# COMPARATIVE STUDY OF THE KINEMA PREPARED FROM THREE VARIETIES OF SOYBEANS USING PURE CULTURE OF *BACILLUS SUBTILIS*



*by*

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# Comparative Study of the Kinema Prepared from three Varieties of Soybeans using Pure Culture of *Bacillus Subtilis*

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## Approval letter

**Dissertation entitled *Comparative Study of the Kinema Prepared from three Varieties of Soybeans Using Pure Culture of Bacillus Subtilis,* presented by *Sanjay Kumar Nepali* has been accepted as the partial fulfillment of the requirements for the degree of Bachelor in Food Technology**.

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## Abstract

The purpose of the study was to compare the three varieties of kinema in its chemical composition and organoleptic quality. The proximate composition of all the kinema samples were determined and sensory evaluation was carried out. The three varieties of soybeans (black, brown and white) were collected from the local market of Dharan. Kinema, an indigenous fermented soybean food of Nepal, was prepared by inoculating pure culture of *Bacillus subtilis* which was isolated from the old kinema sample. All the three varieties of kinema were prepared by incubating at 37°C for 48 hours. pH of all the fresh samples were determined . All the samples were dried at 60±5°C for 10 hours for its chemical analysis. Preparation of kinema, determination of chemical constituent and sensory evaluation was carried out in three different lots making gap of 15 days each in similar process and condition so as to assure the reliability of the result obtained and viability of the culture organism. Chemical analysis of kinema showed the significant increase of protein and fat in all the kinema samples. Color, flavor, texture, and overall acceptability of black kinema , brown kinema and white kinema of all the three lots were not significantly different at $p \leq 0.05$.

Table of Contents

# Part I

## INTRODUCTION

### 1.1 General background

A rich cultural background is an ornament of a society and a nation. Culture is a conglomerate of people, their glorious customs and the various rites and rituals that thrive among them, and of these, one indispensible element is the food habit they treasure.

Soybean (Glycine *max* L.) is a leguminous crop that was originated in China. Soybean is nutritively richest natural vegetable food known. Because of very high percentage of protein (40%) and fat(19%) and less carbohydrate(33.3%) than almost all pulses, the soybean is called as King of legume (Sharma,1997). The soybean has many names depending on the country where it is grown and used. It is generally reported that the name was derived from Chinese Chiang yiu which means soy sauce; in Japanese it would be pronounced Sho yu. Rather recent names include soybean, sojabean, soy, so-yu, Chinese pea, Manchurian bean and soia (Smith and Circle,1978). In Nepali, it is called Bhatmas(Katawal,1984).

Kinema is a common food of Nepal. It is traditionally prepared by natural fermentation of boiled soybeans and is covered with a sticky, colorless material accompanied by pungent odor of ammonia. The major organism responsible for kinema fermentation is *Bacillus subtilis* (Karki,1986 and Tamang and Sarkar, 1994). It resembles with other oriental fermented foods like natto of Japan, thua-nao of Thailand and tempeh of Indonesia(Tamang et al,1998 and Nikkuni et al , 1995). The other similar products are akhoni of Nagaland, troombai of Meghalaya, hawaijar of Manipur, and bekang-um of Mizoram(Tamang 1996). Consumption of kinema is done in Darjeeling, Sikkim, eastern part of Nepal and Bhutan.

Like its close companion, natto of Japan, because of its characteristic odor, slimy appearance and low appearance and low shelf life, kinema , even though it is well known in Eastern Nepal and certain parts of India, is not so popular nor so widely consumed like other fermented foods. No industrial manufacture, even at small level, of kinema has been tried. Production figure of kinema is not available. One obvious reason for limited kinema production is due to the virtual ignorance of this product. Another probable reason could be the typical ammonical odor which is not acceptable to other ethnic people living in other parts of the country (G.C, 1994).

Kinema can be prepared by traditional method using banana leaves or sal leaves or powdered straw as the source of fermenting organism mainly Bacillus subtlis. Kinema can also be made from pure culture method making the isolation of Bacillus subtilis from old kinema samples. Dhungel (2000)has concluded that kinema prepared from pure culture method is better in its quality than that prepared by traditional fermentation method. Previously , in all the studies about kinema , yellow seeded variety (white)soybean was used as the raw material. But if soybean is the raw material for kinema , question arises about the other varieties of soybean as the raw material. So this study was done so as to compare the varieties of kinema in its nutritional and sensory aspects.

**1.3 Significance of the study**

The physical and psychological consequences of child malnutrition are causing widespread concern and this concern has brought about emergency feeding programs. In many malnourished children's diet , protein is one of the major nutrients in inadequate quality and quantity. Hence , there is a global need to identify, explore and provide a practical mean to solve this problem. Traditional fermented food have a tremendous potential for alleviating protein energy malnutrition particularly in third world , as they are nutritious , cheap , and easy to prepare .

Indigenous foods are the reflection of tradition and heritage of a nation. Indigenous people had by experience and experimentation, evolved food items and techniques that were optimally suited to them. There is a lot of wisdom that underlies their practices and this warrants serious scientific study before the race for commercialization introduces pan global culture and food preferences and these indigenous foods are lost for ever.

This study will help the people for making the kinema from any variety of soybeans as per the availability. The other contemporary fermented foods like natto , tempeh and thua- nao has the large market and is eaten by many people around the globe. These products are commercialized in large scale and are major exchange of foreign currency. So, the kinema should also be made in a commercial scale so that there is access of this product to wider population inside Nepal as well as internationally. The production of kinema in large scale needs the technological innovation as well as the reasonable market for its sustenance.

## 1.3 Objectives

The objectives of this study were as follows:

### A. General objective

1. To compare the nutritional and sensory parameters of kinema prepared from different varieties of soybeans by using pure culture of *Bacillus subtilis* .

### B. Specific objectives

1. To assess the physical and chemical properties of different varieties of soybean (Black, Brown and White).
2. To assess the chemical, nutritional and sensory properties of kinema.
3. To study the fermentation pattern and compare the degree of changes in different varieties of kinema from its original source i.e. soybean.
4. To motivate the commercialization of kinema so as to consolidate its identity.

# PART II

# LITERATURE REVIEW

## 2.1. Soybean

Soybean is the most popular legume of orient. It has a long history of use in this subcontinent with records of cultivation in China as early as 2838 B.C. It is exceptionally high source of protein. The current global production of soybean during 1994-1995 was 115 million metric ton. Almost half of the world's soybean is produced by U.S.A, followed by Brazil (18%), China (11%), and Argentina (10%) (Hulse, 1996). Nepal produced 13,630 metric ton in the year 1993-1994 (Table 2.1). Whereas, the Eastern region of Nepal produced 2480 metric ton in the year 1993, 35% of total production. Soybean is the fifth most important legume in terms of acreage and mainly grown in mid hills and valleys. Both the local and improved varieties are grown (Statistical Pocket book, 2000).

In Nepal soybean is commonly known by the name 'Bhatmas'. The agriculture farms of Kumaltar, Kakani and Rampur collected 138 samples of soybeans from the different districts of height from 500 to 1800 meters and conclusion was derived that most dominant varieties of soybean in Nepal are of white, Brown, Grey and Black colors. It has different local name depending on the varieties , color of seeds and locations like Nepale , Hardi , Saathiya , Darmali, Maily , Kalo , Seto and so on( Shrestha , 1989).

## 2.2. Morphology of the soybean plant

### 2.2.1. Characteristic of the plant

Soybean plant is an erect, bushy and leafy summer annual herb that reaches a height of 25 to 50 inches. It has alternate, trifoliate leaves except the leaves of first two cotyledonary nodes. Its leaves, stems and pods are covered with grey or brown hairs. Flowers are borne in the auxiliary position and are usually either purple or white. Flower is self pollinated and has chromosome no. 40 (2n =40). The pods may be black, brown or tan in color, contains one to four seeds ranging in size 1800 to 22000 seeds per kg (Howell and Coldwell).

Fig 2.1. Mixture of soybean unshelled pods and shelled soybeans to show relative size.

Soybeans grow best in areas having hot, damp summer weather but they can be grown under a great variety of climatic conditions. The growing seasons for soybean varieties is controlled by their response to photo period and temperature. The number of days from planting to maturity can range from 80 to 180 days, depending on the varieties and environmental conditions. Photo periodism also governs the maturity of soybean crop. Yield will vary depending on the growing seasons of the variety concerned.

Soybean being a legume can obtain nitrogen needed for growth from nitrogen fixing bacterium *Rhizobium japonicum* that live in their roots. Soybean can also use the nitrogen from air and leaves in soil. Indeed, soybeans grown for beans can be used to increase nitrogen content of the soil. Mixture of soybean unshelled pods and shelled soybeans to show relative size are given in fig 2.1.

### 2.2.2. Storage and Handling

Soybean is a non perishable food, can be stored for a long period of time if the storage conditions are good. The safe moisture value for soybean storage is in the range of 9 to 13 %. There is no specific information on the effect of storage conditions on the nutritive value of the dry soybeans. However, it is concluded by Bressany et al (1963) that deterioration of the physical, chemical and organoleptical properties of the soybean seed will probably affect their nutritional quality, since dry beans stored for longer period of

time require excessive cooking or heat processing. Such deterioration can affect the protein quality of bean through a decrease in the amino acid availability (Nikkuni et al, 1995).

Proper receiving and cleaning of soybean is important in producing high quality food products. Cleaning is accompanied by magnetic separators, screening and other techniques such as air classification and mechanical separators. It is important to remove all foreign matters as much as possible in order to maintain a pure product and processing condition.

### 2.2.3. Soybean varieties and their development

Varietal development has been very important in establishing the soybean as a major crop in the United States. A primary issue in varietal development is the response of soybeans to length of the photoperiod (light availability). There are few other crops in which this response plays such a major role in limiting areas of adaptation. Varieties grown at higher latitudes have a much narrower band of adaptation than those grown at more southern latitudes. Varieties grown in Northern latitudes flower relatively early and continue to increase in height and dry matter accumulation for several weeks after flowering. These varieties are indeterminate. Determinate types of the Southern latitudes accumulate about 80% of the final dry matter by flowering time. Determinate soybeans flowers late and vegetative growth is sharply curtailed after flowering occurs.

White soybean seed Black soybean seed Brown soybean seed

**Fig 2.2**. Three varieties of soybean generally found in Nepal.

The modern improved varieties have their origin in the several thousand strains of *Glycine max,* introduced from Asia. The varieties *Dunfield* and *Illini*, which were released in the 1920s to producers in the north central China, were selection from strain introduce from Manchuria. The high oiled content of these varieties gave soybeans the impetus needed to become a commercial oilseed crop. Although both *Dunfeild* and *Illini* were tall

and susceptible to lodging, which makes for poor yields and low combining efficiency, they established the standard for oil content of all subsequent varieties.

In the early 1940s, two varieties were released and these proved to put soybean production on a good foundation. *Ogden* variety, released by the Tennessee Agricultural Experiment Station in 1942, had a 30% yield advantage over *Arksoy.* The variety Roanoke was released by the North Carolina Agricultural Experiment Station in 1946 and provided a productive high oil type for the area later in maturity than *Ogden*. Two varieties released in the early 1950s, were among the first to be selected for disease resistance. These were *Lee* selected from cross of *CNS* with *S100* and *Jackson* variety cross of *Volstate(2)* with *Palmetto*. Recent releases that provide increase efficiency of production include the Hampton*, Hardee, Dare, Davis, Bragg, Ramson and Pickett 71.* Three varieties of soybean generally found in Nepal used to make kinema is given in fig . 2.2.

Varieties develop for commercial production generally fall into four broad categories:

1. All purpose varieties – Varieties adopted over a large geographical area and imparting stability to production. Lee is a good example.
2. Specific varieties – Varieties adapted to rather restricted environments and cultural practices and where soil type, prevalent of plant pathogens and insect pest may be unique factor in production. For example, the variety *Semmes* is particularly adapted to the heavy, poorly drained soil of part of the Mississippi valley.
3. Multiple varieties – These are varieties which are similar in yield but because of differences in growth rhythm aid in averaging out production hazards. For example, they provide a range in maturity for date growers in the same geographical area.
4. Heterogeneous varieties – These are cultivars consisting of mixture of pure lines constituted on the basis of some productive interaction between the components which have some capability to adapt to a range of environments due to their heterogeneity.

### 2.3. Structure of soybean seed

Soybean seed differ in size, shape and color depending on variety, weather conditions, and geographical region in which they are produced. They range from small round beans to large oblong, flattened seed of yellow, green, brown, black and a combination of these food colors. The straw- yellow colored soybeans is a most widely grown and consumed throughout the world (Kwon and Song, 1996).

The soybean seed is consisted of three distinct parts: seed coat, hypocotyls, and cotyledons. Kawamura (1967) reported their relative amount as 8%, 2% and 90% respectively (table 2.2). The hull (seed coat) is the least valuable part of the seed due to the high percentage of cellulosic type materials. The percentage of hull varies somewhat with the size of the seed. The larger the seed lower is that the proportion of hull (Smith and Circle, 1978a).

