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Development of Pulverised Starter for *Kinema* Production

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Kinema is a traditional fermented soybean food common to the Eastern Himalayas. Pulverised starter using selected strain of *Bacillus subtilis* KK2:B10 (MTCC 2756), previously isolated from traditionally prepared *kinema*, was developed for *kinema* production. *Kinema* prepared by *Bacillus subtilis* KK2:B10 strain, which was grown and harvested in soybean extract broth, was dried in an oven at 70°C for 10 h and ground aseptically. The 1% of pulverised starter was added aseptically to steamed soybeans and fermented at 40°C for 20 h under 85% relative humidity to get *kinema*. Load of *Bacillus subtilis* in pulverised starter kept in room temperature was constantly maintained at ~10^9 cfu/g, even tested upto 6 months. The consumers' preference trials showed that *kinema* prepared by using pulverised starter was organoleptically more acceptable than market *kinema*. Water-soluble nitrogen and formol nitrogen to total nitrogen contents were higher in *kinema* prepared by using pulverised starter than market *kinema*. Application of inexpensive and ready-to-use pulverised starter may be appropriate for *kinema* processing at household level.

Keywords : *Kinema*, *Bacillus subtilis* KK2:B10, Soybean extract broth, Pulverised starter.

Kinema is a soybean-based fermented sticky, slightly alkaline food with a typical flavour consumed as curry in the Darjeeling hills and Sikkim in India, Eastern Nepal and Bhutan. During *kinema* preparation, overnight-soaked whole soybeans are cooked, soybean extract is drained off, cracked moderately, the grits are then placed in a basket ned with fern (*Athyrium* sp.) leaves and left to ferment naturally for 1-3 days (Tamang et al. 1988). daily per capita consumption of *kinema* was 3.3 g in the Darjeeling hills with annual home production of 829 tonnes, and 2.2 g with annual production of 326 tonnes in Sikkim, respectively during 1997-98 (Yonzan and Tamang 1998). *Kinema* is similar to other fermented soybean products such as *akhoni* of Nagaland, *troombai* of Meghalaya, *hawaijar* of Manipur and *bekang-um* of Mizoram (Tamang 1996) and *natto* of Japan and *thua-nao* of Thailand (Nikkuni 1997).

Bacillus subtlis, *Enterococcus faecium*, *Candida parapsilosis* and *Geotrichum candidum* are associated with *kinema* (Sarkar et al. 1994). However, *Bacilus subtilis* was found to be a sole organism in fermentation of soybeans to *kinema* (Sarkar and Tamang 1994; Tamang 1995). Strain of *Bacillus subtilis* KK2:B10 was selected as the starter culture (Tamang and Nikkuni 1996) and fermentation conditions for laboratory-scale production of *kinema* were optimised (Tamang and Nikkuni 1998). Selling of *kinema* in local market provides income generation to some rural women and the trade protected as hereditary right which passes from mother to daughter (Tamang 1998). The aim of the present work was to develop an inexpensive and ready-to-use pulverised starter for *kinema* production in order to upgrade the traditional process.

Soybean samples : Small (~6mm) with smooth yellow seed coat and dark brown hilum 'local yellow' variety of soybean [*Glycine max* (L.) Merrill] was purchased from Gangtok market.

Microorganism : *Bacillus subtilis* KK-2:B10 [MTCC* 2756 (*Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) was isolated from naturally fermented *kinema* samples, identified as *Bacillus subtilis* (Ehrenberg) Cohn and was selected as best starter cultures for laboratory scale *kinema* production (Tamang and Nikkuni 1996).

Sample collection : *Kinema* samples were collected from Gangtok and Rongli markets in Sikkim aseptically in pre-sterile bags, which were kept in an ice-box and transported immediately to the laboratory for analysis.

Soybean extract broth : Overnight soaked soybeans (100 g) were autoclaved in a beaker containing 200 ml tap water at 121°C for 30 min. Sediment of soybean extract collected in beaker was filtered using Whatman filter paper No. 1. The final pH of filtered soybean extract was adjusted to 7.0 with 1 N NaOH, using pH-meter (Systronics 335, India).

Laboratory-scale kinema *preparation* : Soybeans were cleaned, washed and soaked in tap water overnight at room temperature. Soaked soybeans were autoclaved at 121°C for 30 min and inoculated with cell suspension of *Bacillus subtilis* KK2:B10, harvested in nutrient broth (HiMedia M002), phytone-sucrose broth (phytone peptone 10.0 g, sucrose

10.0 g, sodium chloride 10.0 g, agar 20.0 g, distilled water 1 L, pH 7.0), soybean extract broth and soybean extract-sucrose broth (in soybean-extract, added: sucrose 1.0%, NaCl 0.5%, pH 7.0) at 37°C for 18 h, respectively, at 10^4 cfu/g of cooked soybeans, while the temperature of soybeans was above 80°C. Inoculated soybeans were put into pre-sterile petri-dish (outer lid was replaced by perforated polythene film), and incubated at 40°C for 20 h under 85% relative humidity.

