 2.14) is exclusively practiced by women. The skill of production of this delicacy has been protected as a hereditary right and passed through generations. Small sized (~6 mm dia) soybeans (locally called “bhatamas”) of “yellow” cultivar (“dark brown” variety in Nepal) are washed, dipped in spring water where the level of water is twice than that of the beans, and left overnight (8–12 h) at ambient temperature (10°C–25°C). The beans are drained and cooked by boiling (~95°C) until (2–3 h) they can be crushed easily between fingertips. The beans are drained and crushed lightly using a wooden pestle (locally called “muslo”) and mortar (locally, “okhli”) to make grits of mainly half-cotyledons with a view to increase the surface area of the fermenting substrate resulting in a speedy fermentation by the aerobic sporeformers. The indigenous practice is not to clean the mortar and pestle before undertaking another batch of crushing. The grits are then transferred to a bamboo basket, lined with sack-cloth and a layer of locally available fresh fern (*Athyrium* sp.) fronds or leaves of banana (*Musa x paradisiaca* L.), *Leucosceptum canum* Sm., *Macaranga denticulata* (Blume) Müll.Arg., *Ficus hookeriana* Corner, or

Figure 2.14 Indigenous method of preparing kinema and factors influencing microbial composition in each of the processing stages.

Bauhania vahlia Wight & Arn. At this stage the beans are still quite hot (50°C–60°C). To maintain the condition alkaline, a small amount (~1%) of firewood ash is often sprinkled over the grits which are then wrapped with those leaves and sackcloth to insulate the fermenting beans and left in a warm (35°C–25°C) room, usually kitchen, for 1–3 days. A combination of porous fern fronds with dimensioning of the bean mass are of importance in ensuring appropriate aerobic condition during the fermentation. The desired state of fermentation is determined primarily by a typical kinema flavor dominated by ammonia as well as the development of a rough, white viscous fluid on the beans. This fluid has the property of forming long stringy threads when touched and stretched with thumb and middle finger; the longer the thread, the better is the quality of kinema. Generally speaking, fresh kinema (Figure 2.15a) of good quality gives a nutty flavor accompanied by a mild smell of ammonia, a grayish brown color, and is semi-hard, like raisins. When fresh kinema is fried in oil, within a few seconds, the pungent ammoniacal smell disappears, leaving a persistent nutty odor. After a brief frying, kinema is added with vegetables (grated onion, tomatoes, and green chillies), spices, salt, and a little amount of water and cooked for 5 mins to make a thick curry, which is eaten as a sidedish of steamed rice. Fresh kinema keeps for 2–3 days during summer and 1 week during winter. The shelf life is often lengthened to several months by drying kinema (Figure 2.15b) in the sun or keeping it above an earthen oven in kitchen (Tamang et al., 1988).

Kinema production is a means of marginal income for some rural households. Usually, it is sold by volume measured using a small silver mug containing 150–200 g kinema, which is wrapped with *Ficus* or banana leaves and then tied loosely using a piece of straw. In Darjeeling hills, 1 kg of fresh and sun-dried kinema costs INR 100

Figure 2.15 Fresh (a) and dried (b) kinema, as marketed.

(= US\$1.6) and INR 240 (= US\$3.8), respectively (April, 2014). Per capita daily consumption of kinema was recorded during 1997–1998 as 3.3 g with annual production of 326 tons in Darjeeling hills and 2.2 g with annual production of 326 tons in Sikkim (Yonzon and Tamang, 1998). An average of 5 kg is sold per day by each seller and brings a profit of approximately 40%. Although there is a good market for kinema, processing is still restricted to the individual households as an unorganized sector.

2.1.1.3.3 Changes in the Substrate during Kinema Production

2.1.1.3.3.1 Stage 1: Soaking Soybeans soaked in excess water absorb approximately their own weight of water and undergo a natural microbial acidification. During soaking, there is no significant change in the frequency of coliforms and *Bacillus subtilis* cells present on raw soybeans, but a population of lactic acid bacteria appears, resulting in a decline in pH of soaked beans from 6.6 to 5.3 (Sarkar, 2000). The microbiota residing in soaking vessels acts as a starter for the mixed lactic/acetic acid fermentations, which lower the pH (Nout and Rombouts, 1990). The microbial load and composition of soak water seem to depend on the bean/water ratio, time, and temperature of soaking, and the initial microbial composition of the dry beans (Nout et al., 1987). Soaking of beans leads to a significant decrease in thiamine content; however, the content of riboflavin remains unchanged (Sarkar et al., 1998). The remarkable decrease in thiamine content may be due to increased thiaminase activity or complex formation.