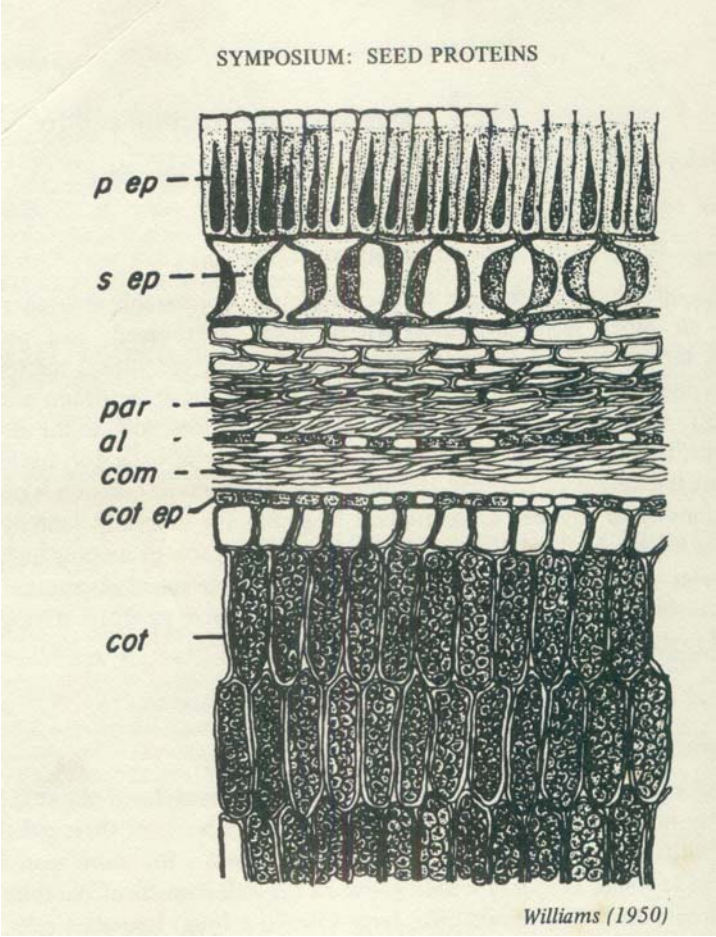



**Fig 2.3.** Cross- section of soybean coat and portion of cotyledon.

In the above figure 2.3 , p ep – Palishade cells; s ep- Hour glass cells; par –parenchyma cells; al – aleurone cells; com-compressed cells; cot ep- cotyledon epidermis ; cot- cotyledon.

The hypocotyls comprise the least amount of all, yellow in color and contain high percentage of oil. Being soybean a dicotyledon contains two cotyledons. It contains high amount of protein as well as other nutrients. So it is the most nutritious and valuable part of soybean and is used for preparing different food products. The hull is made up of an outer layer of palishade cells, smaller compressed parenchyma cells, aleurone cells and finally compressed layer of endosperm cells. The cotyledon is covered with an epidermis and

composed of numerous elongated palishade like cells which contain proteins and oils. The bulk of the proteins are stored in protein bodies which vary from 2 to 20 microns in diameter within the cotyledon cells. The oil is located in smaller structure, 0.2 to 0.5 microns, called spherosomes which are interspersed between the protein bodies (Tombs, 1967 and Wolf, 1975). Cross- section of soybean coat and portion of cotyledon is given in fig 2.3.

**2.4 Soybean production in Nepal**

**Table 2.1** Annual soybean production in Nepal

| Year | 00/01 | 01/02 | 02/03 | 03/04 | 04/05 | 05/06 |
|---|---|---|---|---|---|---|
| Production area (ha) | 20723 | 20968 | 21450 | 22073 | 22559 | 23100 |
| Production (met ton) | 17470 | 18022 | 18681 | 19363 | 19820 | 20580 |
| Yields in (kg/ha) | 843 | 860 | 871 | 877 | 879 | 891 |

Source: CBS, 2006

**2.4. Chemical composition and nutritive value**

Soybeans are well known for variation in their physical as well as chemical properties, depending on varieties and the influence of the climatic conditions in which they are grown (Smith and Circle, 1978a). The detailed composition of soybean of Nepalese variety is shown in the table 2.3. Soybean is the highest source of protein among known food sources. The major fraction of soy protein is termed globulin ( a salt soluble fraction) majority of which can be extracted with water. These are relatively insoluble in pH close to their isoelectric pH (4.5), their solubilities being highest between 2.0 to 3.5 and above 7.0 pH. Being composed of both polar and non polar amino acids, soy proteins can bind to water and to lipids and thus acts as protective colloids and emulsion stabilizers (Snyders and Kwon, 1987).

The major individual proteins of soybean are Glycinin; α, β, and Conglycinin; Hemaglutennins; Trypsin inhibitor, Kunitz trypsin inhibitor; Bowman- Birk trypsin inhibitor; Lipoxygenase ; and other enzymes (Smith and Circle, 1978a). Many of the food uses of soybean products are based on the functional properties of soybean proteins. The functional characteristics include the ability of the proteins to thicken (viscosity), emulsify, form gels, foam, produce films, absorb water and / or fat and creates meat like texturized structures ( Berk, 1992).

Snyder and Kwon (1987) stated that protein of soybean consist almost all amino acids and is a good source of essential amino acids and is a good source of essential amino acids except sulfur containing amino acids . Soybean for human food has been shown to be of highest nutritional value of all the well known plant proteins. The limiting amino acids are those containing sulfur (methionine and cysteine). They are particularly high in lysine which is a limiting amino acid in most of the cereal based diets. Therefore, soybean protein can serve as a valuable supplement to cereal foods.

The lipids of soybeans (crude soybean oil) consist typically of 96% triglycerides, 2%. 1.6% unsaponifiable matters, 0.5% free fatty acids and minute amounts of carotenoid pigments (Berk, 1992). Soybean iol consisits high amount of nutritionally significant polyunsaturated fatty acids (PUFA) such as linoleic acid 50-55%, linolenic acid 5-8% of the total lipid fraction. Soybean oil is a good source of vitamin E, especially α- tocopherol . Raw soy oil contains some β- carotene (Hulse, 1995).

The carbohydrate portion of soybean seeds is substantial, about 30% on a dry weight basis. The carbohydrates in soybean are of soluble and insoluble types. The quantities of the soluble carbohydrates in soybean seeds are of about 10%, with approximately 5% sucrose, 1% raffinose, and 4% stachyose. The latter two are not digested and absorbed as nutrients by humans. However, the intestinal flora in the human digestive tract can digest these oligosaccharides, causing flatulence (Steggarda et al., 1966). These are also responsible for the loose and malodorous stools found in infants. Soybean contains very little starch (Snyder and Kwon, 1987). The crude fiber content of soybean, especially in hull, is relatively high. Though it has no significant nutritional value, crude fiber is found to be effective in lowering plasma triglycerides and cholesterol, and also helps to evacuate the stomach and lower constipation (Kwon and Song, 1996).

Hulse (1996) mentioned that raw soybean possesses a number of anti- nutrients e.g., oligosaccharides, trypsin inhibitors, phytic acid, saponins, isoflavones, hemaglutenins, estrogenic and goitrogenic factors that cause metallic disorders in the body. Their effect is completely eliminated when the soybean components are properly heated. Soybean is hardly considered as a good source of vitamins, and particularly low in vitamin B12 and D. soybean is a rich source of minerals .recent research has shown that soybeans are rich in anti- carcinogens (Kwon and Song, 1996).The chemical composition soybean and its parts are given in table 2.2.

**Table 2.2** Composition of soybean, kinema, natto, and thua-nao

| Parameters | Kinema | Soybean | Thua-nao | Natto |
|---|---|---|---|---|
| Moisture (%) | 15.2±1.6 | 11.9 | 16.5 | 60.0 |
| Ash (%) | 6.02±0.31 | 5.47 | 5.19 | 5.35 |
| Lipid (%) | 22.7±2.3 | 17.0 | 23.0 | 21.7 |
| Protein (%) | 47.63±1.94 | 44.19 | 46.75 | 43.13 |
| Minerals, mg/100g | | | | |
| Potassium, | 1768±130 | 1936 | 1478 | 1697 |
| Magnesium | 252±19 | 240 | 240 | 221 |
| Copper | 1.71±0.18 | 1.31 | 1.48 | 1.46 |
| Iron | 17.7±3.7 | 8.7 | 11.8 | 7.2 |
| Manganese | 5.41±1.87 | 2.70 | 3.41 | 3.63 |
| Zinc | 4.52±0.71 | 3.76 | 6.13 | 4.55 |
| Sodium | 27.7±3.71 | 1.7 | 10.4 | 14.4 |
| Phosphorus | 729±84 | 721 | 778 | 731 |
| Calcium | 432±98 | 186 | 386 | 281 |
| Aminoacids,mg/100g | | | | |
| Asp | 4832±382 | 4842 | 5006 | 4335 |
| Thr* | 1761±110 | 1742 | 1849 | 1593 |
| Ser | 2081±226 | ±2304 | 2353 | 1967 |
| Glu | 9289±769 | 8525 | 8740 | 9363 |
| Gly | 2025±130 | 1923 | 2134 | 1804 |
| Ala | 2156±80 | 1920 | 2220 | 1668 |
| Val* | 2166±192 | 2054 | 2136 | 1971 |
| Cys | 501±84 | 353 | 542 | 492 |
| Met* | 393±90 | 282 | 512 | 420 |
| Ile* | 2157±171 | 2099 | 2186 | 1879 |
| Leu* | 3697±222 | 3564 | 3680 | 3300 |
| Tyr | 1978±157 | 1441 | 1894 | 1903 |
| Phe* | 2670±121 | 2455 | 2384 | 2213 |
| NH3 | 930±58 | 810 | 984 | 963 |
| Orn | 335±69 | 0 | 107 | 91 |
| Lys* | 2951±151 | 2751 | 2628 | 2699 |
| His* | 1016±41 | 1012 | 1026 | 1055 |
| Arg | 2894±273 | 3533 | 3297 | 2601 |
| Pro | 2394±145 | 2047 | 2297 | 1990 |

Data are expressed in mean ± s.d on dry matter basis. * Essential amino acids

Nikkuni *et al.,* (1995)

**Table 2.3 Composition of soybean and its parts**

| Fractons or parts | Protein (%) | Carbohydrate(%) | Lipid (%) | Ash (%) |
|---|---|---|---|---|
| Hull or seed coat (8%) | 8.8 | 86.0 | 1.0 | 4.3 |
| Cotyledon (90%) | 43.0 | 29.0 | 23.0 | 5.0 |
| Hypocotyl(2%) | 41.0 | 43.0 | 11.0 | 4.4 |
| Whole bean | 40.0 | 34.0 | 21.0 | 4.9 |

On moisture free basis, Source: Kawamura, 1967

% Protein= $N_2 \times 6.25$

**Table 2.4** Fatty acid (mg/kg) composition of soybean and kinema

| Fatty acids | Raw bean | Kinema |
|---|---|---|
| C16:0 [Palmitic acid] | 16590[288] | 17943[161] |
| C18:0 [ Stearic acid ] | 4848[58] | 5374[15] |
| C18:1 [ Oleic acid] | 31656[544] | 33856[493] |
| C18:2 [Linoleic acid]* | 87074[2303] | 92203[870] |
| C18:3 [Linolenic acid]* | 12175[337] | 12962[72] |
| C20:0 [Arachidic acid]* | 457[10] | 486[6] |

Values are the means of three replicate sets with SEM in parenthesis.

* Essential fatty acids. Source: Sarkar et al., 1996

**Table 2.5** Biological evaluation of the nutritive value of soybean as determined by rat feeding experiment

| Source of Protein | PER | BV | Digestibility | Chemical score | NPU |
|---|---|---|---|---|---|
| Raw matured soybean | 0.7 | 58 | 82 | 69 | 48-61 |
| Matured autoclaved soybean | 1.3 | 64 | 90 | | |

Source: Kuppuswamy et al (1958)

Where PER= protein efficiency ratio

BV= biological value

NPU= net protein unit

**Table 2.6** Chemical composition of soybean

| Parameter | Soybean brown | Soybean black | Soybean white |
|---|---|---|---|
| Moisture (g) | 8.1 | 12.5 | 10.2 |
| Protein (g) | 43.2 | 33.3 | 33.3 |
| Fat (g) | 19.5 | 15 | 17.7 |
| Mineral(g) | 4.6 | 4 | 5.2 |
| Fiber (g) | 3.7 | 4.3 | 4.2 |
| Carbohydrate (g) | 20.9 | 30.9 | 29.6 |
| Calcium (mg) | 432 | 395 | 400 |
| Phosphorus (mg) | 240 | 313 | 226 |
| Iron (mg) | 11.5 | 9.5 | 8.5 |
| Carotene (mg) | 426 | 10 | 10 |
| Thiamine (mg) | 0.73 | 0.65 | 0.66 |
| Riboflavin (mg) | 0.39 | 0.23 | 0.22 |
| Niacin (mg) | 3.2 | 2.8 | 2.2 |

Source: Nutrient content of Nepalese foods, 1986.