Microbial analysis : Culture of *Bacillus subtilis* KK-2B10 was transferred onto the nutrient broth, phytone broth, soybean extract broth and soybean extract-sucrose broth, separately and incubated at 37°C for 18 h to employ as purified *kinema* starter. Decimal dilution series were prepared in sterile physiological saline (0.85% w/v sodium chloride in water) and 1 ml of appropriate diluted suspension was mixed with molten tryptone soya agar (HiMedia M424) and incubated at 37°C for 24 h. Colonies appeared were counted as colony forming unit per ml (cfu/ml). For viability test, 10 g pulverised starter was mixed with 90 ml of sterile physiological saline for 10 min and decimal series were prepared as described above. The total viable counts of *Bacillus subtilis* in pulverised starter were determined every month till 6 months.

Sensory evaluation : The sensory attributes of *kinema* fermented by *Bacillus subtilis* KK2:B10, harvested in nutrient broth, phytone-sucrose broth, soybean extract and soybean extract-sucrose broth, respectively, were evaluated for flavour, taste, stickiness, texture and colour after sampling by a panel of 15 trained judges, using a 100-point score card as described by Sarkar and Tamang (1994).

Consumer preference trial : Market samples of *kinema* as well as *kinema* prepared by using pulverized starter were served to 100 consumers representing different ethnic groups of people of the Sikkim Himalayas. The 9-point Hedonic scale (IS 1971) used in this study ranged from 'dislike extremely' (score 1) to 'like extremely' (score 9).

Chemical analysis : Total nitrogen and water-soluble nitrogen of samples were determined by micro-Kjeldahl method (AOAC 1990). Formol nitrogen of sample was determined by formaldehyde titration as described by Tamang and Nikkuni (1996). Homogenized samples were mixed with distilled water and centrifuged at 20,000 x **g** for 15 min, 10 ml supernatant was mixed with 20 ml of previously neutralized formaldehyde and titrated against 0.1 N NaOH, using an auto-titrator (TOA, TSB-10a, Tokyo, Japan).

Statistical analysis : Data obtained were analyzed by determining errors of the mean and analysis of variance, using the least square design (Snedecor and Cochran 1989) and SALS software package (version 2.5).

Nutrient broth is conventionally used for development of *Bacillus subtilis (natto)* spores in starter cultures for commercial production of *natto* in Japan (Sulistyo et al. 1988). Nutrient broth is composed of expensive beef extract, which is not acceptable to the majority of the Hindu population in the Sikkim Himalayas, if one introduced the purified starter culture for *kinema* production. Moreover, the soybean extract after cooking soybeans is discarded during *kinema* preparation Instead of discarding the soybean extract, an attempt was made to develop it as an economialy soybean extract broth for production of *B. subtilis* spores. The load of *Bacillus subtilis* KK2:B10 was significantly ($P<0.05$) higher in soybean extract broth (3.2 x 10^8 cfu/ml) as compared to nutrient broth (0.4 x 10^8 cfu/ml) and phytone-sucrose broth (0.2 x 10^8 cfu/ml). However, there was no significant difference ($P<0.05$) in load of *B. subtilis* harvested in soybean extract as compared to soybean extract-sucrose broth. *Kinema* prepared by starter culture harvested in soybean extract broth had significantly ($P<0.05$) higher scores in all sensory attributes than those of *kinema* starter harvested in other broth media (Table 1). Hence, soybean extract after adjusting pH to 7.0 was selected as an inexpensive

TABLE 1. SENSORY SCORES OF *KINEMA* PRODUCED BY *B. SUBTILIS* KK2:B10, HARVESTED IN DIFFERENT BROTH MEDIA

Attributes	Broth			
	NB	PB	SE	SES
Flavour (20)	11.7^{bc} (11.0–12.2)	10.0^{c} (9.0–11.0)	16.2^{a} (15.5–17.0)	13.0^{b} (12.0–14.0)
Taste (20)	11.7^{b} (10.0–14.0)	10.0^{b} (9.0–11.0)	17.1^{a} (16.2–18.0)	12.0^{b} (11.0–13.0)
Stickiness (20)	12.1^{bc} (11.0–13.4)	10.0^{c} (9.0–11.0)	17.0^{a} (16.0–18.0)	13.0^{b} (12.0–14.0)
Texture (20)	11.7^{b} (10.0–14.0)	10.3^{b} (9.0–12.0)	17.2^{a} (16.5–18.0)	13.0^{b} (12.0–14.0)
Colour (20)	15.0^{b} (14.0–16.0)	12.3^{c} (12.0–13.0)	17.3^{a} (17.0–18.0)	14.3^{b} (14.0–15.0)
Total (100)	66.2^{b} (60.5–70.1)	48.4^{c} (45.0–52.4)	84.8^{a} (82.0–87.0)	65.3^{b} (60.5–69.4)

NB, nutrient broth; PB, phytone extract-sucrose broth; SES, soybean extract-sucrose; SE, soybean extract. Data represent the means of three replications. Ranges are given in parentheses. Values bearing different superscripts in each row differ significantly ($P<0.05$)

broth medium for producing *B. subtilis* spores for pure culture fermentation of *kinema* at the laboratory scale.

