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EFFECT OF KINEMA FLOUR ON QUALITATIVE PROPERTIES OF BISCUIT

ABSTRACT

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Kinema was prepared by using the pure culture of *Bacillus subtilis* by the laboratory and traditional method. Now both these two kinema were dried in a batch type of tray drier at (65-70)°C for (8-10) hours and prepared to a fine powder. The chemical analysis of kinema powder prepared by pure culture and traditional method was found as 6.79%, 21.45%, 42.91%, 4.84%, 24.01% and 7.218%, 20.624%, 41.99% and 4.17%, 25.998% in terms of moisture content, fat, protein, total ash, and total carbohydrate respectively. Biscuit fortified at a level of 10% kinema flour powder of particle size 200 µm prepared by both methods showed increased thickness and increased volume. Protein, fat, total ash, moisture content, total carbohydrate, acid insoluble ash and acidity of extracted fat for kinema biscuits prepared by these two methods were found as 17.438%, 12.958%, 1.377%, 4.94%, 63.287%, 0.234%, 0.501% and 17.157%, 12.458%, 1.045%, 5.00%, 64.344%, 0.248%, 0.422% respectively. Finally, kinema biscuits prepared by both these two methods showed better sensorial quality in comparison with a standard biscuit.

Contribution/Originality: Kinema is considered to be a nutritious soybean based fermented food. Kinema when processed to convert flour and incorporate in optimum quantity for biscuit formulation, significantly improves its physical, nutritional and sensorial quality. Therefore, it is a novel method for the improvement of qualitative properties of biscuit.

1. INTRODUCTION

Indigenous food fermentation is one of the oldest 'food biotechnological processes' dependent on the biological activity of microorganisms (Ross, Morgans, & Hill, 2002) from which development of fermented foods is achieved in the cultural history of human being (Geisen & Holzapfel, 1996). During the process locally available ingredient(s) either plant or animal origin are converted biochemically and organoleptically into upgraded edible products called fermented foods (Campbell, 1994) and Steinkraus (1996). A variety of fermented foods and alcoholic beverages, foods produced by microorganisms, are prepared and consumed in the Himalayan regions of India, Nepal, Bhutan and China. The culturally acceptable inexpensive foods provide a basic diet as side-dish, condiment, pickle, confectionery, alcoholic and nonalcoholic beverages, which enhance nutritional quality, palatability, wholesomeness of the product with acceptable flavor and texture. Women using their traditional knowledge of food fermentation technology usually prepare fermented foods, alcoholic and nonalcoholic beverages (Tamang, 2001). Soybean (*Glycine max* (L.) Merr.) belongs to family Leguminosae sub family Papilionoideae in India, soybean is being grown

successfully over a wide range of temperature conditions with 27°C mean maximum temperature for July and August. The soybeans grown in central India were introduced from Japan, south China, and Southeast Asia, and have distinctly different germplasm from those grown in the north (Ren, Liu, Endo, Takagi, & Hayashi, 2006). In most Asian countries, a meat diet was not common until the 19th century, except for a narrow region and an era (Ayyappa, 1997). In Eastern Asia, soybeans have been grown as one of the most important protein sources. Fermentation has been proven to be one of the best methods of improving flavor, texture and nutritional quality of soybean (Snyder & Kwon, 1987).

In Northern Thailand, fermented soybeans, the so-called “*thua nao*”, have been produced and consumed locally for several decades (Chantawannakul, Oncharoen, Klanbut, Chukeatirote, & Lumyong, 2002). Similar fermented soybean products have been described in several countries, i.e. *kinema* in India (Nout, Bakshi, & Sarkar, 1998; Sarkar, Cook, & Owens, 1993) *schuidouchi* in China (Yokotsuka, 1991) and *natto* in Japan (Ohta, 1986). Other related products (i.e. *ugba* and *iru*) made with legume seeds instead of soybeans have also been reported in Western Africa (Odunfa, 1985) and Njoku, Ogbulie, and Nnubia (1990). Several *Bacillus* species have been found to be strongly associated with these fermented soybean products. For example, Ogbadu, Okagbue, and Ahmad (1990) identified a variety of *Bacillus* species (i.e. *B. subtilis*, *B. laterosporus*, *B. pumilus*, *B. brevis*, *B. macerans*, *B. licheniformis*, *B. polymyxa*, and *B. coagulans*) from Nigerian fermented soybean foods. It should also be noted that other bacterial species such as lactic acid bacteria or *Enterococcus* species may exist (Sarkar, Tamang, Cook, & Owens, 1994).