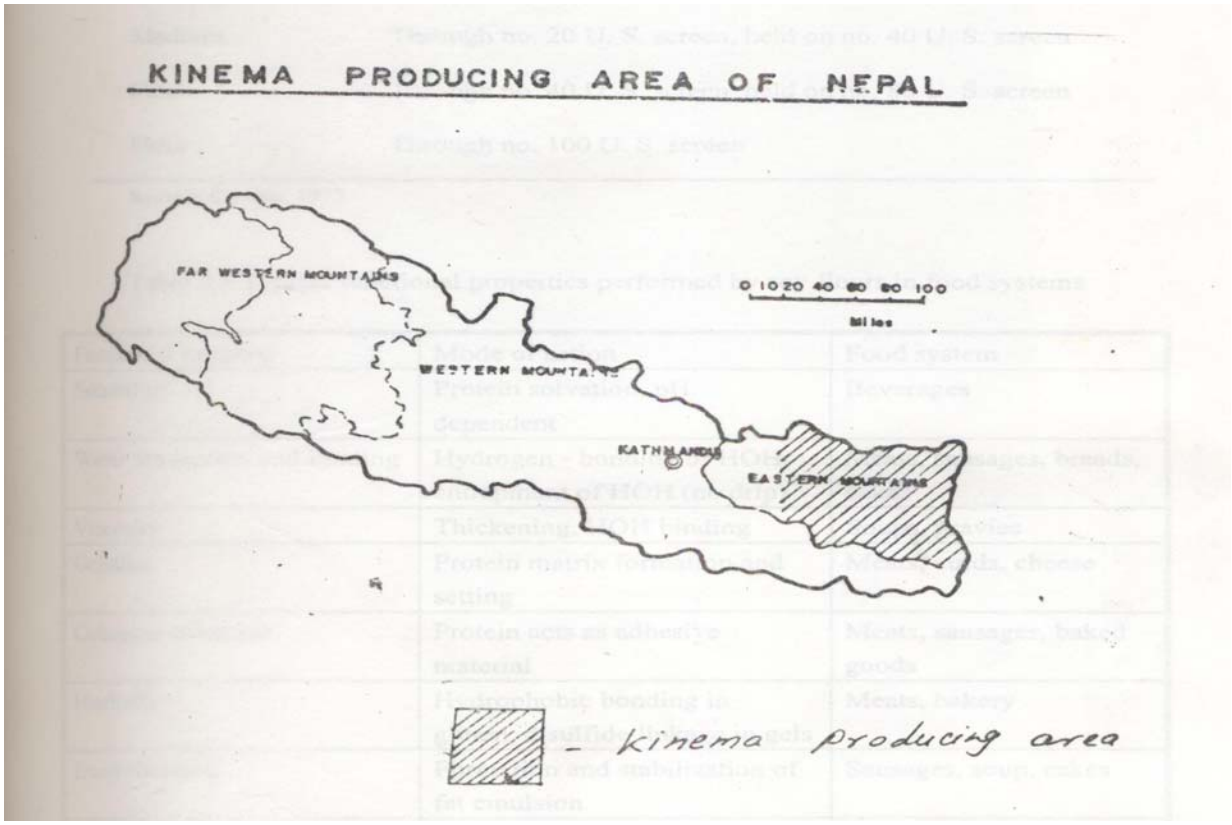



**Fig. 2.4** Kinema producing region of Nepal

## 2.5. Anti- nutritional factors of soybeans

Soybeans contain 40 % protein and 20 % oil and can solve the protein calorie mal nutrition of ever expanding population of the world. However, to make it popular as a food, it is essential that anti – nutritional factors present in soybean be eliminated and simple food products develop to suit the likings and taste of rural masses (Patil and Shukla, 1990).

Some of the important anti- nutritional factors present in soybean are protease inhibitors (Trypsin inhibitor, Kunitz inhibitors, Bowman Birk inhibitors), hemagglutinin, estrogenic factor, saponins , phytic acid and flatus factors(Rackis, 1978). Application of moist heat is very effective and will practiced method for the elimination of anti-nutritional factors of soybean (Wolf, 1978). Fermentation also improves the nutritive value of soybean (Mital and Garg, 1990).

Katawal (1984) in his dissertation work described about these anti-nutritional factor and its inactivation. His conclusion over this was as follows:

A mild heat treatment during the processing of oil meal increases the nutritional value of soybean protein. Soybean is autoclaved for 15 mins at 15 lbs pressure to inactivate the anti-nutritional factor. The importance of soybean is due to its higher protein content. So, soybean must be cooked in special way before they are eaten. A good way to eat them is to leave them to sprout in the dark before they are cooked and eaten.

## 2.6. Soybean: A multipurpose food

Soybean has been as food from the time immemorial. It is speculated that roasting or boiling were the methods first used by man to prepare soybean for eating. These methods are still practiced in many developing countries and also in Nepal. In our country, roasted soybean is eaten with roasted maize for breakfast; powdered chutney is prepared from soybean by roasting, powdering or splitting the beans and mixing with spices, chili and some sour citrus juice.

In the United States and many other countries a great interest in food uses of soybeans has been stimulated by the high nutritive value of their proteins and their high content of polyunsaturated fatty acids. Despite the great interest in soybean foods, progress in their development has been slower than anticipated. This is attributed to the characteristic flavor of most soy products which is very difficult to remove.

The flavor problem in the oriental countries was resolved through the development of fermentation products which contributed acceptable flavors to the soybean. However, the

food used in the orient has not been generally accepted in other countries and it is developed into soybean flour, concentrates and isolated with a blend flavor to obtain the wider acceptance they deserve. The bulk of world soybean crop is processed into edible oil and soybean meal. Soybean oil (refined and hydrogenated) is used for cooking, salad dressing, shortening and for making margarine. The defatted cakes are used to produce soyflour, grits, protein products like protein concentrate (more than 70% protein), protein isolate (more than 90% protein), texturized vegetable protein (30 to 50% protein), spun protein (over 90% protein), meat analogue and so on. The use of soyprotein for supplementation of cereal and other nutritional purpose is exceptional.

The following are the use of soy protein product for food:

1. Baking goods like breads, rolls, buns, doughnuts, sweets, cakes and mixes cookies and special crackers.
2. Meat products like different types of sausages, luncheon loaves, ground meats and meat analogues.
3. Breakfast cereals like infant and junior foods, confectionary items and dietary foods.
4. Vegetable toned milk.

   Due to the versatile physiochemical properties of soybean protein isolate, it is used as dough forming moisture holding, fat binding, emulsifying, foaming, film forming, thickening, stabilizing, jelling, cohesiveness and adhesiveness agent (Circle and Smith, 1978).

### 2.7. Effect of heating on soybean protein

The condition and extent of heating procedure may effect food adversely or beneficially with respect to physical and organoleptical characteristics as well as utilization of nutrient they content. The nature of these changes depend mainly on the temperature, heating time, exposure and moisture content (Ricardo et al. 1974). The heat treatment applied to soybean does improve their texture and palatability by removing the undesirable beany odor and increasing cooking quality. The denaturation of soybean protein by moist heat helps to destroy or inactivate the anti-nutritional factor present tin raw soybean.

It has been observed that maximum nutritive value of soybean protein is achieved by heat treatment with live steam for 30 minutes or by autoclaving at 15 lbs for 15-20 minutes. Heat processing can effect adversely the protein quality of bean through the chemical

reaction among the amino acid themselves or with carbohydrates (Liener, 1978). Cysteine is particularly sensitive to heat and as much as half to one- third of the cysteine content of soybean protein may be destroyed by excessive heat. Lysine not only undergoes destruction when soybean protein is overheated but much of the lysine also rendered unavailable. Besides these number of other amino acids including arginine, tryptophan, histidine and serine are either partially destroyed or inactivated by the excessive heating of soybean meal (Liener , 1978).

The heat treatment can also affect the activity of the enzymes, flavor, color, nutritional value and dispersibility of protein in water. Heat treatment done to soybean causes the denaturation of soy proteins which is responsible in functionality of soyprotein e.g. . loss in solubility , an increase in viscosity, loss of biological activity (anti-nutritional factors), changes in binding characteristics and increase susceptibility to proteolytic degradation (Lapanje , 1978). Usually, as the water content decrease, the amount of heat require to denature a protein significantly increases ( Jagerdahl and Lofquist, 1978).

**2.8. Water absorption and solubility of soy protein**

There are different factors which affect the water absorption in soybean. Hsu et al (1983) found that water uptake of soybean during soaking was influenced by seed size. Small seeds are observed to absorb more water than large size seeds and this was explained as being due to the fact that smaller kernels provide more surface area per unit mass for mass transfer. Calero et al (1981) found that the capacity to absorb in soybean was dependent on the shape and size of functional pores on the seed coat and amount of waxy materials embedded in the epidermis. Small seeds were found to have near round and functional pores whereas larger seed were found to have distorted pores due to the size effect, thus enabling small seeds to absorb more water than larger seeds. Mwandemele et al (1983) reported that the influence of temperature or seed size on water uptake was not same as all the line tested. He further found cookability to be negatively associated with water uptake during cooking. Generally small sized seed soy bean variety may help to overcome the problem of cookability encountered in soybean. Some soybean lines showed an increase in water uptake with increased seed size while other showed decrease in water uptake with increase in seed size.

Soybean proteins are sensitive to physical and chemical treatments that have little or no effect on other food components. The most important soy protein component is globulin

which are characterized by being insoluble in water at their isoelectric point (pH 4.6) but soluble in presence of salts such as sodium or calcium chloride. If the pH is above or below the isoelectric point, globulins will dissolve in aqueous solutions in the absence of salt. Thus, soy proteins have minimum solubility between the pH 3.75 and 5.25, whereas they have maximum solubility at pH 1.5. to 2.5 on the acid and above pH 6.3 on the alkaline side of their isoelectric point.

### 2.9. Fermented Soybean Foods

Fermented soybean is a common diet of oriental people for centuries. Several workers reported the beneficial effects of fermentation on soybean which are enlisted below (Snyder and Kwon, 1988; Hasseltine and Wang, 1978; Hayashi, 1961; and Marshall, 1990):

1. Proteins and carbohydrates are partially hydrolyzed into smaller units and become nutritionally available. The flavor undergoes change; derive mainly from specific microorganisms but partly from the substrate and partly from the process. It helps to manufacture products with a variety of flavors. The reduction of bitterness of soybean is an important feature of fermentation.
2. The hydrolysis of macromolecules like protein and oligosaccharides increases the digestibility, protein efficiency ratio, and biological value of the bean. This also reduces the cooking time of the products.
3. There is accumulation of B- vitamins such as folic acid, B12, and growth factors.
4. Bound minerals in raw soybean are made available by fermentation process.
5. Fermentation accompanied by heat processing inactivates or decrease the anti-nutritional and toxic factors present in raw soybean e.g., reduction of ricin, ricinoleic acid, trypsin inhibitors etc. This brings down the diarrheal effect of soy based food product.

### 2.10. Kinema

Kinema is an indigenous non salted fermented soybean food commonly consumed in eastern hilly region of Nepal and in North Eastern Hills of India bordering Nepal as a seasoning agent. Yoshida (1988) reported the origin of kinema in Southern part of China. While it spread, this food settled into a niche as seasoning in Nepal, Burma, Thailand, and Korea and as a food in Japan and Indonesia. While it is fermented food to those who eat it and may appear to be merely a “rotten bean” who don’t. This is because kinema has

powerful odor and slimy appearance like a rotten food. But to those who eat it the smell is delightful and its consistency or texture part of pleasant eating experience. It is eaten in the fresh form or as a fried curry dish along with boiled rice, and sometimes as soup, pickle, or mixed with other vegetables. It is sold in all markets of these regions by some rural women who are economically dependent upon this product (Shrestha, 1989 and Chhetri, 1994). The chemical composition of kinema is given in the table 2.3.

The method of preparation of kinema differs from home to home, depending upon the localities, family convenience and materials available. General method of household preparation involves soaking, cooking, splitting beans, mixing with firewood ash, pack in bamboo basket lined with plant leaves, and overnight fermentation in warm place. The final product has a sticky texture, typical musty flavor and a detectable ammonical odor. Kinema is considered to be of good quality if longer mycelium is formed when beans are pulled apart (Tamang, 1996 and Karki, 1986). After fermentation, fresh kinema is sun dried and stored for months. Ash is believed to increase the pH of boiled sou bean to inhibuit the growth of Bacillus and increase the undesirable organisms like fungus while facilitate the growth of Bacillus and increase the mineral level in the final product (Nikkuni et al., 1995). However, Karki(1986) and Shrestha (1989) reported no significant of ash in sensory quality of the final product. A variety of leaves e.g., banana (Musa *paradisica* (L), smith leaves (Leucosceptrum *canum*) and sal leaves (Shorea *robusta*) are used to wrapped the boiled beans before fermentation (Tamang et al., 1988).

Kinema prepared by traditional method contains a large amount of microorganisms besides Bacillus *subtilis* e.g., Enterococus *faecium*, Candida *Parapsilosis*, Geotrichum *candidum* , many yeast and mold strains in traditionally made kinema (Karki , 1986 an dSarkar and Tamang, 1994). Unhygienic method of preparation, poor storage condition, substrate itself and materials used may influence the possibility of contamination of product causing health risks. The quality of kinema also inconsistent; products differ from method of preparation, raw materials used, and person making the product. The product appearance is rough as it wrapped in plant leaves and possesses distinct odor of that leave. Whereas the kinema prepared by using the pure culture is free of these shortcomings and better in overall quality (Shrestha, 1989 and Chhetri, 1994).