2.1.1.3.3.2 Stage 2: Cooking Most of the bacteria in soaked beans are killed during the subsequent process of steaming, which leaves a near pure culture of *Bacillus*, which survives as spores and can grow without competition (Nout and Rombouts, 1990; Leejeerajumnean et al., 2000). Because of draining of soak water and cooking the soaked beans in fresh water, the pH of the cooked beans rises from 5.3 up to 6.2–6.6. However, soaking is found to be essential because bypassing this hydration step yields kinema of poor organoleptic quality due to significantly lower sensory scores in the body and texture attributes (Sarkar, 2000). The acidification of beans must also be considered important in restricting the growth of spoilage bacteria (Ashenafi and Busse, 1991). Cooking has no significant influence on thiamine content, although

riboflavin and niacin levels increase (Sarkar et al., 1998). The increase in riboflavin contents after cooking (Table 2.4) may result from a more complete extraction of coenzyme forms, e.g., FMN, FAD.

2.1.1.3.3.3 Stage 3: Fermentation

Microbiota: Fermentation of cooked soybeans to produce kinema is natural, i.e., no deliberate inoculation of the substrates with starter cultures is practiced. It is likely that the environment

Table 2.4 Nutrient Contents of Unfermented Soybeans and Kinema

PARAMETER	RAW BEANS	COOKED BEANS	KINEMA
Moisture (g/kg)	121b ± 0	749a ± 4.0	752a ± 0
Essential amino acids (free) (mg/kg dry wt)			
Valine	—	0.08b ± 0	7.05a ± 0.12
Leucine	—	1.0b ± 0	9.50a ± 0.13
Isoleucine	—	0.06b ± 0	6.22a ± 0.33
Threonine	—	0.13b ± 0	2.79a ± 0.19
Lysine	—	<dl (0.01)	8.23 ± 0.28
Histidine	—	0.09b ± 0	4.52a ± 0.07
Arginine	—	0.27a ± 0	0.22b ± 0.02
Phenylalanine	—	0.15b ± 0	11.82a ± 0.09
Tryptophan	—	<dl (0.01)	<dl (0.01)
Methionine	—	0.04b ± 0	3.15a ± 0.06
Essential fatty acids (free) (mg/kg dry wt)			
C18:2 (linoleic acid)	3838b ± 42	3758b ± 28	4774a ± 5
C18:3 (linolenic acid)	511b ± 11	524b ± 13	580a ± 13
Phytosterols (mg/kg dry wt)			
Campesterol	169b ± 11	170b ± 9	255a ± 14
Stigmasterol	146b ± 10	144b ± 7	225a ± 14
β-Sitosterol	492b ± 30	488b ± 30	788a ± 29
B-group vitamins (mg/kg dry wt)			
B ₁ (thiamine)	16.8a ± 0.4	5.8b ± 0.3	8.4b ± 0.6
B ₂ (riboflavin)	3.4c ± 0.2	6.8b ± 0.5	11.6a ± 0.3
B ₃ (niacin)	<dl (14.7)	36.4 ± 4.8	44.8 ± 5.6

Source: Sarkar, P.K., and Tamang, J.P., *Food Microbiology*, 11, 317–325, 1994; Sarkar, P.K., and Tamang, J.P., *Food Microbiology*, 12, 317–325, 1995; Sarkar, P.K. et al., *Letters in Applied Microbiology*, 24, 337–339, 1997a; Sarkar, P.K. et al., *Food Chemistry*, 59, 69–75, 1997b; Sarkar, P.K. et al., *Journal of the Science of Food and Agriculture*, 71, 321–328, 1996; Sarkar, P.K. et al., *Journal of the Science of Food and Agriculture*, 78, 498–502, 1998.

Note: dl, detection limit. Values with standard error were obtained from three replicates. Means within a row sharing a common letter are not significantly different ($p < 0.05$).

and utensils become highly contaminated with a “house flora” that functions as an inoculum. Market samples of kinema contain six species of *Bacillus*, namely *B. subtilis*, *B. licheniformis*, *B. cereus*, *B. circulans*, *B. thuringiensis*, and *B. sphaericus* (Sarkar et al., 2002). In addition, *Enterococcus faecium*, *Candida parapsilosis*, and *Geotrichum candidum* occur in 100%, 50%–80%, and 40%–50%, respectively, of kinema samples (Sarkar and Tamang, 1994). A study with 83 isolates of *Bacillus* from kinema revealed *B. subtilis* as the most abundant among the six species (Figure 2.16) (Sarkar et al., 2002). Further, in pure culture experiments *B. subtilis* has been shown to be capable of the fermentation as the sole organism; the members of the accompanying flora are probably opportunists (Sarkar and Tamang, 1994; Sarkar et al., 1994).