B. subtilis, a Gram-positive, endospore forming bacteria, has usually been found as the predominant microorganism in these fermented soybean foods. The ability of *B. subtilis* to grow over a wide pH range with an active growth between (5.5 to 8.5) and to produce several enzymes (i.e. proteases) and other useful biological compounds (Sneath, 1986) seems a likely reason for its superiority in the soybean fermentation. Kinema is an indigenous traditional fermented soybean food with characteristic amour and stickiness. It is commonly consumed in the local diet as a low-cost source of high protein food by the people of the eastern Himalayan regions of the Darjeeling hills and Sikkim in India, Nepal and Bhutan. Kinema curry is delicious eaten with boiled rice in main meals. Traditionally kinema is prepared by cooking overnight-soaked whole soybeans, wrapped in leaves and fermented naturally for 1±3 day sat ambient temperature (Tamang, 1996; Tamang, Sarkar, & Heseltine, 1988). It is similar to itohiki-natto of Japan in respect of texture and aroma. The purpose of the utilization of soy products is to increase the quantity and quality of protein in food which is low in these respects.

The many applications include meat products, baked goods, infant foods, breakfast cereals, beverages, snack foods etc (Wolf & Cowan, 1971). Kinema is a high protein based product based on soybean fermentation. One of the potential use of kinema powder as well as flour is in cookies, nutro biscuits etc. Soy cookies have been developed by 25% of full fat soy flour (FFSF) to replace wheat flour. These are well accepted in the market place (Maneepaan, 1995). Kinema powder has been consumed in the form of soup along with green vegetables. To increase the acceptability of a product to the larger group of population, diversification or modification of the product is necessary. One of the best alternatives is to make it in the powder form and fortify with biscuit. Biscuit industry is growing day by day in India due to its increasing demand.

An experiment in India reported that biscuit prepared with tempeh, a similar type of fermented soy based food, showed significant improvement in health among malnourished children compared with defatted soy flour blended biscuits fed group (Vaidehi, 1995). Soy protein cookies is prepared by adding soy flour up to 25% which added up the protein content of (10- 12) % and fat content about 20 % (Vichai, 1995). Man (1996) and Arimoto (1961) reported that powdered natto at the level of 15.5% in biscuits, 20% in crackers, and 5% in the soup was acceptable to school children. The objective of my research work was to analyze the nutritional characteristics of kinema powder prepared by both pure culture and traditional methods and also to study its suitability by fortifying with wheat flour for the preparation of biscuits in context to nutritional and organoleptic characteristics.

2. MATERIALS AND METHODS

2.1. Selection of Kinema Producing Strain

Microorganisms responsible for kinema fermentation were isolated from traditional kinema and screened according to Pelczar and Reid (1972) by incubating at 37°C for (24-36) hours on the nutrient agar (Himedia M087, India) plate and nutrient broth (Himedia M088, India). Now those strains that result in desirable changes in soaked and steamed soybeans within 24 hours of incubation at 37°C were considered to be kinema-producing organisms. A loop full of the pure culture of *Bacillus subtilis* from the stock culture was aseptically inoculated into nutrient agar and kept for 24 hours at 37°C. It was further transferred to the nutrient broth and incubated under similar stationary conditions. 0.001l of cell suspension was then aseptically inoculated to 0.01kg of soaked and sterilized soybean in a 0.25l conical flask plugged with cotton and incubated at 37°C for 24 hours. Finally, this can be used as a seed culture for further study.

2.2. Preparation of Kinema from a Pure Culture

Soybean seed selected for this study was purchased from the local market of Kokrajhar, Assam, India, just adjacent to the institute. Weigh 10gm of soybeans and then sort and clean for the removal of undesirable materials. Soybean seed was then heated at 80°C for two hours. The soybean was dehulled by dehulled and the removal of husk was carried out by winnowing. Hence the dehulled soybean was soaked in excess water for about 3 hours and drained off excess water. The beans were steamed at 15lb pressure for about ½ hour, drained, and cooled at 40°C. It was transferred to a clean and wide aluminum tray and mixed with seed culture of pure *Bacillus subtilis*. The inoculated beans were then incubated at 40°C for 48 hours. Fresh kinema was prepared.