### 2.10.1. Consumption pattern of kinema

Because of the unique flavor and appearance like natto and thua- nao, kinema is produced and consumed only by certain localities where its taste is favorite. It is mostly favorite among Kirati tribes who are predominant in the Eastern mid hills of Nepal. It is attaining fresh form or as fried curry dish. It is sold in all markets of these regions by some rural women who are economically dependent upon this product (Shrestha, 1989).

### 2.10.2. Physio- chemical changes during Kinema fermentation

The kinema organism produces strong proteolytic enzymes which hydrolyze the protein to peptides, amino acids, ammonia and other flavoring compounds. Proteolysis increases the solubility of protein and improves other functional properties as well. The release of ammonia increases pH from neutral to 8.0. The combined effect of higher pH and natural toxicity, ammonia and other metabolites inhibit the growth of other organisms in kinema (Karki, 1986 and Hasseltine and Wang, 1978). The sticky mucilaginous gum on the surface of the soybean substrate is impotant physical change brought about by fermenting organisms. These gummy substances are exoplysaccharides of D-isomeric glutamic acid and having structure glutamyl- glutamyl polyglutamic acid. The extent of production mucilaginous gum depends upon the strain of fermenting organism (Chhetri, 1994).

A great increase in water soluble ammonia nitrogen was noted during fermentation and storage. The amino acid composition remains same. There is significant increase in the level of thiamine, riboflavin, and vitamin $B_{12}$( Hayashi, 1959).

During traditional kinema fermentation the moisture content remain same throughout the period of fermentation (Karki, 1986). Several workers have reported a significant increase in the pH, protein, and reducing sugars and decrease in crude fiber and total sugar content (Chhetri., 1994; Tamang, 1996; and Karki, 1986). They found no significant change in the level of fat and mineral content. (Sarkar and Tamang,1994) , reported a 33 times increase in free fatty acid value in kinema as compared to raw soybean suggesting release of lipase during fermentation process.

### 2.10.3. Microbiology of kinema

*Bacillus subtilis*, Enterococcus *faecium*, Candida *parapsilosis* and Geotricum *candidum* are associated with kinema. However, *Bacillus subtilis* was found to be the sole organism in fermentation of soybean to kinema (Sarkar and Tamang , 1994) .

Kinema prepared by traditional method contains a large number of microorganism besides *Bacillus subtilis* (Karki , 1986). Unhygienic method of preparation, poor storage condition, substrate itself and material used may influence the possibility of contamination of product causing health risks. The quality of kinema also becomes inconsistent, products depending upon the method of preparation, raw material used and person making the product. The product appearance is rough as it is wrapped in plant leaves and possesses distinct odor of the leaves. Whereas, the kinema prepared by using pure culture is free of those shortcoming and better in overall quality (Shrestha, 1989 and G.C., 1994).

### 2.10.4. Bacillus subtilis

*Bacillus subtilis* is the species of a group of related strains, a type species of the genus, under the family Bacillaceae (Richard et al., 1989). It is also called hay bacillus because it is easily isolated from hay by enrichment culture (Bryan et al., 1962). It is an aerobic, endospore forming, gram positive rod. This bacteria is largely non pathogenic, grows quickly inexpensive media. When cultivated in liquid medium, organisms often accumulate on the surface giving rise to a skin or pellicle (Hugo, 1972). The colonies are irregular and have a curled or hair like structure giving what is sometimes called "Medusa head" appearance. On microscopic examination tangled coils of long chains of bacilli may be found (Burrows, 1973).

Priest (1989) reported the amylolytic, proteolytic and lipolytic (de-esterification) activity of this organism. It has ability to secrete high levels of protein into the growth medium (Ohmiza et al., 1983). Marshall (1990) reported *Bacillus subtilis* as one of few organisms that has been used to remove the bitter taste of soybean. The lack of pathogenicity among strains of *B.subtilis* of any of its close relatives has resulted Food and Drug Administration granted the organism GRAS (Generally Regarded As Safe) genus (Richard et al., 1989).

Colonies on other media are round or irregular, surface dull, become thick and opaque, may be wrinkled and may become cream colored or brown. Features of colonies vary greatly with composition of the medium. Active spreading occurs on agar with moist surface. Cell material grown on agar does not disperse readily in liquids. In 1 % glucose nutrient agar stab surface growth becomes thick often rugose and brown. A disk of reddish pigment may form below the growth. Deep growth starts but soon comes to stand still. Weak acid formation occurs to the bottom and neutralization proceeds from the top. In

broth, dull , wrinkled , coherent pellicle, little or no turbidity is seen. Energy yielding metabolism is predominantly respiratory, oxygen being the terminal electron acceptor. Anaerobically , in complex media containing glucose, growth and fermentation are weak , admission of oxygen permits abundant growth with formation of 2, 3 – butanediol , acetoin and carbon dioxide as measure products.

Pectin and polysaccharides of plant tissues are decomposed and some strains produce a rot in live potato tubers. Levan is formed extracellularly from sucrose and raffinose , the yield varying with strength. The chief endo cellular storage product is a glycogen like carbohydrate. Pigment which in particular case has been identified as pulcherrimin or melanin, may be produced in colonies or adjacent medium. In many strains they are brown or red, in fewer orange or black. Occurrence of each pigment is dependent on composition of medium. Gordon et al (1973) reported the shape of Bacillus subtilis to be rod shaped, 0.7 to 0.8 micrometer width, and 2 to 3 micrometer length.

### 2.10.5. Products similar to kinema

Kinema is an alkaline fermented food and it has great resemblance with many other fermented foods in the world. However, they are named differently. The most notable food products are natto of Japan and Thua-nao of Thailand (Tamang et al., 1988). Some other kinema like foods are Nigerian “Dawadawa”, Ivory Coast’s “Soumbara”, and West African “Iru” made by fermentation soaked, cooked locust beans (Parkia biglobosa) seeds with Bacillus. Nigerian “Ugba” , made by fermentation of the oil bean (Pentaclethra macrophylla) and Nigerian “Ogiri”, made by fermentation of castor oil bean (Ricinus communis) seeds, are also similar food products to Kinema. Malaysian also ferments locust beans to produce similar product (Chhetri, 1994).

Natto is a traditional fermented soy food of Japan. The bacteria, identified as *Bacillus subtilis* (B.natto) is the organism responsible for this fermentation. Thus, natto possesses the characteristic odor and persistent musty flavor of this organism and is also covered with the viscous, sticky polymers that this organism produces. Making natto is a simple operation, can be done at home. The chemical composition of natto is given in table no 2.3. Muto et al., (1963) tested natto as a source of protein in the diets of infants and concluded that it could substitute, at least in part, for animal protein with no adverse effects on growth, digestibility, and nitrogen retention.

Thua-nao traditional fermented soybean food of Northern Thailand. Thailand Institute of Science and Technology Research (TISTR) have developed improved method for making thua-nao using pure culture of *Bacillus subtilis* N-8. It has also used thua-nao powder with added flavoring agent to make a low cost high protein food "ferm soy mix" (Prathavat, 1986). Yaovadee et al. (1966) evaluated chemical and nutritional properties of thua-nao. They found the protein quality, in terms of digestibility, of thua-nao was better than raw and boiled bean. Other chemical constituents change only slightly. They also found vitamin $B_{12}$ in thua- nao which is almost absent in boiled soybean. The chemical and nutritional properties of soybean are given in table 2.3.

A food related to natto and kinema , called as tempeh , exist in Indonesia while the various type of natto in other countries are made by fermentation of boiled soybean with Bacillus natto , tempeh is made by allowing white mold to form around the beans . Tempeh lacks the characteristic smell and slimy consistency of ordinary natto.

**2.10.6. Technology of kinema**

Kinema is a fermented soybean food product indigenous to Nepal. It is mostly prepared and consumed by Limbus of eastern Nepal especially in the hills. The preparation is limited to household level. Methods followed for its preparation are often subjected to variation. Some of the more important factors contributing to variation are locality, convenience, availability of raw materials and processing steps. Kinema preparation is a relatively a simple process. General method of preparation of kinema as learned from kinema vendors and other experience Limbu peoples can be described in the following steps:

1. Selection of soybean: soybean with a larger seed size and brown or white color variety is preferred.
2. Cleaning: soybeans are sorted out for homogenous physical characteristics such as size, color and shape. The immature and shriveled grains with foreign materials are removed. Beans are washed in tap water.
3. Soaking: soybeans are soaked for 5 to 8 hours till the beans become soft and attains sufficient volume rise. Long time soaking should be avoided as it produces to soft bean which is unsuitable for making kinema.
4. Cooking and steaming: soybeans are steamed for 1 to 2 hours in a well covered vessel till the beans become soft enough to be splitted by the use of two fingers.

Again care must be taken up for avoiding too much cooking as it renders splitting of beans impossible. Excess water is drained off and immediately preceded for splitting.

5. Splitting of bean: steamed beans are splitted into two cotyledons with the help of pestle. It must be done at hot condition and should not involve flaking of beans. Generally, ash is incorporated at this stage. Splitting , sprinkling of ash and mixing should be done at the same time.
6. Racking: small bamboo baskets (Dalo) are covered thoroughly with green leaves of either banana or Sal. The splitted are then placed over the Dalo and covered with same variety of leaves. It is further covered by some fine cloth.
7. Fermentation: the Dalo is then placed in places inside the house such as kitchen so that the temperature during fermentation is maintained around 40oC. It should not be kept at the close space which may cause development of some unpleasant flavor in the product. The fermentation is completed between 18 to 36 hours. The completion of fermentation process is checked from time to time until a viscous product with characteristic flavor is developed. If roasted variety of kinema has to be prepared then roasted soybean is taken as raw material. The roasted soybean is cleaned, washed and soaked for 2 to 3 hours. It is followed by steaming half to one hour and splitted with or without ash. It is then fermented as before keeping it wrapped in Dalo. The kinema resulted now may have physical or sensory characteristic like beany odor, slimy texture and musty flavor as that of natto. Due to its characteristic flavor, taste and appearance which are quite rough, it is not liked by many people a restricted to only a part of Nepalese people.
8. Drying: freshly made kinema has high moisture content and is readily perishable and is consumed within 2 to 3 days after its preparation. Its shelf life can be increased by drying. In our country the village people spread the kinema on the bamboo mat (Nanglo) and dry it in sunlight or in oven (Chulo). This dried kinema bean is devoid of the unpleasant musty flavor and slimy texture but taste almost same as raw kinema when made into curry and soup.