A number of interesting changes occurs during processing of soybeans to produce kinema. Spores of *B. subtilis*, which are normal inhabitants of soybeans, pass through soaking and cooking treatments to initiate and carry out fermentation. The mean viable counts of *B. subtilis*, *E. faecium*, and *C. parapsilosis* increase in fermenting beans. In monoculture fermentation (Figure 2.17), the count of *B. subtilis* cells increases from an initial 10^5 /g up to 10^9 /g wet beans after 48 h at 37°C (Sarkar et al., 1993, 1994).

Figure 2.16 Relative abundance of *Bacillus* spp. in marketed kinema. Values within parentheses indicate %occurrence out of 83 isolates. (Modified from Sarkar, P.K. et al., *International Journal of Food Microbiology*, 77, 175–186, 2002.)

Figure 2.17 Changes in *Bacillus subtilis* cell count (*Bs*), pH, and contents of free fatty acid (FFA), non-protein nitrogen (NPN), and hexanal accompanying fermentation of soybeans to produce kinema. (Modified from Sarkar, P.K., *Journal of Food Science and Technology*, 37, 477–483, 2000.)

Proximate Composition: It is interesting that before 16 h of fermentation there is no significant increase in the contents of free fatty acids (FFA) and non-protein nitrogen, suggesting that sugars, not fats or proteins, are initially used as substrates for metabolism and growth of *B. subtilis*. This conjecture gets support from the fact that while the sucrase activity sharply rises from the onset of fermentation and reaches its peak after 12 h, amylase activity starts after 6 h and then continues to increase until the end of fermentation (Figure 2.18). The lipase activity is maximum after 24 h only (Sarkar, 2000).

The average moisture content of market kinema is 615 g/kg. Kinema contains 477 g protein, 169 g fat, 281 g carbohydrate, 72 g ash, and 20 MJ energy per kg dry weight (Sarkar and Tamang, 1994). Although the titratable acidity (0.1%) in kinema is about 10 times higher than in raw soybeans, the product has a high pH value (>7.8; even up to 8.5). This is due to the high buffering capacity of the legume beans and the proteolytic activities of *B. subtilis* leading to ammonia release, characteristic of most vegetable protein fermentations (Hesseltine, 1965). The pH drops from an initial 6.9 to 6.4

Figure 2.18 Changes in the content of some hydrolases accompanying fermentation to produce kinema. (Modified from Sarkar, P.K., *Journal of Food Science and Technology*, 37, 477–483, 2000.)

after 8 h, and then rises to 8.6 at the end of fermentation (Figure 2.17), with a coincident increase in proteolytic activity (Sarkar et al., 1993).

Amino Acids: The very high acidic amino acid content of kinema is of particular interest. Glutamic acid is the most abundant amino acid, followed by aspartic acid, together representing 31% of total residues (Sarkar et al., 1997b). The strong proteolytic activity results in a significant decline in protein nitrogen content, with a concomitant increase in non-protein and soluble nitrogen contents (Sarkar et al., 1993; Sarkar and Tamang, 1995). The free amino acid content of unfermented soybeans, which is only 0.18% of the total dry mass, increases 60-fold in kinema. The percent liberation of amino acids (based on the ratio of sum of the free amino acid contents to that of the total amino acid contents) at the time of fermentation was 37 (Sarkar et al., 1997b). Although *E. faecium* has no influence, the presence of yeasts (*C. parapsilosis* and *G. candidum*) significantly reduces the levels of several free amino acids, suggesting their preferential uptake by the yeasts. So, in case of traditionally prepared kinema the percent liberation of amino acids was 11.4 only (Nikkuni et al., 1995). The net decrease in some individual amino acid levels after fermentation suggests

that they are metabolized to a greater extent than they are replaced by proteolytic activity. The decrease is particularly pronounced for arginine and cyst(e)ine. In addition, levels of some amino acids (alanine, isoleucine, serine, aspartic acid, asparagine, arginine, tyrosine, methionine, and hydroxyproline) are significantly depleted when yeasts contribute to the *Bacillus* fermentation of soybeans (traditional fermentation). Interestingly, kinema proteins are a good source of almost all the essential amino acids (EAA; Table 2.4) (Sarkar et al., 1997b). Kinema has EAA₇ and EAA₁₀ values of 41.4% and 52.2%, respectively, of total protein. The EAA profile of kinema is comparable with two high-quality protein foods (eggs and cow's milk) and also with that of the reference pattern established by FAO/WHO/UNU (1985) and FAO/WHO (1990).

