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HACCP model of *kinema*, a fermented soybean food

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The Hazard Analysis Critical Control Points (HACCP) system basically applies on food processing to identify specific hazards and measures for their control to ensure the safety of foods. *Kinema* is a naturally fermented soybean food of the Eastern Himalayas. The present study on HACCP of *kinema* revealed that marketed *kinema* has higher microbial load as compared to the one prepared under laboratory condition and home-made *kinema*. Furthermore, the Critical Control Point (CCP) was checked during *kinema* preparation in both traditionally prepared method and *kinema* prepared under laboratory condition. HACCP model for optimised production of *kinema* has been proposed.

Keywords: Fermented foods, *Kinema*, HACCP

Introduction

Kinema is a sticky, ammonia-flavoured, naturally fermented soybean food produced by the Himalayan women of Sikkim, Darjeeling hills, Eastern Nepal and Bhutan and is eaten as curry[1]. *Bacillus subtilis* is the dominant and functional bacterium in *kinema* along with *Enterococcus faecium, Candida parapsilosis* and *Geotrichum candidum*[2,3]. The Hazard Analysis and Critical Control Points (HACCP) system is the science based and systematic which identifies specific hazards and measures for their control to ensure the safety of foods[4,5,6]. HACCP is used in the food industry to identify potential food safety hazards so that key actions called Critical Control Points (CCPs) can be taken to reduce or eliminate the risk of the hazards being realized, and the system is used at all stages of food production and preparation processes including packaging, distribution, etc[4]. HACCP was introduced to foodservice by Bill Vomvoris in 1987[5]. In 1993, the Codex Alimentarius Commission endorsed the HACCP system as the most cost-effective approach for ensuring the safety of food[7]. The aim of this study is to focus on assessment of microbiological safety and to formulate possible application of HACCP system in both traditionally prepared and laboratory-prepared *kinema*.

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Materials and Methods

Survey

Aao village, near Pakyong in East Sikkim was selected for the study. A survey was conducted in fifteen households who practices *kinema* preparation by traditional method. Most of them belong to Limboo community. They prepare *kinema* weekly and sell to the nearby market.

Sample Collection

The sample was collected from in and around Gangtok. For determination of CCP samples were obtained from different stages of *kinema* processing. Collected samples were analyzed for microbial counts. Approximately 100 g of unfermented soybean cotyledon, soaked soybean cotyledons and freshly fermented condiments were collected in sterile poly-bags and transported to laboratory for analysis. Tap water was collected from laboratory for analysis. *Kinema* was prepared under laboratory condition following the guidelines mentioned elsewhere Tamang[8].

Microbiological analysis

10 g of sample was homogenized with 90 ml of 0.85 % (w/v) sterile physiological saline in a stomacher lab-blender (400, Seward, UK) for 1 min. A serial dilution (10^{-1} to 10^{-8}) in the same diluents was made. Spore-forming bacilli were isolated on nutrient agar (MM012, HiMedia), after inactivation of vegetable cells by heating at 100 ^{0}C for 2 min[9] and then incubated at 37 ^{0}C for 24 h. Enumeration of pathogenic bacteria from the food samples was done in selective media such as *Bacillus cereus* agar base

(M833, HiMedia) for *Bacillus cereus*, Violet Red Bile Glucose agar w/o lactose (M581, HiMedia) for enterobacteriaceae[10].

Selective enumeration of *Staphylococcus aureus* was carried out on spread plates of Baird-Parker Agar Media (MM043, HiMedia), with appropriate addition of Egg Yolk Tellurite Emulsion (FD046, HiMedia)[11]. *Salmonella-Shigella* Agar (M108, HiMedia) was used for the detection of *Salmonella* and *Shigella* and *Listeria* identification agar base (M1064, HiMedia) with *Listeria* selective supplement (FD 061, HiMedia) for *Listeria* in the samples following the standard method of Metaxopolous *et al*.[11] Most Probable Number (MPN) counts of coliforms was determined as described by Harrigan[12]. The water samples were tested for MPN which included presumptive, confirmatory and completed. Microbiological data obtained were transformed into logarithms of the numbers of colony forming unit (cfu) per g of sample.