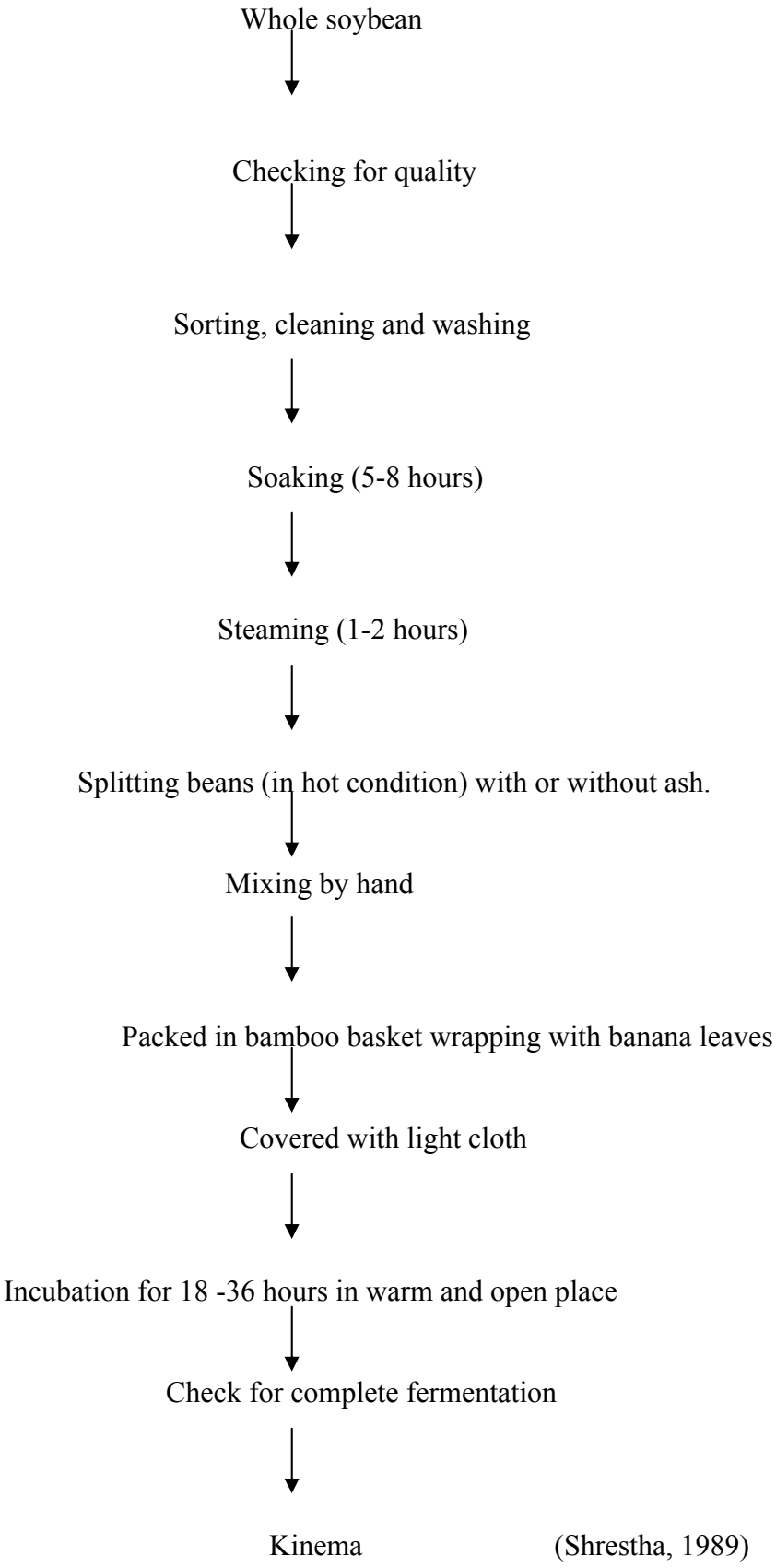
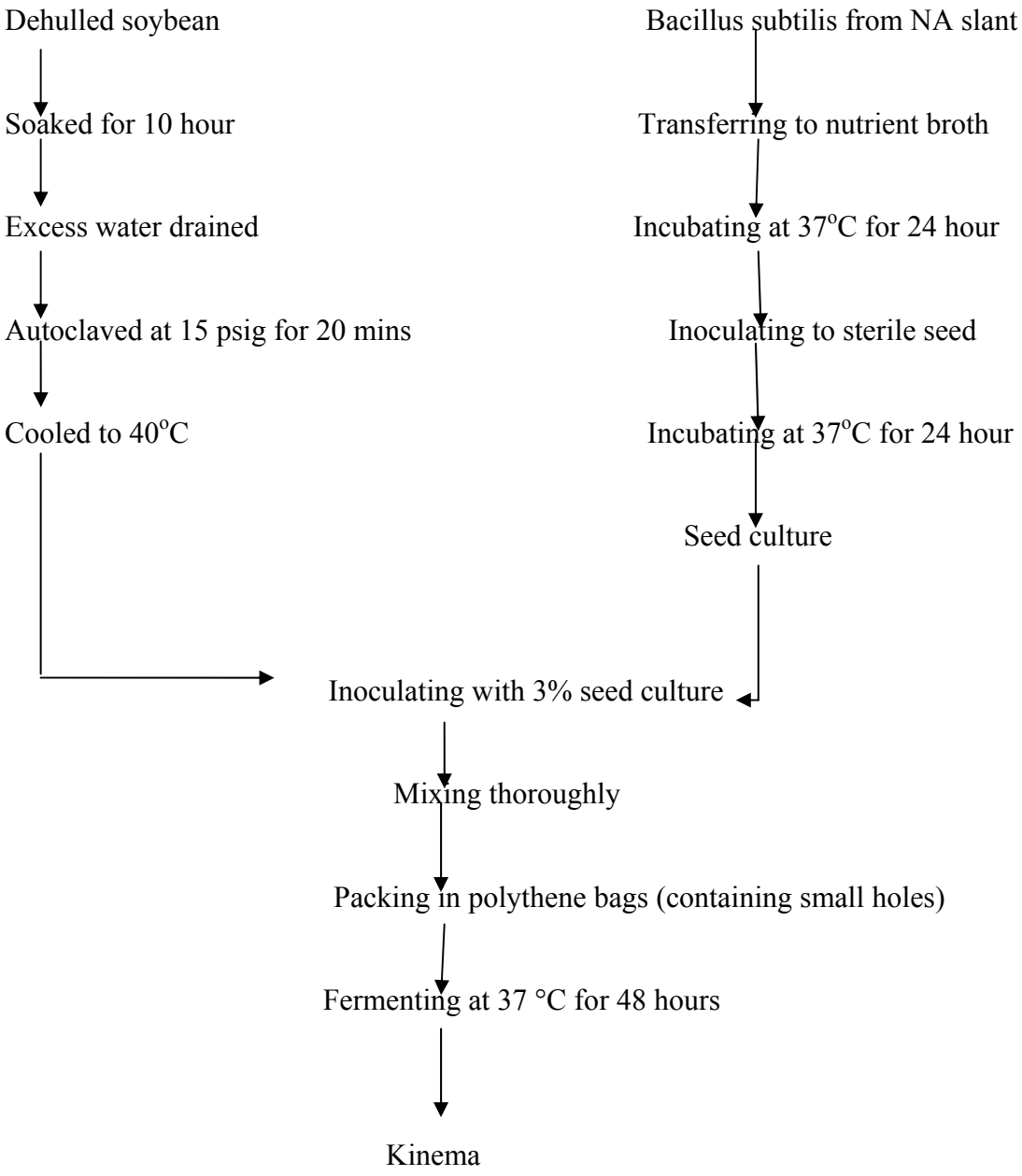



**Fig. 2.5** Flow sheet of kinema preparation by traditional method

Kinema can also be prepared by pure culture method. The method as described by Dhungel (2000) can be shown in the following flow sheet.




Source: Dhungel (2000)

**Fig 2.6** Flow sheet for preparation of kinema by pure culture method

### 2.10.6. Work carried out by others in Kinema

Shrestha (1989) made a comparative study on the preparation and quality evaluation of natto and kinema. In his study, natto was prepared in scientific way using pure culture of B. natto. While traditional method using banana leaves as the source of fermenting

organism was used for making kinema. After the statistical analysis, natto was found to be more acceptable than kinema as a food.

Dhungel (2000) made a study on quality evaluation of starter culture for kinema production. In his study kinema was prepared by traditional method using dehulled soybean of white variety. B. subtilis was isolated from this kinema. Using third pure culture of B. subtilis another lot of kinema was prepared in the laboratory. Sensory evaluation of the pure culture kinema (PCK), traditional method kinema (TMK) and market sample kinema (MSK) was carried out. Pure culture kinema was found to be significantly superior in quality ( $p<0.05$).

Moktan (2001) made a study on preparation of soy-maize kinema using pure culture of B. subtilis. In his study, kinema was prepared by inoculating pure culture of B. subtilis on several blends of soybean and maize grits. All the blends were incubated at 40°C for 48 hrs. From statistical treatment ($p<0.05$), when control (100% soybean) was excluded, kinema with 50% maize grits and 50% soybean was concluded as the best. Moisture, crude fat, crude protein, total ash, crude fiber and carbohydrate contents in the best product were 9.13%, 13.71%, 20.5%, 2.13%, 2.1% and 52.43% respectively.

# Part III

## Materials and Methods

### 3.1. Materials

#### 3.1.1. Selection of soybeans

The three varieties of soybeans i.e. black, brown and white were collected from the market (*Sabjimandi*) of Dharan. Reportedly, white variety was brought from Panmara, Sunsari; brown variety from Dandabazar, Dhankuta and black variety from Dhankuta. Soybeans were packed in polythene bag and stored until used.

#### 3.1.2. Pure culture of *Bacillus subtilis*

*Bacillus subtilis* was isolated from the previously prepared kinema sample brought from the local market of Dharan. Kinema sample was prepared from white variety of soybean by the local people of Machhabhoudi, Dharan -1. Kinema was prepared by traditional method.

### 3.2. Methodology

#### 3.2.1. Isolation of pure culture of *Bacillus subtilis*

All the glass wares required for preparation of media were cleaned with soap and brush using tap water and finally with distilled water. All the glass wares were sterilized at 15 psig for 20 minutes. For nutrient agar media, 1.25 gm peptone, 3.75 gm agar agar and 0.75 beef extract were taken in a conical flask and 250 ml distilled water was added. The mixture was heated so as to enhance the proper dissolution of media constituents. Nutrient broth (without agar agar) and nutrient agar media in conical flask were cotton plugged and autoclaved for 20 mins at 121$^{o}$C for 15 psig in autoclave (SS Scientific Service, India , Volts-230 to 440 V and frequency -60 Hz). Media was then cooled and transferred aseptically into sterile petridishes and test tubes for culturing of *Bacillus subtilis*.

Ten gram of powdered kinema sample brought from the local market of Dharan was taken in a conical flask and mixed with 250 ml of distill water. The inoculating loop (Platinum) was sterilized in a burning flame. A loopful of broth was taken and streaked on nutrient agar plate and incubated at 37$^{o}$C for 24 hour. Streaking and incubation process was repeated for obtaining a well separated and a spreading nature of colony. Then well separated and spreading nature colony was taken and prepared a stock culture in nutrient

broth by incubating at 37$^{o}$C for 24 hour. The colonies formed were streaked on nutrient agar media and NA slant and incubated at 37$^{o}$C for 24 hour.

### 3.2.2. Colonial test of *Bacillus subtilis*

Well separated colonies on nutrient agar media, which must probably originated from single cell from the stock of pure culture was taken and examined for their colonial characteristics as described by Pelczar (1999). The dimension i.e. length and breadth was determined by using ocular micrometer. The ocular micrometer is a circular disk graduated into several small parts i.e. division marked from 0 to 100. It was placed inside the eyepiece of the microscope. The distance varies according to the objective of the microscope which can be easily determined by using stage micrometer. Then, dimensions were determined by adjusting the ocular and stage micrometer (Dubey and Maheshwari, 2005).

### 3.2.3. Biochemical test of *Bacillus subtilis*

Biochemical test of *Bacillus subtilis* that were done include catalase test , Gram reaction , growth on 7% Nacl, mannitol test and anaerobic growth. All the biochemical test of *Bacillus subtilis* were carried out by the method described in Bergy's manual of determinative bacteriology (1974).

### 3.2.4. Maintenance of pure culture of *Bacillus subtilis*

The pure culture of *Bacillus subtilis* was maintained by growing on nutrient agar slant and preserving it under refrigerated condition. Successive sub culturing was carried out in each 15 days. The refrigerator (Kelvinator Refrigerator, manufactured by Kelvinator, India ltd.) used for the preservation of pure culture.

### 3.2.5. Preparation of Kinema by pure culture method:

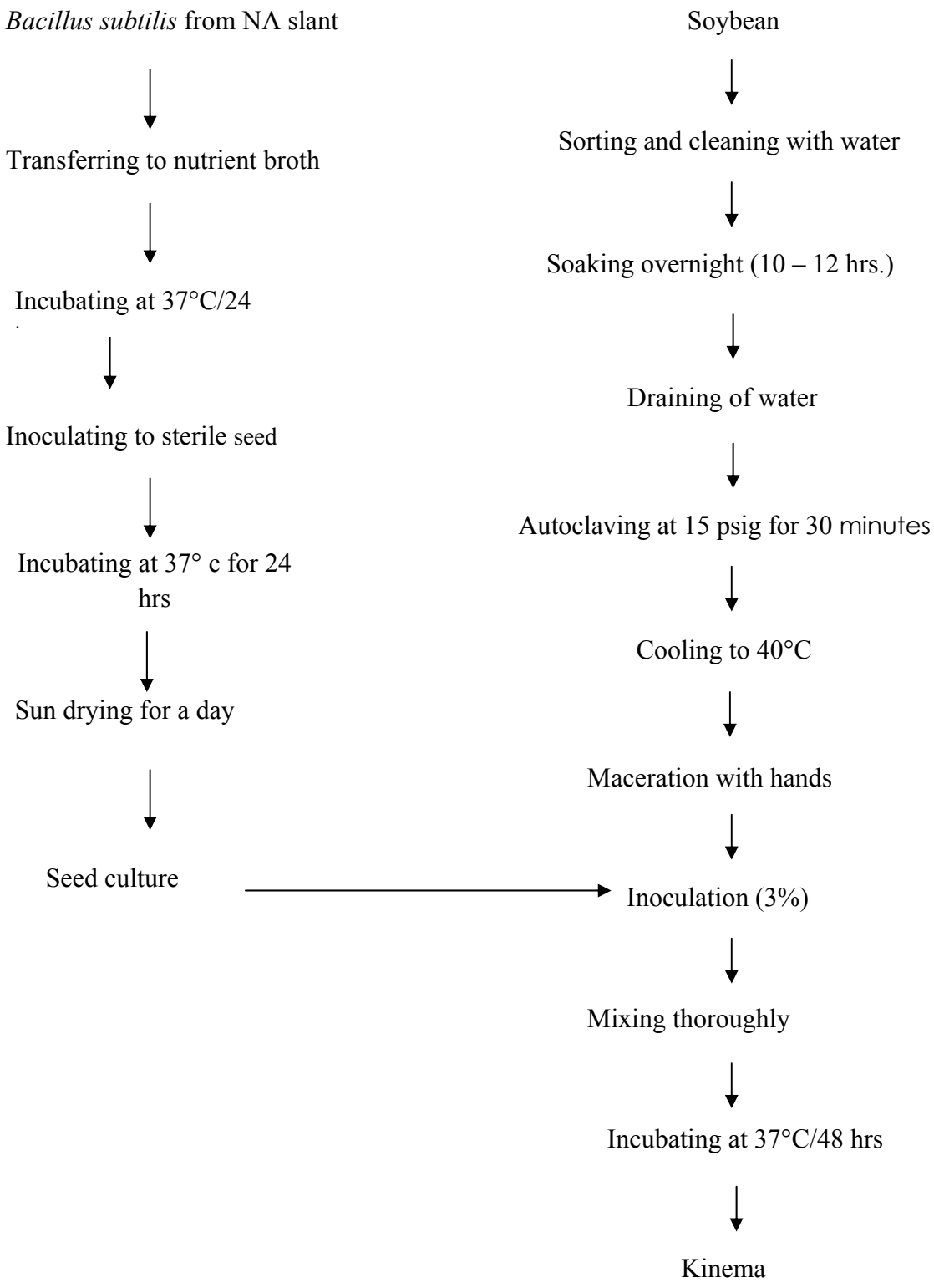



**Fig. 3.1** Flow diagram for Kinema preparation by pure culture method

### 3.2.5.1. Preparation of starter culture or seed culture

10g of kinema samples bought from the local market of Dharan was powdered and was mixed with 250ml of distilled water in a conical flask aseptically. A loopful of growth was taken and streak on nutrient agar plate. The plates were incubated at 37°C at 24 hrs in incubator (Heraeus Company). Streaking and incubation process was repeated for obtaining characteristic white, circular and spreading nature colony. To determine that colonies were that of *B. subtilis* colonial test was carried out as described by Pelczar (1999) and Dubey and Maheshwari (2005).