2.3. Preparation of Kinema by the Traditional Method

0.01kg of previously selected soybean was weighed and then sorted and cleaned for removing undesirable material. The pretreated soybean was heated at 80°C for two hours and dehulled. Winnowing was carried out to remove the husk. Now it was treated with boiling water for 2 hours followed by drainage of excess water and finally cooled to 40°C. The beans were wrapped, covered with clean banana leaves, and placed on the small tray. Finally, it was incubated at 40°C for 48 hours. Kinema was developed with characteristic flavor and texture.

2.4. Drying and Powdering of Kinema

Kinema was dried at (65-70)°C for about (8-10) hours till the final moisture content of the product reached about 10%. Grinding of dried kinema into a fine powder, conventionally known as kinema flour was carried out on the laboratory model disc mill.

2.5. Biscuit Formulation and Preparation

Soft wheat flour was used for this purpose. Biscuits were prepared in the food processing laboratory of the food technology department of the institute. The dough was prepared by creaming together the shortening agent as vegetable fat (0.2kg), sugar (0.3kg), sugar syrup (0.06kg), skimmed milk powder (0.02kg), glycerol monostearate (0.02kg), and soy lecithin(4gm) for (5-10) minutes in a laboratory model high speed sigma blade mixer (Reliance Industries, Kolkata, India).

Wheat flour (1kg) and the mixture of wheat flour (1kg) and kinema flour (0.150kg) blend along with baking soda (0.006kg, ammonium bicarbonate 0.916kg), common salt (0.01kg), flavoring agent as vanilla essence (0.02l) and water (0.3l) were added to the creamy mixture for the development of soft dough for standard biscuit and kinema flour fortified biscuit respectively. The final mixing will be continued till desirable consistency was obtained. The finished dough was shaped into an appropriate one by biscuit molder and baked on a laboratory-type batch bakery oven (Reliance Industries, Kolkata, India). The average temperature of baking was 160°C and the

entire operation was performed for (35-40) minutes. Finally, the biscuits were cooled to room temperature, weighed, and packed in an aseptically sealed plastic packet. Now the prepared kinema flour fortified biscuits were analyzed for physical, chemical, and sensory characteristics.

2.6. Analysis of Physical Parameters

Weight (kg), length (m), breadth (m), bulk density (kg/m^3), and the husk content (%) of selected soybean seeds were determined as averages of five replicates for each sample and the spread ratio was calculated as the diameter to thickness ratio. Physical characteristics of kinema fortified biscuits prepared by both methods were analyzed in terms of thickness (m), volume (m^3), the weight of a single biscuit, and weight/volume ratio (kg/m^3) before baking and after baking respectively.

2.7. Analysis of Chemical Parameters

All the whole soybean and dehulled soybeans in duplicate were analyzed for moisture content, crude fat (Soxhlet oil extraction apparatus, Suan Scientific Instruments co, Kolkata), and total carbohydrate content. The total ash was determined according to the method recommended by Pearson (1976). The crude protein as $\text{Nx}6.25$ was estimated by the Microkjeldahl method (AOAC Official Method, 1980).

The water absorption capacity (WAC) of both the wheat flour and kinema flour were analyzed according to Whiteley (1971). Moisture content, crude fat content, crude protein content, and total ash content were also estimated for wheat flour and kinema flour prepared by both methods. Kinema fortified biscuits prepared by both methods were also determined for moisture, crude fat, crude protein, and total ash. The acidity of the extracted fat and acid insoluble ash were determined according to Ranganna (1986). The total carbohydrate was calculated by the difference method.

2.8. Sensory Evaluation of Kinema Biscuits.

A total of twenty two panelists including faculty members and students of Central Institute of Technology Kokrajhar, Kokrajhar, Assam, India rated the biscuits for preference of color, texture, palatability, crispiness, and overall acceptability of the products. Evaluation of organoleptic properties was done by nine points hedonic rating scale from like extremely to dislike extremely according to Ihekeronye and Ngoddy (1985).