Preparation of *kinema* under laboratory condition

Kinema was prepared in laboratory using monoculture of *Bacillus subtilis* (KK-2B10), previously isolated and identified[8]. Strain of *B. subtilis* (KK-2B10) was cultured on a nutrient broth at 37°C for 24 h. Yellow varieties of soybeans (*Glycine max*) were purchased from Gangtok. A 100 g of soybean was washed and soaked overnight (~12 h) at the ratio of 1:10 (w/v). The soaked soybean was autoclaved for 45 min to soften the seeds. A 0.1ml of fresh inoculum was inoculated on autoclaved soybeans, and then slowly stirred with a sterile glass rod and incubated at 50°C for 24 h.

Preparation of *kinema* by traditional method

100 g of soybean was washed and soaked overnight (~12 h) with clean water at the ratio of 1:10 (w/v). The soaked soybean was boiled for 45 min to soften the seeds. Cooked soybeans was cracked by using pestle and added 1% of wood ash then packed in *nevara (Ficus hookeriana)* leaves and kept in warm place for 2-3 days for fermentation[1].

Identification of bacterial isolates

Initial characterization of bacterial isolates included colony and cell morphology, Gram staining and other standard biochemical tests[3]. Rapid biochemical identification test kits (HiMedia) were used to identify the bacterial isolates of *kinema*. Biolog (Biolog Inc., USA) was also used for identification.

HACCP

HACCP (Hazard Analysis and Critical Control Points) was determined following the method of Gupta *et al*.[4] with slight modification. For the purpose of this study, HACCP team was constituted which comprised individuals having adequate knowledge and work experience viz., food microbiologist, research scholars and local personnel regarding *kinema*.

Result and Discussion

Microbial load in laboratory-made and home-made *kinema* was shown in Table 1. In market sample of *kinema* the bacterial load of *E.coli* ranged from 5.2×10^3 cfu/g to 7.5×10^8cfu/g. *Enterobacteriaceae* was found with 10.1×10^5cfu/g in *kofta,* 5×10^4 cfu/g in sausage and 37.8×10^4 cfu/g in *shawerma*[13,14]. Microbial population of *Shigella* in market sample was found to be 7×10^5cfu/g to 5.2×10^7cfu/g. It was reported that *Shigella* can easily be transmitted by person to person contact, food and water to create adequate exposure for infection[15]. The microbial count of *Salmonella* in market kinema was 6×10^4 cfu/g to 7.3×10^4 cfu/. It was reported that

Table 1 - Microbial count of laboratory-made and home-made *kinema*

Sample	Lab-made *Kinema* (Monoculture)			Home-made *Kinema* (Traditionally)		
	cfu/g (x 10^4)					
	E. coli	*Staphylococcus*	*Bacillus*	*E. coli*	*Staphylococcus*	*Bacillus*
Raw soybeans	3.7	1.6	0.05	3.7	1.6	0.05
Tap Water collected from Tadong	0.01	0.02	ND	0.5	0.02	ND
Autoclaved Soybean	ND	ND	ND	ND	ND	0.02
Fresh *kinema*	0.04	300	ND	0.01	120	15

ND = not detected.
Enterobacteriaceae, Shigella, Salmonella, Listeria and *Vibrio* were not detected in any sample.
Data shows a mean average of 3 sets of experiment