To confirm the colonies were that of *B. subtilis*, different biochemical test described in Bergy's Manual of Determinative Bacteriology (1974) were carried out. After the confirmation of *B.subtilis* colonies, streaking and incubation at 37°C for 24 hrs in NA slant was carried out. Then *B. subtilis* from NA slant were transferred aseptically to nutrient broth and incubation done at 37°C for 24 hrs. Soybean seed were cooked in autoclave (SS Scientific Service, Kolkata-73, India, Volt- 230 to 440 and frequency -60 Hz). Soybean seeds were macerated and inoculated with culture broth and incubation was done at 37°C for 24 hrs. Seeds were then sundried for a day covering with muslin cloth from top. Seeds were then grinded in mixer grinder (Yamaha) and were used as the seed culture.

### 3.2.5.2. Preparation of Kinema

Soybeans (Black, white and brown) were collected from the local market of Dharan. The soybeans were then sorted manually so as to remove any straw, large soil and stones, wires, metals, and other visible foreign matters. Then cleaning with water was done so as to remove any adhered dirt, dust and mud. All the varieties of soybeans were soaked overnight in water 10-12 hrs. Excess water was drained after the completion of soaking. Soaked soybeans were autoclaved at psig for 30 minutes in autoclave (Heraeus Company). The seeds were then cooled to temperature around 40°C. Seeds were then macerated with hands so as to expose the cotyledons from seed coat. Inoculation was carried out with previously prepared starter culture and mixing was done thoroughly with hands. The inoculated seeds were then incubated at 37°C for 48 hrs. After 48 hrs of fermentation kinema was ready with nutty and musty odor.

### 3.2.6. Drying of Kinema

The fresh kinema was dried in cabinet drier at 60± 5$^{o}$C for 10 hour to bring down the moisture content to about 7%. Kinema was then packed in polythene bag, sealed and stored at ambient condition.

### 3.2.7. Sensory evaluation of Kinema

The main objective of this evaluation was to compare the color, flavor, texture and overall acceptability of the kinema samples and choose the best among them. For sensory evaluation, five semi-trained panelists familiar with kinema comprising of teachers and students of CCT were selected. Sensory evaluation was done by observing and tasting the samples. Appearance, smell, and texture of raw kinema samples were evaluated. For organoleptic acceptability, all kinema samples were deep fried in soybean oil with equal proportion of spices (chili, fenugreek, onion, garlic and ginger) and salt. Hedonic rating test (Ranganna, 1999) was used to measure the panelists' acceptability of food products.

## 3.3. Analytical method

### 3.3.1. Physical properties

#### 3.3.1.1. Color, shape and surface

Color, shape and surface were determined by visual inspection method. The soybean seeds were spread on wooden tray and color, shape and surface were meticulously examined.

#### 3.3.1.2. Length /Breadth ratio

Length and breadth of soybean seed were measured by micrometer screw gauge. A replicate of 20 different samples were taken and length/breadth ratio was determined.

#### 3.3.1.3. 1000 kernels weight

1000 sound kernels were taken and weight was determined on electronic balance, model – MP 2002. Following are the specifications:
Weighing capacity – 200 gm.,Readability – 0.01 gm.,Repeatability - ≤ ±0.01 gm. Linearity - ≤ ± 0.02 gm.

#### 3.3.1.4. Bulk density

Bulk density was determined by taking the weight by soybean seeds using a 100 cc measuring cylinder.

### 3.3.2. Analysis of chemical component of soybean and kinema

#### 3.3.2.1. Moisture content

Moisture content of the sample was determined by heating in an oven (Ambassador electric oven, 966550) at 130 ± 2$^{o}$C to get constant weight (Ranganna, 1999). Weighing of the sample was done on tared petriplates using electronic balance , model – MP 2002.

#### 3.3.2.2. Crude fat content

Crude fat content of the samples was determined by solvent extraction method using Soxhlet apparatus (Corning Company) and solvent petroleum ether (Ranganna, 1999).

#### 3.3.2.3. Crude protein content

Crude protein content of the samples was determined indirectly by measuring total nitrogen content by micro Kjeldahl method. Factor 6.25 was used to convert the nitrogen content to crude protein (Ranganna, 1999).

$$\% \text{ protein} = \% \text{ nitrogen} \times 6.25$$

#### 3.3.2.4. Total ash

Total ash content of the samples was determined by following the method given by Ranganna (1999) using Ambassador Muffle furnace..

#### 3.3.2.5 Crude fiber content

Crude fiber content of the samples was determined by the method given by Ranganna (1999).

#### 3.3.2.6 pH

For the determination of pH, digital pH meter (Model 152K) was used. Soybeans were powdered into fine particles with the help of mixer grinder (Yamaha). Then solution of 25% of this fine powder was made. pH meter was then calibrated by using buffer of known pH values. A standard pH meter having glass electrode was inserted into the solution and pH was determined.

For kinema , the pH was determined directly by inserting standardized pH electrode into fresh kinema.

### 3.3.2.7. Carbohydrate

The carbohydrate content of the sample was determined by difference method (James, 1995, as cited by Shrestha, 1997) as follows:

%carbohydrate (dry basis) = 100-% protein - % fat- % ash- %crude fiber

## 3.4. Statistical analysis

To obtain the reliability of the data and assuring the viability of the culture organism, three different varieties of kinema were prepared. The chemical components of kinema were determined. After fifteen days gap of the first preparation, second lot preparation of kinema was done in the similar process and condition. Similarly, chemical properties were determined. Again after fifteen days, third lot of kinema preparation was done in the similar process and condition and chemical properties were determined. Then, the result obtained is expressed as mean of the three lot analysis. Then, sensory evaluation was done. Data of chemical composition, sensory scores and ANOVA of sensory evaluation of all the three lots are given in the appendix 1 to appendix 6.

Statistical analysis was carried out by following the method given by Gomez and Gomez, (1984) and Gupta, (1994).

# Part IV

## Result and discussion

### 4.1. Physical properties of soybean seed

It's obvious that the quality of finished product is dependent upon the quality of raw material used. Morphological analysis is a tool that provides essential information regarding the soundness of kernels. Hence, selection of suitable variety of raw materials can be done with the help of morphological analysis. Result of analysis of raw materials has been tabulated in table 4.1.

**Table 4.1** Physical properties of three varieties of soybeans

| Parameter | Black soybean | White soybean | Brown soybean |
|---|---|---|---|
| Color | Black | Yellowish white | Brown |
| Shape | Oblong | Oblong and elliptical | Spherical and oblong |
| Surface | Smooth and Glossy | Smooth | Smooth |
| Length | 8.4 mm | 8.63 mm | 8.53 mm |
| Breadth | 6.9 mm | 6.86 mm | 7.26 mm |
| L/B ratio | 1.2 | 1.26 | 1.17 |
| 1000 kernel weight | 231.3 gm | 192.33 gm | 186.67 gm |
| Bulk density | 0.81 gm/cc | 0.81 gm/cc | 0.8 gm/cc |

The L/B ratio gives the idea about shape (sphericity) of the seed (Shrestha, 1997). This ratio was found to be 1.2 for black soybean, 1.26 for white soybean and 1.17 for brown soybean. 1000 kernels weight gives the information about the size of the seed. Higher the 1000 kernel weight, greater is the size of the seed. 1000 kernel weight for black soybean was 231.3 gm, for white soybean was 192.33 gm and for brown soybean was 186.67 gm. This indicates that black soybean is greater in its size than other two varieties. The value for white soybean was quite similar, 190 gm, as determined by Dhungel (2000). The bulk density observed for black soybean, white soybean and brown soybean were 0.81 gm/cc, 0.81 gm / cc and 0.8 gm/cc respectively. The bulk density of white soybean was quite higher than that obtained by Shrestha (1989) and Dhungel (2000).

### 4.2. Test of Bacillus subtilis

**Table 4.2** Colonial test of *Bacillus subtilis*

| Parameter | Observation |
|---|---|
| Color | White |
| Shape | Circular |
| Length | 2.0437 μm |
| Breadth | 0.7846 μm |

White, circular or round colonies were observed in nutrient agar media which are the colonial characteristics of *Bacillus subtilis*. Length and Breadth of the organism determined with the help of ocular micrometer were 2.0437 μm and 0.7846 μm respectively. Gordon et al (1973) reported the length of *Bacillus subtilis* to be in the range of 2 to 3 μm and that of breadth in the range of 0.7 to 0.8 μm. So the above dimension obtained for the organism was comparatively in the range given by Gordon et al (1973).

**Table 4.3** Biochemical test of *Bacillus subtilis*

| Parameter | Observation |
|---|---|
| Catalase test | Positive |
| Gram reaction | Positive |
| Growth on 7% Nacl | Positive |
| Mannitol test | Positive |
| Anaerobic growth | Negative |

The entire biochemical test that should be done to check the confirmation of the organism *Bacillus subtilis* were obtained as the positive test. Anaerobic growth was not shown as *Bacillus subtilis* are aerobes. Stainer et al( 1987) reported that *Bacillus subtilis* unlike most of other species cannot grow anaerobically because it cannot reduce triosephosphate to glycerol and in the presence of air this species metabolizes glucose with the formation of large amount of 2, 3- butanediol. As described by Bergey's Manual of Determinative Bacteriology ($8^{th}$ edition) , *Bacillus subtilis* are Gram positive , catalase positive , shows growth on 7% NaCl, gives positive mannitol test and shows no growth in anaerobic agar.

Both colonial test and biochemical test confirms the isolated organism as *Bacillus subtilis* .

### 4.2. Chemical properties

The raw materials for the preparation of kinema were analysed and proximate composition of soybeans were comparatively equal to that given in nutrient content of Nepalese food, 1986. The proximate composition of soybeans are given in table 4.3

Three varieties of kinema were prepared from soybeans. Nutritional value and sensory property of food prepared is effected by composition of the product. Hence, the composition of kinema was determined. The analytical result of the proximate composition of the kinema is given in table 4.4.

**Table 4.4** The results of proximate analysis of the raw materials

| Parameter | Black soybean | White soybean | Brown soybean |
|---|---|---|---|
| Moisture(%) | 9.15±0.28 | 9.89±0.115 | 10.24±0.216 |
| Crude protein (%) | 38.7±0.195 | 39.2±0.177 | 40.1±0.224 |
| Crude fat (%) | 16.8±0.189 | 19.42±0.441 | 21.1±0.511 |
| Total ash (%) | 5.5±0.216 | 5.43±0.182 | 5.34±0.205 |
| Crude fiber (%) | 4.4±0.115 | 4.55±0.295 | 3.9±0.302 |
| Carbohydrate (%) | 34.6±0.451 | 31.4±0.398 | 29.56±0.315 |
| pH (uncooked) | 6.85 | 6.88 | 6.91 |

Data are expressed in mean ± s.d. on dry matter basis.

**Table 4.5** Mean chemical properties of kinema samples

| Parameter | Black kinema | White kinema | Brown kinema |
|---|---|---|---|
| Moisture (%) | 7.03±0.28 | 7.4±0.115 | 6.62±.216 |
| Crude protein (%) | 47.54±0.451 | 48±.121 | 45.94±0.273 |
| Crude fat (%) | 22.69±0.519 | 23.02±0.431 | 24.2±0.415 |
| Total ash (%) | 5.76±0.123 | 5.5±0.155 | 5.57±0.141 |
| Crude fiber (%) | 3.55±0.135 | 3.49±0.062 | 3.35±0.091 |
| Carbohydrate (%) | 20.46±0151 | 19.99±0.192 | 20.49±0.119 |
| pH (fresh) | 8.2 | 8.1 | 8.1 |

Data are expressed in mean of triplicate analysis ± s.d. on dry matter basis

The moisture content of all the varieties of kinema was reduced. The moisture of black soybean,white soybean and brown soybean were 9.15%, 9.89% and 10.14% respectively. The moisture content of black kinema, white kinema and brown kinema were 7.03 %, 7.4% and 6.62% respectively.