Ready-to-use starter culture for *kinema* production was prepared (Fig. 1). *Kinema* prepared by using *B. subtilis* KK2:B10 strain, which was harvested in soybean extract broth, was dried in an oven at 70°C for 10 h and ground aseptically to make pulverised starter. The 1% of pulverised starter (instead of *B. subtilis*, as in Fig. 1) was added aseptically to autoclaved soybeans and fermented to get *kinema* (Fig. 1, Stage A). The total viable counts of *B. subtilis* in pulverised starter were found constantly maintained at the level of ~10^9 cfu/g till 6 months. This was due to survival of endospores of *B. subtilis* for longer period at room temperature. No other microorganisms were recovered from pulverised starter kept in pre-sterile polythene bags at room temperature.

The consumers' preference trials showed that *kinema* prepared by using pulverised starter under optimized conditions was more acceptable than market *kinema*. Market *kinema* was liked extremely (score, 9) by 15%, very much (score, 8) by 30% and moderately (score, 7) by 55%, while *kinema*

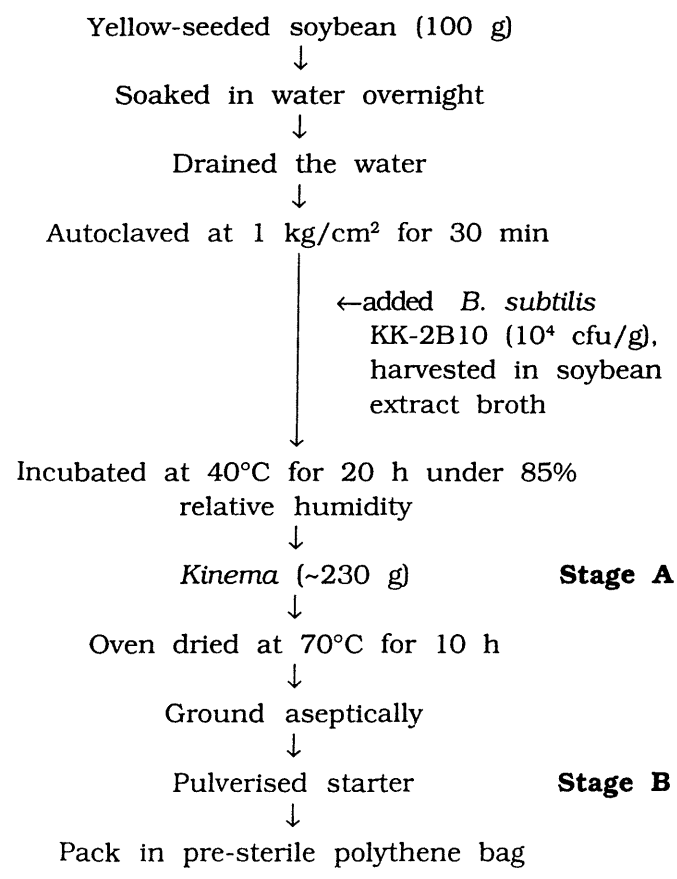

Fig. 1. Flow sheet of pulverised *kinema* starter production

TABLE 2. WATER-SOLUBLE NITROGEN AND FORMOL NITROGEN CONTENTS OF *KINEMA*

Parameter	Cooked soybean	Gangtok *kinema*[a]	Rongli *kinema*[b]	Laboratory-*kinema*[c]
Water-soluble nitrogen, % of TN	15.4 (12.2–17.0)	62.0 (54.0–68.0)	60.2 (57.0–64.0)	73.0 (68.0–78.0)
Formol nitrogen, % of TN	1.5 (1.2–1.7)	6.0 (4.0–7.2)	7.0 (4.0–8.7)	10.5 (8.5–12.5)

Data represent the means of four replications. Ranges are given in parentheses. TN, total nitrogen; [ab] *kinema* collected from Gangtok and Rongli markets in Sikkim, respectively; [c] *kinema* prepared by using pulverised starter

prepared by using pulverised starter was liked extremely by 30%, very much by 40% and moderately by 30% of the consumers. Water soluble nitrogen and formol nitrogen contents were higher in *kinema* prepared by using pulverised starter than market *kinema* (Table 2.). Increased water soluble nitrogen in *kinema* helps in digestibility and high amount of formol nitrogen, which contains free amino acid supplements that impart better taste to *kinema* (Nikkuni et al. 1995).

Application of ready-to-use pulverized starter may appear appropriate in *kinema* production at household level, since it is cost-effective. *Kinema* prepared by using pulverised starter had more advantages over traditional method due to shorter fermentation time that eliminates the chance of growth of contaminants, hygienic conditions, maintaining consistency with better quality and flavour.

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