60.8% of *Salmonella* spp. are the contaminating agent in retail raw foods[16]. About 10^7 -10^8 cfu/g of population of *Staphylococcus* spp was determined in market *kinema* samples. Species of *Staphylococci* survive in a wide variety of food especially those require manipulation during processing including fermented food products like cheeses[17]. The growth of *Bacillus* spp. ranged 6.0×10^4 cfu/g to 9×10^7cfu/g in market sample. *Doenjang* , one of the most common soybean fermented food of Korea was found contaminated with *B. cereus*[18]. It was mentioned that ingestation of more than 10^5 cfu of *B.cereus* per gram of food may cause food poisioning[19]. The load of *Vibrio* was 10^2 - 10^4cfu/g in market *kinema*. The present study highlighted the prevalence of higher bacterial population in market *kinema* as compared to the one prepared under laboratory condition and homemade (Fig 1). Preliminary identification of isolates was done by colony morphology, simple staining and Gram staining. The bacteria isolates were identified based on standard microbiological methods[20]. For further differentiation of isolates a rapid test was performed by using biochemical test kits. The results of test kits were compared with the given biochemical test kit chart, the isolates were 50-80% positive similarity and identified as *E.coli, Salmonella enteritidis, Shigella sonnei*, *Staphylococcus aureus*, *Staphy. chromogens, Vibrio parahaemolyticus, V. orientalis* and *V. fluvialis.* For taxonomic identification of *Bacillus* spp. from *kinema*, the methods described by Cowan[21] were followed and isolated strains were identified as *Bacillus cereus.* Unidentified isolates were inoculated at Biolog, and were identified as *Serratia liquefaciens/grimesii, Proteus penneri/vulgaris*, *Providencia rettgeri.*Multiple tube fermentation (MTF) technique was performed to detect coliforms. For presumptive test MPN index of coliforms from tap water of Tadong contained 13/100 ml of coliform with reference to standard MPN index chart which ranged from 4-35 colonies. Similarly, the water collected from Entel area of Gangtok contained 1600/100ml of coliform with respect to standard chart which had confidence limit ranging from 400-4600 colonies. Biochemical tests were performed to confirm *E. coli*[22]. It was reported that the characteristic colouration of *E.coli* colonies on EMB agar was green metallic sheen and production of gas bubbles on lactose broth indicated positive test[23]. Most of the isolates from water sample showed positive result to indole and methyl red (data not shown).

Implementation of HACCP in this investigation identified the point of contamination during processing of *kinema* and tries to reduce the contamination load in it. Only the microbiological hazards i.e. bacterial contaminations were taken for current study. During implementation of HACCP, CCP was applied at four processing steps during preparation; they are (i) raw cotyledons (ii) water (iii) during cooking (iv) freshly prepared *kinema*. Fig 2 and 3 red box indicate the possible site of contamination during *kinema* preparation at

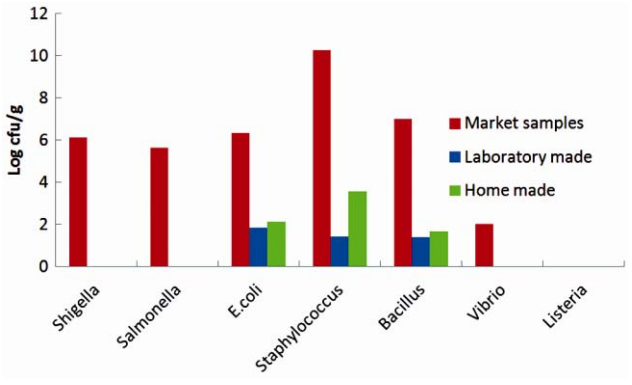

Fig 1 Graphical representation of comparison of microbial load of market, laboratory-made and home-made *kinema*.