The pH of black,white,brown variety of kinema are 8.2, 8.1 and 8.1 respectively. The release of ammonia during fermentation increases the pH from almost neutral to 8 (Karki, 1986). This shows that there was equivalent amount of ammonia production during fermentation in all the varieties of kinema. The pH of white variety of kinema was not very much different from that obtained by Tamang (1994) ,Moktan (2001) and Dhungel (2000).

Not much significant change in ash content was observed in all the varieties of kinema .It lied almost in between that of the raw materials (Table 4.3 and 4.4) . Chhetri (1994), Tamang (1996) and Karki (1986) reported that there is no significant change in the level of mineral content. The ash content of white variety (5.5%) was not much different from that obtained 5.4% by Shrestha (1989). But it was quite lower from that obtained 5.8% by Dhungel (2000). The ash content of black and brown variety of kinema was found to be 5.76% and 5.57% respectively (Table 4.3).

Slight decrease in crude fiber content was noticed in all the varieties of kinema in comparison against the respective raw materials. According to G.C.(1994), crude fiber content decreased significantly during kinema fermentation by *Bacillus subtilis* and this decrease is governed by the enzymatic activity of the kinema fermenting organism. The decrease in the amount of crude fiber is from 4.4% in black soybean to 3.55% in black kinema, from 4.55% in white soybean to 3.49% in white kinema and from 3.9% in brown soybean to 3.35% in brown kinema( Table 4.3 and 4.4). The crude fiber content of white kinema (3.49%) was lower than that obtained by Dhungel (2000) which was 3.8%.

The crude fat content of all the varieties of kinema was higher than their respective raw materials (Table 4.3 and 4.4). Sarkar et al (1996) reported significant increase in crude fat during kinema fermentation by *Bacillus subtilis*. The increase in crude fat might be due to the active assimilation of carbohydrate and limited consumption of fat resulting in an enrichment of crude fat at the end of fermentation. There was 5.89% increase in crude fat from 16.8% in black soybean to 22.69% in black kinema , 3.6% increase in crude fat from 19.42% in white soybean to 23.02% in white kinema and 3.1% increase in crude fat from 21.1% in brown soybean to 24.2% in brown kinema. The crude fat content of white kinema

(23.02%) was lower than that obtained by Dhungel (2000) which was 24% and Shrestha (1989) which was 23.88%.

After 48 hours of fermentation, protein content in all the varieties of kinema was found to increase (Table 4.3 and 4.4). The increase in crude protein content might be due to the increased amount of nitrogen, as Hayashi (1972) has observed the nitrogen fixing capacity of *Bacillus subtilis*. Similarly Ohmiza et al (1983) reported the ability of *Bacillus subtilis* to secrete high level of protein into the growth media. G.C. (1994) reported the significant increase in crude protein during fermentation by *Bacillus subtilis*. Karki (1985) observed the increase in protein by 3 to 6% during kinema fermentation. The protein content increases from 38.7% in black soybean to 47.54% in black kinema , from 39.2% in white soybean to 48% in white kinema and from 40.1% in brown soybean to 45.94% in brown kinema ( Table 4.3 and 4.4). The protein content of white kinema was not much different from that obtained by Shrestha (1989) which was 48.94% but it was higher than that obtained by Dhungel (2000) which was 43.46%. There was significant decrease of carbohydrate in all the varieties of kinema . According to Hayashi (1959), fermentable carbohydrate of soybean almost totally disappears in 24 hours of fermentation. Total carbohydrate content of black kinema , white kinema and brown kinema were 20.46%, 19.99% and 20.49% respectively. The decrease in carbohydrate in black kinema, white kinema and brown kinema was 14.14%, 11.41% and 8.62% respectively. This shows that black and white soybean has fairly large amount of fermentable carbohydrate whereas that for the brown soybean is less.

The technology of kinema was grown and nurtured in our homeland. The technology has been so devised and developed so as to optimally suite the Nepalese status. Hence, the factor responsible for the confinement of its commercialization is not its cost , rather it is the typical flavor it possesses that governs the concept . Hence, the technology should be so evolved as to make it more appealing to the masses without much affecting its cost. An attractive packaging aided with strong promotional activities could surely help in establishing kinema as a household name and projecting it as a cosmopolitan commodity.

### 4.3. Sensory evaluation

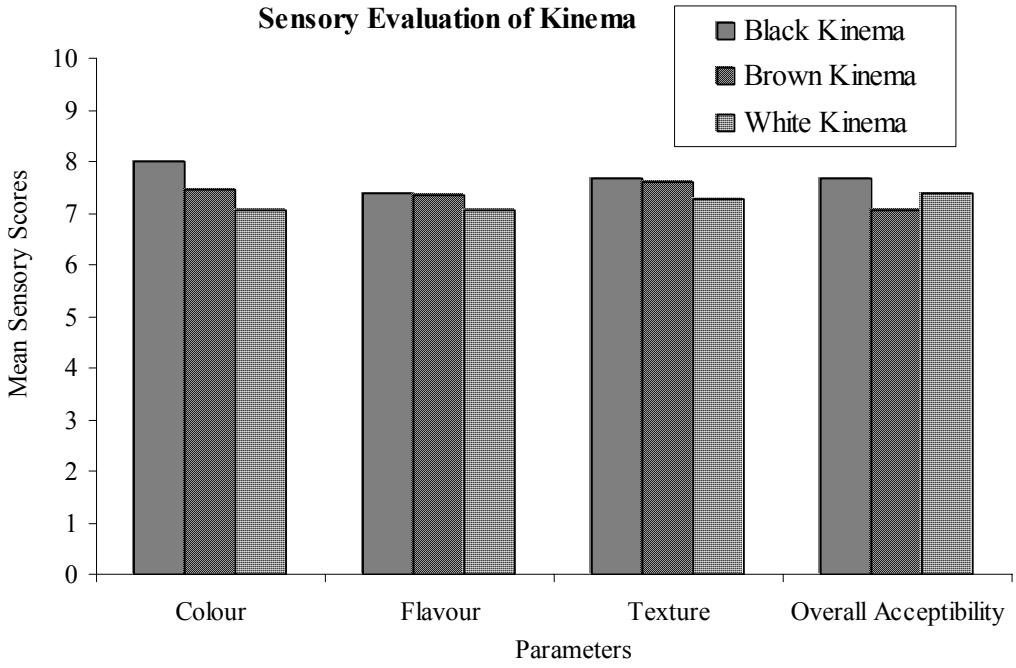



**Fig 4.1** Histogram showing the sensory evaluation of kinema

Upon sensory evaluation of kinema, color of black kinema, white kinema and brown kinema were not significantly different at $p \leq 0.05$ (Appendix 3). Black kinema was superior in color than other kinema (Fig 4.1). The mean sensory scores of black, brown and white kinema were 8, 7.43 and 7.07 respectively (Fig 4.1).

Upon sensory evaluation of kinema, flavor of black kinema, white kinema and brown kinema were not significantly different at $p \leq 0.05$ (Appendix 4). Flavor of black kinema was superior to other kinema (Fig 4.1). The mean sensory scores of black, brown and white kinema were 7.4, 7.33 and 7.07 respectively (Fig 4.1).

Upon sensory evaluation of kinema, texture of black kinema, white kinema and brown kinema were not significantly different at $p \leq 0.05$ (Appendix 5). Texture of black kinema was superior in texture to other kinema (Fig 4.1). The mean sensory scores of black, brown and white kinema were 7.67, 7.6 and 7.27 respectively.

Upon sensory evaluation of kinema, overall acceptability of black kinema, white kinema and brown kinema were not significantly different at $p \leq 0.05$ (Appendix 6). Overall acceptability of black kinema was superior to kinema (Fig 4.1). The mean sensory scores of black, brown and white kinema were 7.67, 7.073 and 7.4 respectively.

# Part V

## Conclusions and Recommendations

### 5.1. Conclusions

On the basis of study conducted, following conclusion can be drawn:

1. All the kinema from different varieties of soybean with good appearance, flavor, texture and organoleptic quality can be prepared using pure culture of *Bacillus subtilis.*
2. Maximum increase in fat and protein Percentage was shown by black kinema which is 5.89% and 8.84% respectively. The decrease in amount of carbohydrate in black kinema was 14.14% which is maximum.
3. Color, flavor, texture and overall acceptability of black kinema , brown kinema and white kinema of all the three lots were not significantly different at $p \leq 0.05$.
4. The protein content of white kinema was found to be maximum i.e. 48% in comparison against 47.54% in black kinema and 45.94% in brown kinema.

### 5.2. Recommendations

1. Optimization of time and temperature for soaking and steaming of the beans should be done to minimize the processing time and detrimental effect on quality. The standardization of process of kinema preparation by pure culture in pilot plant scale is needed to make it feasible for large scale production.
2. A combination of different time and temperature on drying of kinema should be tried to assess the effect of drying on the quality of kinema.
3. Kinema should also be studied for incorporation as a protein supplement for production of cereal based diets like macaroni, noodles, soup, bread and other baked products, infant foods and snack food.
4. Storage life of kinema should also be studied with packaging materials e.g. aluminum foils, laminated packages etc.
5. To develop the methods to make kinema more acceptable among the larger part of the population by removing its flavor especially of the odor.

# Part VI

## Summary

Kinema is an indegenous food of Nepal. It is traditionally prepared by natural fermentation of boiled soybeans and is covered with a sticky, colorless material accompanied by pungent odor of ammonia. The major organism responsible for kinema fermentation is Bacillus subtilis (Karki, 1986 and Tamang and Sarkar, 1994). Consumption of kinema is done in Darjeeling, Sikkim, Eastern Nepal and Bhutan. The major organism responsible for kinema fermentation is *Bacillus subtilis*(Karki,1986 and Tamang and Sarkar, 1994). It resembles with other oriental fermented foods like natto of Japan, thua-nao of Thailand and tempeh of Indonesia (Tamang et al, 1998 and Nikkuni et al, 1995). The other similar products are akhoni of Nagaland, troombai of Meghalaya, hawaijar of Manipur, and bekang-um of Mizoram (Tamang 1996).

Many research works in the past has been done which had tremendously helped in knowing about the basic characteristics of kinema. Dhungel( 2000) studied about the starter culture for kinema production, Shrestha (1989) carried out a comparative study of kinema and natto, Moktan (2001) studied about the blends of soy-maize kinema and again Shrestha (1997) studied about the kinema flour and its utilization. Dhungel (2000) has concluded that the kinema prepared by pure culture method was superior in quality than that produced by traditional fermentation method. Shrestha (1989) has concluded that natto was good in quality than kinema as a food. Moktan (2001) concluded that equal blends of soybean and maize used for kinema preparation gave the best quality.

In all the previous works, kinema was prepared from white variety of soybean. In my work, three different varieties of soybeans (black, brown and white) were utilized for kinema production so that comparative study could be carried out. Soybean samples were collected from the local market of Dharan. Pure culture of *Bacillus subtilis* was isolated from the old kinema sample brought from the local market of Dharan. All the varieties of soybean were soaked overnight for 10 – 12 hours, autoclaved at 15 psig for 30 minutes, cooling to around 40 $^{o}$C temperatures, maceration of beans with hands and inoculation with 3% of starter culture was done. Kinema was prepared by incubating at 37$^{o}$C for 48 hours.

After 48 hours of fermentation, kinema was dried at 60±5$^{o}$C for 10 hours to the moisture content about 7%. Kinema samples were then subjected to chemical analysis and

subsequently to sensory analysis. All the kinema samples were prepared in three different lots each differing of 15 days gap. This was done so as to obtain the reliability of the result and viability of culture organism used.

In all the varieties of kinema, there was significant increase in pH from almost neutral to 8 and protein and crude fat are also significantly increased. There was decrease in total carbohydrate and crude fiber content. The level of total ash remains similar to that of raw material. Maximum increase of crude protein and crude fat was found in black kinema which was 9% and 5.8% respectively.

From statistical analysis of sensory evaluation at $p \leq 0.05$, Color, flavor, texture and overall acceptability of black kinema, brown kinema and white kinema of all the three lots were not significantly different.