Lipids: Although there is no significant change in the crude lipid (CL) content of soybeans after soaking and cooking, the increase (20%–30%) is significant after fermentation. This is probably due to active assimilation of carbohydrates and limited consumption of lipids, resulting in an enrichment of CL at the end of fermentation. In terms of CL content, neither the *Enterococcus* nor the yeasts have any influence on the *Bacillus* fermentation of soybeans (Sarkar et al., 1996).

In both raw soybeans and kinema, the content of unsaturated fatty acids (oleic, linoleic, and linolenic) is six times higher than that of saturated fatty acids, and linoleic acid (the essential fatty acid in animal diets) is the predominant fatty acid, comprising 57% of the total fatty acids (Table 2.4). The major fatty acids are liberated during fermentation by microbial lipases. The overall level of FFAs does not change significantly upon soaking and cooking but increases significantly after fermentation. The increase in FFA is 16%–27% during fermentation. In kinema, the FFA content is 3.1%–3.7% of CL. The increased fatty acid levels in fermentation products may be due to the synthesis and accumulation of extra lipids or hydrolysis of glycerides during fermentation. Another possibility is dissociation of lipoprotein complexes in soybeans during fermentation, resulting in the release of

ether-extractable FFAs (Wang et al., 1975). Since soybeans are heated (inactivating the intrinsic enzymes), liberation of FFAs during fermentation is most likely due to the action of lipases of the kinema microorganisms. A possible negative aspect of kinema is that FFAs can inhibit glycolytic and gluconeogenic enzymes, some hydrolases including trypsin, and fatty acid synthesis.

The unsaponifiable lipids of unfermented soybeans, identified as campesterol, stigmasterol and β -sitosterol (1.2:1:3.4) increase by 58% in kinema (Table 2.4), which have a cholesterol-lowering effect (Sarkar et al., 1996). Mattson et al. (1977) found a 30% reduction in cholesterol absorption by fortifying dietary fat (containing 10 g cholesterol/kg) with twice this amount of phytosterols.

Vitamins: *Bacillus* fermentation (37°C, 48 h) enhances thiamine, riboflavin, and niacin levels by 45, 71, and 23%, respectively (Table 2.4). This shows that *B. subtilis* has a great synthetic capacity for all these vitamins. These levels decline by 31, 18, and 74%, respectively, in the presence of *E. faecium*, indicating that this bacterium uses readily available vitamins for growth and metabolism. *Enterococcus* requires vitamin-rich media for growth and would be expected to decrease vitamin levels in foods when it is present during fermentations. Since thiamine is susceptible to heat, kinema prepared at 45°C leads to 33% decrease compared with that prepared at 37°C (Sarkar et al., 1998). In terms of overall B-group vitamin content, *Bacillus*-fermented kinema produced at 37°C for 48 h is superior to that prepared by other fermentation conditions. Increase in these vitamins as a result of fermentation has important nutritional implications in communities where this food contributes to the formation of the only side dish of rice.

Minerals: Kinema contains significantly lower levels of minerals than found in raw beans. In the kinema production process, soaking as well as cooking water is discarded, and this may be responsible for the 6-fold depletion in mineral levels. Of course, the mineral content is influenced by that of the water used for soaking beans. Despite such a large loss in minerals during processing, kinema (prepared in laboratory using

distilled water for soaking and cooking and without adding firewood ash) still contains appreciable quantities (4, 10, 13, 18, 683, 494, 1257, and 2077 mg/kg dry weight) of copper, manganese, zinc, iron, calcium, magnesium, phosphorus, and potassium, respectively. It is noteworthy that potassium is the most abundant mineral in kinema, and sodium content in kinema is below its detection limit (0.5 mg/kg dry weight) (Sarkar et al., 1998). Since in practice spring water is used for soaking and firewood ash is often added, mineral content of traditional kinema may not be less than that observed and may even be higher in some instances than in raw beans. In fact, Nikkuni et al. (1995) estimated the amount of copper, manganese, zinc, iron, calcium, magnesium, phosphorus, and potassium as 17, 541, 45, 177, 4320, 2520, 7290, and 17,680 mg/kg dried kinema collected from Nepal. In industrialized countries, people generally consume too much sodium and not enough potassium. The high ratio of sodium to potassium (Na/K) has been linked to high blood pressure, heart disease, and stroke (Cook et al., 1998). Higher ratios of Na/K were independently linked to a higher risk of cardiovascular diseases and to higher all-cause mortality (Yang et al., 2011). Public health recommendations have stressed that a reduction in sodium intake should be accompanied by a simultaneous increase in potassium intake. The net effect would be to reduce the ratio of Na/K in the diet. Hence, kinema is not only a valuable source of minerals particularly where intake from other sources is marginal but also an important potassium-rich diet.