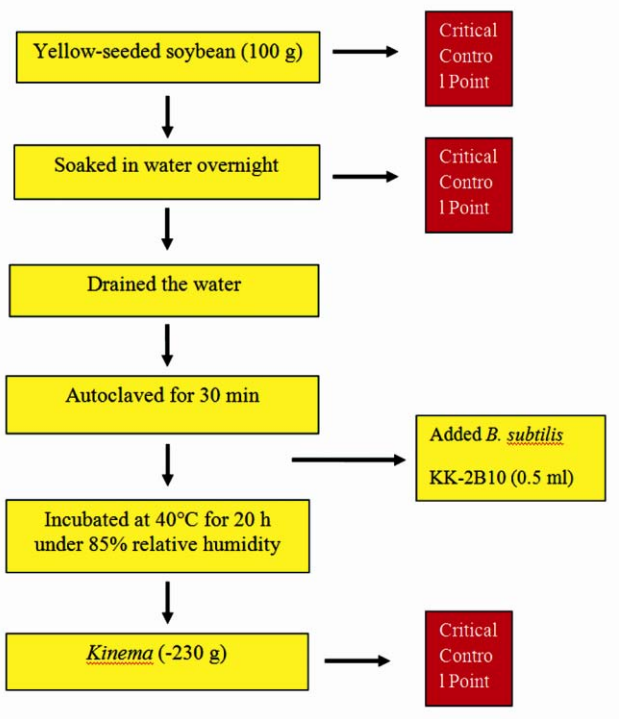

Fig 2 Determination of CCP in monoculture used *kinema* at laboratory.

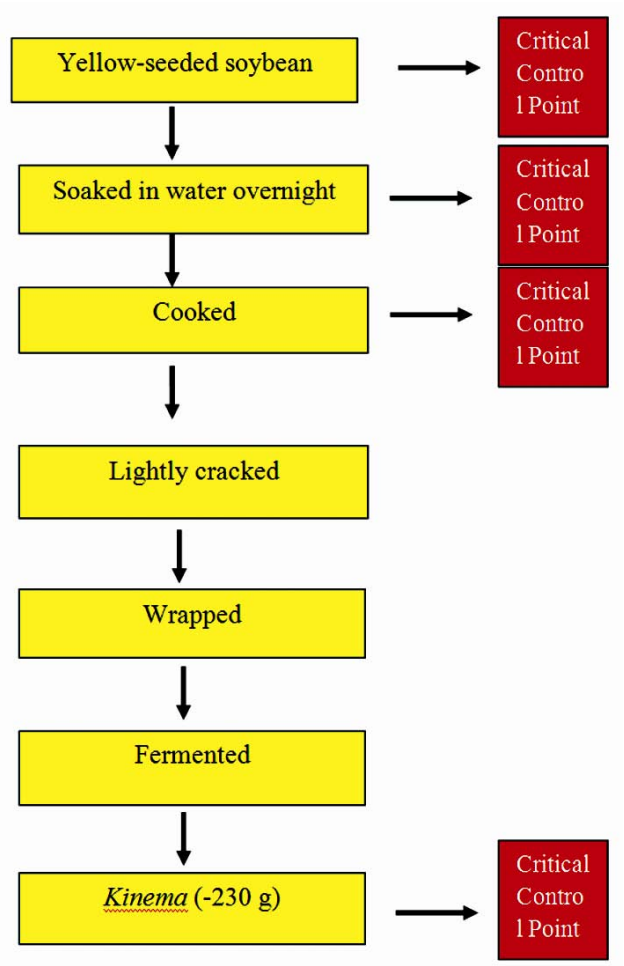

Fig 3 Determination of CCP in traditionally processed *kinema*

laboratory and at home. During analysis raw soybean was found contaminated with *Bacillus*, probably contaminated from soil. To eliminate or reduce the hazards to an acceptable level, proper cooking of soybean is required as in this study cooked cotyledons (autoclaved) did not contain any microorganism (Table 1). Similarly, tap water was found contaminated with coliform and to reduce this hazard, boiled water should be used instead of tap water. Freshly prepared *kinema* was found contaminated with *Enterobacteriaceae, Staphylococcus* and *Bacillus* (Table 1). Handling during *kinema* processing may add the microorganisms to the *kinema* sample. It was stated that personal hygiene was imperative because humans are the largest sources of contamination in food[24]. If proper handling and self hygiene is maintained throughout the processing it may help in reduction of microbial hazards to an acceptable level.

Conclusion

Implementation of HACCP during *kinema* processing may help to reduce the pathogenic load to an acceptable level. This current study was able to find four critical control points during processing and also found existence of preventive control measures. General awareness of self hygiene during preparation should provide to the handlers.

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