2.9. Statistical Analysis

Data were analyzed using the analysis of variance (ANOVA). The multiple range test (Duncan multiple range tests) with a significance level ($P \leq 0.05$) was applied to the results to test the significant variance (Shrestha & Noomhorm, 2002).

3. RESULTS AND DISCUSSIONS

In this study, the fermenting organism for kinema transformation was identified as *Bacillus subtilis*. A report has already been shown according to Sarkar and Tamang (1994) that the microorganism responsible for kinema fermentation was *Bacillus subtilis*. Kinema prepared from isolated strain was darker, tender, a pleasant flavor, and characteristic limy texture than prepared by traditional method.

3.1. Physical Characterization

The physical characteristic of the selected soybean seed was analyzed and the result was shown in Table 1. It was evident from the experimental study that the spread ratio was consistent for the characteristic growth of the organism. Moreover, the bulk density and weight of soybean seed were quite significant for kinema preparation.

Table 1. Physical parameters of soybean seed.

P	V
Diameter (m)	8.806 x10 ⁻³
Thickness (m)	6.130 x10 ⁻³
Spread ratio	1.314
Weight of seed (kg)	2.170 x10 ⁻³
Bulk density (m ³)	0.690 x10 ⁻⁶
Husk (%)	9.0

Note: Legend: P – parameter, V – value.

3.2. Physical Characteristics of Biscuits

The value added kinema powder fortified biscuits prepared by both these methods showed characteristic physical properties. The analytical values were shown in Table 2. Results showed that the baking process improves the thickness (m) and volume (m³) of both kinds of biscuits. Moreover, there was a significant rise in weight (kg) and volume (m³) ratio (kg/m³) of biscuits prepared by the pure culture method in comparison to the traditional method. It was observed that there is no such significant difference between the weight of biscuit, biscuit volume, and prebaking weight but the only characteristic difference in post baking weight and prebaking size prepared from traditional kinema flour and pure culture kinema flour.

Table 2. Physical analysis of kinema flour fortified biscuit.

Type of Method	T(m)	V(m ³)	W(kg)	W/V (kg/m ³)	PB (kg)	PB Size (m)	PoB (kg)
Pure culture Method	3x10 ⁻³	3.66 x10 ⁻⁶	0.67 x10 ⁻³	0.183 x10 ³	6.47 x10 ⁻³	0.0635	5.312 x10 ⁻³
Traditional Method	3x10 ⁻³	3.50 x10 ⁻⁶	0.66 x10 ⁻³	0.188 x10 ³	6.40 x10 ⁻³	0.0571	4.928 x10 ⁻³

Note: Legend: BT- biscuit type, T- thickness, PB- pre baking, PoB- post baking, v- volume, W- the weight of single biscuit, W/v- weight/volume.

3.3. Chemical Analysis of Soybean

Different forms of soybean viz. whole soybean and dehulled soybean were undergoing chemical characterization. The result was shown in Table 3. The result of the analysis revealed that dehulled soybean was significantly superior to the whole soybean due to reduced moisture content (%) but the enhanced level of protein content (%), crude fat (%), and total ash content (%). So, it was selected for kinema preparation. This study is important to evaluate the nutritional potential of two forms of soybean viz. whole and dehulled soybeans. Experimental results revealed that dehulled soybean was suitable for kinema fermentation due to enhanced nutritional potential and considered as kinema flour development for biscuit formulation in further studies.

Table 3. Chemical analysis of whole and dehulled soybean.

ST	M (%)	CP (%)	CF (%)	TA (%)	TC (%)
Whole soybean	9.389	39.620	19.063	4.420	27.508
Dehulled soybean	6.61	40.31	20.399	4.488	28.233

Note: Legend: ST- soybean type, M- moisture, CP- crude protein, CF- crude fat, TA-total ash, TC- total carbohydrate.