Digestibility: An in vitro model was used by Kiers et al. (2000) to demonstrate a change in the digestibility of soybeans during fermentation by using *B. subtilis* strains from kinema. During fermentation for 48 h of autoclaved soybeans the quantity of soluble and dialyzable matter increased from 22% and 6% up to 65% and 40%, respectively. The *Bacillus* strains were able to degrade soybean macromolecules to a large extent resulting in water-soluble low-molecular-weight compounds, being small enough to pass the dialysis membrane. Soybeans contain two main storage proteins, β -conglycinin (consisting of

three components – α , α' , and β subunits) and glycinin (consisting of two components – an acidic [A] and a basic [B] polypeptide). A complete breakdown of all the subunits from these proteins was observed after fermentation of cooked soybeans by a kinema strain (*B. subtilis* DK-W1). After 24 h of fermentation, substantial quantities of polymeric carbohydrate matter were degraded into compounds <5 kDa. In vitro digestibility was increased from 29% to 33%–43% after 48 h of fermentation. The ratio between absorbability and digestibility was more than 80% after fermentation for 24 h increasing up to 95% after fermentation for 48 h. In vitro digestion of *Bacillus*-fermented soybean using gastrointestinal enzymes only slightly increased the amount of dialyzable matter. The results clearly demonstrated that after 18–24 h of fermentation considerable substrate modification had occurred leading to kinema with high nutrient availability in which the need for degradation of nutrients by gastrointestinal enzymes is minimal.

Flavor: In the course of fermentation, the objectionable beany flavor in cooked soybeans gradually diminishes and finally disappears. Gradually, a nutty flavor begins to develop and eventually becomes masked by the smell of ammonia (Sarkar and Tamang, 1995). The volatile compounds detected in cooked soybeans include 1-hexanol, 1-octane-3-ol, hexanal, nonanal, decanal, 2-heptanone, 3-octanone, decylbutanoate, low concentrations of several pyrazines, 2-pentylfuran, vinylbenzene, benzaldehyde, 2-methoxyphenol, 4-vinyl-2-methoxyphenol, and indoline (Owens et al., 1997). The content of hexanal (the major cause of beany flavor), which accounts for 7.5% (quantitatively the second highest) of the total volatiles of unfermented soybeans, decreases steadily and goes below the limit of detection within 24 h of fermentation (Figure 2.17). On the other hand, the ammonia content of unfermented soybeans shows a 34-fold increase in kinema (Owens et al., 1997; Sarkar et al., 1997b; Sarkar, 2000).

Antioxidative Activity: Fermentation of soybeans with *B. subtilis* to kinema causes 144% increase of total phenol content (TPC) and enhances free radical-scavenging activity, metal-chelating

ability, reducing power, and lipid peroxidation inhibitory activity. All these antioxidant activities of kinema are significantly higher than those of unfermented soybean, suggesting the role of fermentation in enhancing these attributes, which are positively correlated with the TPC. While unfermented soybean shows 36% inhibition of linoleic acid peroxidation, kinema exhibits antioxidant activity with 44% inhibition. However, the peroxidation inhibition of kinema extracts declines with time and reaches merely 26% after 72 h. Thus, the kinema strain (*B. subtilis* DK-W1) is an effective producer of antioxidant activities. The sharp decline of the peroxidation inhibition in kinema beyond 24 h can be attributed to the shelf life of the product. After about 12 h of traditional fermentation, the surfaces of the beans are covered with a rough, white viscous mass when kinema is ready for cooking. Kinema can be shelved for only two days, after which a rapid softening along with a strong off-flavor develops in the product, rendering it unacceptable for consumption. In unfermented soybean, the highest correlation is between the radical-scavenging activity and the metal-chelating activity, while in kinema, it is a radical-scavenging activity with lipid peroxidation inhibitory activity, being closely followed by metal-chelating activity (Moktan et al., 2008). The higher level of antioxidant activity in kinema compared with unfermented soybean could be attributed to the extensive hydrolysis of proteins and an increase of 58% in the overall content of phytosterols that happens during the fermentation (Sarkar et al., 1996; 1997b). Because the sequence and composition of amino acids in the peptides are critical, several peptides in soy protein hydrolysate have good antioxidant activity while the others have marginal activity (Quinn and Tang, 1996).