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# Appendices

**Appendix 1**

Sample A= Black kinema

Sample B= Brown kinema

Sample C = White kinema

**Appendix 2**

**Overall scoring table of sensory evaluation for kinema samples**

**A. First lot**

| Parameters | Color | | | Flavor | | | Texture | | | Overall acceptability | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples<br>Panelists | A | B | C | A | B | C | A | B | C | A | B | C |
| 1 | 8 | 9 | 7 | 7 | 8 | 7 | 8 | 8 | 7 | 8 | 7 | 7 |
| 2 | 8 | 7 | 6 | 8 | 7 | 7 | 7 | 7 | 8 | 7 | 7 | 7 |
| 3 | 9 | 8 | 7 | 8 | 9 | 7 | 9 | 8 | 7 | 8 | 7 | 6 |
| 4 | 9 | 8 | 9 | 9 | 9 | 9 | 6 | 7 | 7 | 9 | 9 | 9 |
| 5 | 8 | 9 | 8 | 9 | 8 | 8 | 8 | 8 | 7 | 9 | 8 | 8 |
| Total | 42 | 41 | 37 | 41 | 41 | 38 | 38 | 38 | 36 | 41 | 38 | 37 |
| Mean score | 8.4 | 8.2 | 7.4 | 8.2 | 8.2 | 7.6 | 7.6 | 7.6 | 7.2 | 8.2 | 7.6 | 7.4 |

**B. Second lot**

| Parameters | Color | | | Flavor | | | Texture | | | Overall acceptability | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples<br>Panelists | A | B | C | A | B | C | A | B | C | A | B | C |
| 1 | 7 | 8 | 6 | 7 | 8 | 6 | 8 | 9 | 8 | 9 | 7 | 6 |
| 2 | 8 | 8 | 7 | 7 | 8 | 7 | 7 | 6 | 7 | 7 | 8 | 7 |
| 3 | 9 | 7 | 8 | 8 | 6 | 9 | 6 | 7 | 6 | 8 | 7 | 9 |
| 4 | 7 | 6 | 7 | 6 | 6 | 6 | 9 | 8 | 7 | 7 | 6 | 7 |
| 5 | 7 | 6 | 6 | 7 | 6 | 6 | 7 | 7 | 7 | 8 | 6 | 7 |
| Total | 38 | 35 | 32 | 35 | 34 | 34 | 37 | 37 | 35 | 39 | 34 | 36 |
| Mean score | 7.6 | 7 | 6.8 | 7 | 6.8 | 6.8 | 7.4 | 7.4 | 7 | 7.8 | 6.8 | 7.2 |

**C. Third lot**

| Parameters | Color | | | Flavor | | | Texture | | | O. A | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples | A | B | C | A | B | C | A | B | C | A | B | C |
| Panelists | | | | | | | | | | | | |
| 1 | 8 | 7 | 8 | 8 | 7 | 7 | 8 | 7 | 8 | 8 | 7 | 8 |
| 2 | 8 | 7 | 7 | 7 | 7 | 8 | 9 | 9 | 8 | 8 | 7 | 8 |
| 3 | 7 | 7 | 6 | 6 | 6 | 6 | 7 | 8 | 7 | 6 | 7 | 6 |
| 4 | 8 | 7 | 7 | 6 | 6 | 7 | 8 | 8 | 7 | 7 | 6 | 8 |
| 5 | 9 | 8 | 7 | 8 | 9 | 6 | 7 | 8 | 8 | 6 | 7 | 8 |
| Total | 40 | 36 | 35 | 35 | 35 | 34 | 39 | 40 | 38 | 35 | 34 | 38 |
| Mean score | 8 | 7.2 | 7 | 7 | 7 | 6.8 | 7.8 | 8 | 7.6 | 7 | 6.8 | 7.6 |

O.A- Overall acceptability

**Appendix 3**

**Analysis of variance for difference in color for three different lots**

| Source of variation | Degree of freedom | Sum of squares | Mean squares | F- value | |
|---|---|---|---|---|---|
| | | | | Calculated | Tab(P<0.05) |
| 1. For black kinema | | | | | |
| Lot | 2 | 1.6 | 0.8 | 1.12 | 0.374 |
| Panelist | 4 | 0.6667 | 0.1667 | 0.23 | 0.912 |
| Error | 8 | 5.7333 | 0.7167 | | |
| Total | 14 | 8 | | | |
| 2. For brown kinema | | | | | |
| Lot | 2 | 4.1333 | 2.0667 | 2.82 | 0.118 |
| Panelist | 4 | 1.7333 | 0.4333 | 0.59 | 0.679 |
| Error | 8 | 5.8667 | 0.7333 | | |
| Total | 14 | 11.7333 | | | |
| 3.Forwhite kinema | | | | | |
| lot | 2 | 0.933 | 0.467 | 0.44 | 0.656 |
| Panelist | 4 | 1.6 | 0.4 | 0.38 | 0.817 |
| Error | 8 | 8.4 | 1.05 | | |
| Total | 14 | 10.933 | | | |

L.S.D . test of color for black kinema at 5% level of confidence( LSD value = 1.235)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 8.4 | a-b =0.8 <1.235 | Not significantly different |
| b (second) | 7.6 | a-c = 0.4<1.235 | Not significantly different |
| c (third) | 8 | b-c =0.6<1.235 | Not significantly different |

Conclusion : color of black kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of color for brown kinema at 5% level of confidence( LSD value = 1.249)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 8.2 | a-b =1.2 <1.249 | Not significantly different |
| b (second) | 7 | a-c = 1<1.249 | Not significantly different |
| c (third) | 7.2 | b-c =0.2<1.249 | Not significantly different |

Conclusion : color of brown kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of color for white kinema at 5% level of confidence( LSD value = 1.494)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 7.4 | a-b =0.6 <1.494 | Not significantly different |
| b (second) | 6.8 | a-c = 0.4<1.494 | Not significantly different |
| c (third) | 7 | b-c =0.2<1.494 | Not significantly different |

Conclusion : color of white kinema for first lot, second lot and third lot are not significantly different among themselves.

**Appendix 4.**

**Analysis of variance for difference in flavor for three different lots**

| Source of variation | Degree of freedom | Sum of squares | Mean squares | F- value | |
|---|---|---|---|---|---|
| | | | | Calculated | Tabulated (P<0.05) |
| 1. For black kinema | | | | | |
| Lot | 2 | 4.8 | 2.4 | 2.67 | 0.130 |
| Panelist | 4 | 1.6 | 0.4 | 0.44 | 0.774 |
| Error | 8 | 7.2 | 0.9 | | |
| Total | 14 | 13.6 | | | |
| 2. For brown kinema | | | | | |
| lot | 2 | 5.733 | 2.867 | 1.87 | 0.216 |
| Panelist | 4 | 1.333 | 0.333 | 0.22 | 0.921 |
| Error | 8 | 12.267 | 1.5333 | | |
| Total | 14 | 19.333 | | | |
| 3. For white kinema | | | | | |
| lot | 2 | 2.133 | 1.067 | 0.76 | 0.498 |
| Panelist | 4 | 1.6 | 0.4 | 0.29 | 0.879 |
| Error | 8 | 11.2 | 1.4 | | |
| Total | 14 | 14.933 | | | |

L.S.D . test of flavor for black kinema at 5% level of confidence( LSD value = 1.384)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 8.2 | a-b =1.2 <11.384 | Not significantly different |
| b (second) | 7 | a-c = 1.2< 1.384 | Not significantly different |
| c (third) | 7 | b-c =0< 1.384 | Not significantly different |

Conclusion : Flavor of black kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of flavor for brown kinema at 5% level of confidence( LSD value = 1.806)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 8.2 | a-b =1.4 <1.806 | Not significantly different |
| b (second) | 6.8 | a-c = 1.2< 1.806 | Not significantly different |
| c (third) | 7 | b-c =0.2< 1.806 | Not significantly different |

Conclusion : Flavor of brown kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of flavor for brown kinema at 5% level of confidence( LSD value = 1.806)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 8.2 | a-b =1.4 <1.806 | Not significantly different |
| b (second) | 6.8 | a-c = 1.2< 1.806 | Not significantly different |
| c (third) | 7 | b-c =0.2< 1.806 | Not significantly different |

Conclusion : Flavor of brown kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of flavor for white kinema at 5% level of confidence( LSD value = 1.726)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 7.6 | a-b =0.8 <1.726 | Not significantly different |
| b (second) | 6.8 | a-c = 0.8< 1.726 | Not significantly different |
| c (third) | 6.8 | b-c =0< 1.726 | Not significantly different |

Conclusion : Flavor of white kinema for first lot, second lot and third lot are not significantly different among themselves.

**Appendix 5.**

**Analysis of variance for difference in texture for three different lots**

| Source of variation | Degree of freedom | Sum of squares | Mean squares | F- value | |
|---|---|---|---|---|---|
| | | | | Calculated | Tabulated (P<0.05) |
| 1. For black kinema | | | | | |
| Lot | 2 | 0.4 | 0.2 | 0.13 | 0.88 |
| Panelist | 4 | 0.933 | 0.233 | 0.15 | 0.957 |
| Error | 8 | 12.267 | 1.533 | | |
| Total | 14 | 13.6 | | | |
| 2. For brown kinema | | | | | |
| lot | 2 | 0.9333 | 0.4667 | 0.48 | 0.634 |
| Panelist | 4 | 0.6667 | 0.1667 | 0.17 | 0.946 |
| Error | 8 | 7.7333 | 0.9667 | | |
| Total | 14 | 9.3333 | | | |
| 3. For white kinema | | | | | |
| lot | 2 | 0.9333 | 0.4667 | 2.15 | 0.179 |
| Panelist | 4 | 2.2667 | 0.5667 | 2.62 | 0.115 |
| Error | 8 | 1.7333 | 0.2167 | | |
| Total | 14 | 4.9333 | | | |

L.S.D . test of texture for black kinema at 5% level of confidence( LSD value = 1.806)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 7.6 | a-b =0.2<1.806 | Not significantly different |
| b (second) | 7.4 | a-c = 0.2< 1.806 | Not significantly different |
| c (third) | 7.8 | b-c =0.2< 1.806 | Not significantly different |

Conclusion :Texture of black kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of texture for brown kinema at 5% level of confidence( LSD value = 1.434)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 7.6 | a-b =0.2<1.434 | Not significantly different |
| b (second) | 7.4 | a-c = 0.4< 1.434 | Not significantly different |
| c (third) | 8 | b-c =0.6< 1.434 | Not significantly different |

Conclusion :Texture of brown kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of texture for white kinema at 5% level of confidence( LSD value = 0.679)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 7.2 | a-b =0.2<0.679 | Not significantly different |
| b (second) | 7 | a-c = 0.4< 0.679 | Not significantly different |
| c (third) | 7.6 | b-c =0.6< 0.679 | Not significantly different |

Conclusion :Texture of white kinema for first lot, second lot and third lot are not significantly different among themselves.

**Appendix 6.**

**Analysis of variance for difference in overall acceptability for three different lots**

| Source of variation | Degree of freedom | Sum of squares | Mean squares | F- value | |
|---|---|---|---|---|---|
| | | | | Calculated | Tabulated (P<0.05) |
| 1. For black kinema | | | | | |
| Lot | 2 | 3.7333 | 1.8667 | 1.96 | 0.202 |
| Panelist | 4 | 2 | 0.5 | 0.53 | 0.72 |
| Error | 8 | 7.6 | 0.95 | | |
| Total | 14 | 13.3333 | | | |
| 2. For brown kinema | | | | | |
| lot | 2 | 2.1333 | 1.0667 | 1.31 | 0.323 |
| Panelist | 4 | 0.2667 | 0.0667 | 0.08 | 0.986 |
| Error | 8 | 6.5333 | 0.8167 | | |
| Total | 14 | 8.9333 | | | |
| 3. For white kinema | | | | | |
| lot | 2 | 0.4 | 0.2 | 0.15 | 0.866 |
| Panelist | 4 | 2.267 | 0.567 | 0.41 | 0.794 |
| Error | 8 | 10.933 | 1.367 | | |
| Total | 14 | 13.6 | | | |

L.S.D . test of overall acceptability for black kinema at 5% level of confidence( LSD value = 1.422)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 8.2 | a-b =0.4<1.422 | Not significantly different |
| b (second) | 7.8 | a-c = 1.2< 1.422 | Not significantly different |
| c (third) | 7 | b-c =0.8< 1.422 | Not significantly different |

Conclusion : Overall acceptability of black kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of overall acceptability for brown kinema at 5% level of confidence( LSD value = 1.318)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 7.6 | a-b =0.8<1.318 | Not significantly different |
| b (second) | 6.8 | a-c = 0.8< 1.318 | Not significantly different |
| c (third) | 6.8 | b-c =0< 1.318 | Not significantly different |

Conclusion : Overall acceptability of brown kinema for first lot, second lot and third lot are not significantly different among themselves.

L.S.D . test of overall acceptability for white kinema at 5% level of confidence( LSD value = 1.705)

| Lots | Mean score | Difference | Remark |
|---|---|---|---|
| a (first) | 7.4 | a-b =0.2<1.705 | Not significantly different |
| b (second) | 7.2 | a-c = 0.2<1.705 | Not significantly different |
| c (third) | 7.6 | b-c =0.4< 1.705 | Not significantly different |

Conclusion : Overall acceptability of white kinema for first lot, second lot and third lot are not significantly different among themselves.

**Appendix-7. Sample card for sensory evaluation of kinema samples by Hedonic Scale**

Specimen Evaluation

Hedonic rating test

**Name:**

**Product:** **Date:**

| Parameter | A | B | C |
|---|---|---|---|
| Color | | | |
| Flavor | | | |
| Texture | | | |
| Overall acceptability | | | |

**Scale**

Like extremely- 9

Like very much-8

Like moderately-7

Like slightly-6

Neither like nor dislike- 5

Dislike slightly-4

Dislike moderately- 3

Dislike very much -2

Dislike extremely -1

**Comments** (if any):

_____

**signature**