Anti-Nutritional Factors: Although soaking and subsequent cooking of soybeans cause a 73%–88% reduction in the content of raffinose family oligosaccharides (RFOs) in raw soybeans, monoculture fermentation to produce kinema lowers the levels below their detection limit (Table 2.5), thus eliminating a potential cause of flatulence for consumers (Sarkar et al., 1997a).

Table 2.5 Anti-Nutrient Contents of Unfermented Soybeans and Kinema

PARAMETER	RAW BEANS	COOKED BEANS	KINEMA
Moisture (g/kg)	121b $\pm$ 0	749a $\pm$ 4.0	752a $\pm$ 0
RFOs (g/kg dry wt)			
Raffinose	19.2a $\pm$ 1.0	2.3b $\pm$ 0.1	<dl (1.0)
Stachyose	43.2a $\pm$ 0.6	10.6b $\pm$ 0.4	<dl (1.0)
Phytic acid (g/kg dry wt)	8.4a $\pm$ 0.6	4.7b $\pm$ 0	1.9c $\pm$ 0.3
Trypsin inhibitor activity (U/g dry wt) ^a	3373a $\pm$ 139	1907b $\pm$ 5	1139c $\pm$ 83
Hemagglutination activity (U/g dry wt) ^b	427a $\pm$ 106	20b $\pm$ 4	0c

Source: Sarkar, P.K. et al., *Letters in Applied Microbiology*, 24, 337–339, 1997; A. Sharma and P. K. Sarkar (unpublished data).

Note: dl, detection limit. Values with standard error were obtained from three replicates. Means within a row sharing a common letter are not significantly different ($p < 0.05$).

^a One unit is defined as the amount of trypsin inhibitor required to cause 50% inhibition of trypsin (50 μ g) under the assay condition.

^b One unit is defined as the least amount of hemagglutinin, which produces positive evidence of agglutination under the assay condition.

Phenolic compounds and phytate in plant foods have been shown to interfere with the availability of minerals such as iron (Svanberg et al., 1993). Low bioavailability of iron from vegetable diets is recognized to contribute to anemia. Among the phenolic compounds, galloyl, resorcinol, and catechol groups are known to have an inhibitory effect on iron availability. Several simple processing methods, such as cooking and fermentation, were found to reduce the amounts of phenolic compounds in soybeans during kinema production (Table 2.5).

Phytic acid, widely distributed in mature legume grains, stores most of the grain phosphorus. Phytic acid chelates several minerals and thereby reduces their bioavailability (Nolan and Duffin, 1987). During kinema production, after soaking and cooking there was 44.9% reduction in the phytic acid content of raw soybeans (8.44 g/kg dry weight); monoculture fermentation caused a further reduction of the content in cooked beans by 58.4% (Table 2.5). This indicates that the reduction in phytate content during soaking and cooking was due to its hydrolysis by endogenous phytases in seeds, leaching out in the medium, and thermal degradation, and during fermentation was due to the phytases produced by *B. subtilis* (A. Sharma and P.K. Sarkar, unpublished data).

Trypsin inhibitor (TI) has been implied to be one of the factors responsible for reducing protein digestibility, pancreatic hypertrophy, and poor growth performance in rats, mice, and chicks (Gumbmann et al., 1989). Therefore, reduction of TI activity is useful in improving nutritional quality of soybean with respect to protein digestibility. During kinema production, the TI activity reduced by 43.5% after soaking and cooking, and 66.2% after monoculture fermentation (Table 2.5). A reduction in TI activity during *Bacillus* fermentation of soybeans has been reported by other investigators (Teng et al., 2012).

Soybean agglutinin (SBA, a tetramer with a 30-kDa subunit) belongs to the lectin family and has the unique property of binding to carbohydrates. SBA, by binding itself with the carbohydrate moiety of cell surfaces, causes cells to agglutinate. This activity is referred to as hemagglutination activity (Liener, 1994). Ma and Wang (2010) demonstrated after heat denaturation at 100°C for 20 min, SBA had 60% of hemagglutination activity remaining; after single enzyme (such as trypsin, chymotrypsin, and thermolysin) hydrolysis, SBA still had 50%–60% activity, and the activity of SBA was fully eliminated by multienzyme hydrolysis. They explained that on heating the tetramer SBA is denatured, the activity is reduced due to a three-dimensional structure change; however, hydrolyzing by single enzymes, bioactive peptides are released, displaying some activity; after hydrolyzing by a combination of enzymes, the active peptides are fully destroyed, losing active sites and their binding capacity. During the production of kinema only 5% of the hemagglutination activity remained after soaking and cooking soybeans, and the activity was fully destroyed after fermentation (Table 2.5).

Biogenic amines are toxic nitrogenous compounds and produced mainly by microbial decarboxylation of amino acids in many foods, especially in protein-rich foods. The major biogenic amines found in food products are putrescine, cadavarine, histamine, tyramine, tryptamine, 2-phenyl-ethylamine, spermine, agmatine, and spermidine. Common intoxication symptoms of biogenic amines in human are nausea, respiratory

distress, hot flushes, sweating, heart palpitation, headache, a bright red rash, oral burning, and hypotension or hypertension. Oral intake of biogenic amines generally provokes no adverse reactions, because amine oxidases in the intestine rapidly metabolize and thereby detoxify them. However, the capacity of amine oxidases can be saturated by ingesting high doses of biogenic amines, which may lead to food intoxication. Therefore, upper limit of total biogenic amines for human consumption has been suggested by Ten Brink et al. (1990) as 1000 mg/kg food. During the production of kinema although the level of total biogenic amines in raw soybeans increased significantly in the product, the final level is below the safe upper limit prescribed (Table 2.5).

2.1.1.3.4 Safety of Kinema Like any other food, kinema harbors some food-borne bacterial pathogens, including enterotoxigenic strains of *B. cereus* and fecal coliforms. In the presence of functional bacterium (*B. subtilis*), the growth of *B. cereus* is adversely affected and the production of enterotoxin is ceased. Kinema is free from *Staphylococcus aureus*. However, the presence of high numbers of Enterobacteriaceae in fresh kinema is an indication of preparing and/or selling the product in an unhygienic condition. It can serve as a vehicle for contamination of other foods by cross-contamination routes. Since *B. cereus* enterotoxin is heat-sensitive, the traditional way of making kinema and its culinary use in curries is safe. However, if we consider the utilization of kinema in novel applications, e.g., as a dried powdered ingredient for protein or flavor enrichment of starchy foods, adequate measures must be taken to avoid the carry-over of enterotoxin or *B. cereus* endospores, which might germinate, proliferate, and produce enterotoxin in fabricated foods (Nout et al., 1998).

2.1.1.3.5 Plasmid for γ -Polyglutamate Production A γ -polyglutamate (γ -PGA)-producing *Bacillus* strain, isolated from kinema, was found to harbor a plasmid species (Hara et al., 1995). The hybridized region on the kinema plasmid, pNKH, with the γ -PGA production-stimulating factor (PSF) gene (*psf*) on natto plasmid pUH1, which is responsible for γ -PGA production in *B. subtilis* (natto), was identified, and the nucleotide sequence was determined. There was found

only one possible 1281-bp-long open reading frame (ORF) that could potentially encode a polypeptide consisting of 427 amino acids with a molecular weight of 50,251 Da. The polypeptide was found to be 7 residues longer than that of pUH1 PSF. The percent match of the amino acid sequence between pNKH ORF and pUH1 PSF was 92. Their results suggested that the plasmid pNKH of kinema *Bacillus* might be responsible for γ -PGA production in kinema. Hara (1990) revealed that pUH1 resembled pNKH in partial sequences more than pTNH4, isolated from γ -PGA-producing *Bacillus* strain responsible for thua nao production. Based on pattern similarity of free amino acid compositions of kinema, thua nao, and natto using the complete linkage cluster analysis, Nikkuni et al. (1995) also indicated that kinema is similar to natto rather than thua nao. However, they suggested that the hydrolytic products of the soybean proteins of kinema, thua nao, and natto are affected by their fermentation process rather than the characteristics of the *Bacillus* strains.

2.1.1.3.6 Process/Product Improvisation Organoleptically, the monoculture fermentation of soybeans by *B. subtilis* DK-W1, isolated from kinema from the market of Darjeeling, produced the best quality kinema (Sarkar and Tamang, 1994; Sarkar et al., 1994). In this study, *B. subtilis* DK-W1 was selected for further improvement of kinema quality. Since the optimum temperature for the growth of this strain is 45°C (Sarkar and Tamang, 1994), an attempt was made to determine the optimum time for fermentation at that temperature following *Bacillus* inoculation. The 18-h period was the optimum time for fermentation at 45°C. That kinema had a very pleasant nutty flavor associated with a mild ammonia odor and a highly sticky texture, scoring “excellent.” During the production of natto, which is also produced by *B. subtilis*, the fermentation lasts for 18–20 h at 40°C–43°C (Fukushima, 1979).

The monoculture fermentation of soybeans has many advantages over the conventional natural fermentation. First, the load of spore-formers in monoculture fermentation can be manipulated to make a good start. Second, the desirable fermentation is completed within a much shorter time with the organoleptically excellent product development. Third, at high temperature and short period of incubation, the chance of the growth of contaminants is remote. Fourth, because of controlled fermentation, the biochemical changes up to 18 h

are desirable. The pH initially goes down significantly within 6 h of incubation and then increases significantly up to 7.5 at the end of fermentation. This indicates that the production of ammonia during monoculture fermentation is not so high as in natural fermentation. The FFA content in kinema produced by monoculture fermentation is 140% less than that in kinema produced by the conventional method. Monoculture fermentation results in the formation of kinema without undesirable odor. The desirable increase in non-protein and soluble nitrogen content within an 18-h fermentation time, compared with that of the conventional method, makes the product acceptable and probably contributes to the improved digestibility.

2.1.1.3.7 Development of Kinema Starter Tamang (1999) attempted to develop pulverized starter using a selected strain of *B. subtilis*, previously isolated from traditionally prepared kinema. Kinema prepared using this strain, harvested after growing in soybean extract broth, was dried in an oven at 70°C for 10 h and ground aseptically. The pulverized starter was packed aseptically in a polyethylene bag, where it can be kept for at least 6 months.

Attempts have been made to search for high-quality kinema starter strains (Tamang and Nikkuni, 1996; Sarkar et al., 2002); however, these were only preliminary studies. Strain selection should depend on individual performance of strains under practical conditions, also considering additional factors, such as the production of desirable aroma and competitiveness in the presence of contaminating microbiota in case of natural fermentations (Sarkar et al., 2002).

2.1.1.3.8 Development of Kinema-Fortified Cookies Although highly nutritious, fresh kinema has some limitations, especially its ammoniacal odor and slimy appearance, which deter its acceptability to a broader dimension of public. Hence, product diversification has been attempted for better acceptance and usefulness of kinema. Biscuits, supplemented with kinema flour, were prepared by Shrestha and Noomhorm (2002). Kinema, prepared using dehulled soybeans and a *B. subtilis* isolate from kinema, was dried at 65°C–70°C for 10–12 h and ground to flour, which was incorporated at the 15% level into biscuit formulation. Kinema supplementation resulted in decreased hardness but increased weight and spread ratio (an indicator of cookie density)

in fortified red colored biscuits, which had greater organoleptic acceptability compared with full-fat soy-flour-supplemented biscuits. Protein content of the product was more than 17%.

2.1.1.3.9 Conclusion Traditional knowledge of tribal women in preparing kinema has relevance to the sustainable food and nutritional security and subsistence economy of the kinema-practicing societies. To promote commercialization of kinema, the product must maintain superior quality. It is necessary to increase efforts to introduce consumer preferences in terms of taste and produce high-quality products, packaging, and design as well as safety management. Given the concerted efforts of academia, industry, and government and private organizations, kinema has a potential to become an enjoyable part of the menu.

2.1.1.4 Doenjang

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Korea has a long history of eating fermented soybean foods, and doenjang is one of the representative Korean traditional fermented soybean foods. Historically, soybean and processed soybean foods have been one of the most important protein sources in the Korean diet because Koreans mainly eat vegetables. Doenjang has also been used as a seasoning for enhancing the taste of foods (Park and Jung, 2005). As a traditional Korean fermented soybean food, doenjang has unique qualities and characteristics in terms of its flavor and taste compared with those of other similar products from Asian countries. This is due to the fermentation process and Korea's unique natural environment (Park et al., 2000; Kim, 2002).

Doenjang is recognized as a nutritious food that provides essential amino acids that are lacking in cereal and vegetable diets as well as fatty acids, organic acids, minerals, and vitamins, which are highly related to its quality and diverse nutritional benefits (Lim et al., 1999). Proximal composition of doenjang is 54.7% water, 13.8% crude protein, 8.0% crude lipid, 14.4% titratable acidity, and 11.8% salt. Free amino acids are comprised 3.8% of the nitrogen content, of which glutamic acid content (25%) is the highest, but leucine, alanine, histidine, lysine, proline, and valine are also present in relatively high quantity. Lactic acid was the most abundant organic acid, but acetic,