3.4. Chemical Characteristics of Wheat Flour and Kinema Flour

Chemical characterization of wheat and kinema flour was basically explained by the evaluation of proximate composition or nutritional profiling in terms of estimating moisture, crude fat, crude protein, total carbohydrate, total ash, or mineral contents. Wheat flour and kinema flour prepared by both methods were analyzed for chemical parameters. The result was shown in Table 4. The experimental result showed that kinema flour prepared by pure culture technique was superior in major, nutritional aspects viz. crude protein (%), crude fat (%), and total ash (%). It has been established by Nikkuni et al. (1995) that an increase of 7.8% protein level during the production of soybean fermented kinema. Reduced moisture proves its better acceptance for biscuit preparation. As nitrogen is the chief

component of organic nitrogen in protein, Sarkar and Tamang (1995) has been established an increase in nitrogen level in kinema prepared from both methods. WAI of wheat flour was found maximum in comparison to a pure culture-based kinema flour and traditional kinema flour which indicates the maximum water binding capacity. It has been reported that biscuit formation is closely correlated to the WAI of the flour (Doeschler, Hoseney, Milliken, & Rubenthaler, 1987). Sarkar, Jones, Gore, Craven, and Somerset (1996) has been established a significant rise of thirty three times in free fatty acid level (FFA) of fresh kinema as compared with crude soybean due to the liberation of lipolytic enzyme lipase during fermentative production of kinema.

Table 4. Chemical analysis of flour and kinema flour.

Flour variety	M (%)	CP (%)	CF (%)	TA (%)	TC (%)	WAI
Wheat flour	14	9.0	1.0	0.5	75.5	52.54
Kinema flour (Pure)	6.79	42.91	21.45	4.84	24.01	28.46
Kinema flour (Traditional)	7.218	41.99	20.624	4.17	25.998	29.92

Note: Legend: M- moisture, CP- crude protein, CF- crude fat, TA-total ash, TC- total carbohydrate, WAI- water absorption index (gm of water/ gm of the sample).

3.5. Chemical Characterization of Different Biscuits

Chemical characterization or evaluation of the nutritional composition of biscuits prepared by wheat flour, pure culture kinema flour, and traditional kinema flour was so significant in this study. Now, the proximate composition, as well as nutritional constituents of biscuit prepared by pure culture kinema flour, traditional kinema flour, and wheat flour, were represented in Figure 1. The higher moisture content of wheat biscuit than kinema fortified biscuit may be due to the greater water absorption index of wheat flour. The water binding abilities of wheat flour, pure culture kinema flour, and traditional kinema flour are 5.254×10^{-3} , 2.846×10^{-3} and 2.992×10^{-3} kg of water per kg of sample. The protein content of both categories of fortified biscuits was almost double that of conventional soft biscuits having about 8% protein content (Egan, Kirk, & Sawyer, 1981; Singh, Singh, & Chauhan, 2000). Biscuits from pure culture kinema flour were found to have a higher protein value because of the higher initial protein content of the original one. The acidity of the extracted fat was about 0.5% for all categories of kinema biscuits excluding wheat biscuit i.e. of 0.61%. So, there was no such significant danger of rancidity in those biscuits (Arimoto, 1961). Here the total ash and acid insoluble ash (AIA) content of standard quality biscuits were less than both those categories of kinema biscuits (Singh et al., 2000).

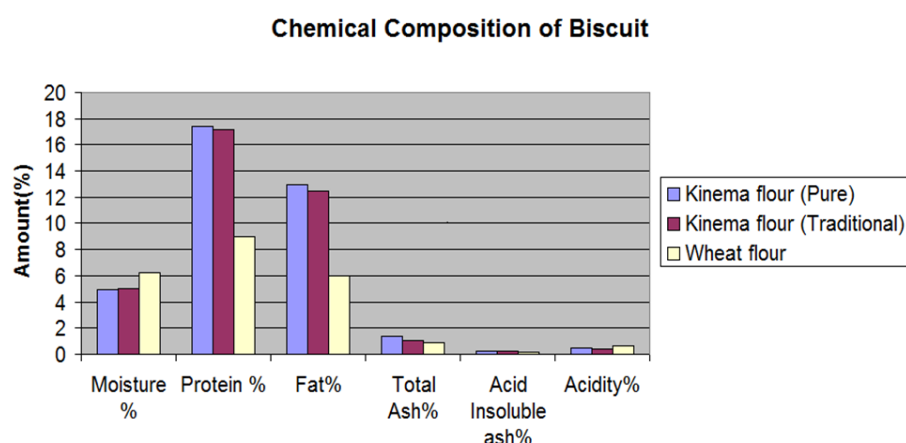


Figure 1. Comparative evaluation of chemical characteristics of kinema biscuit and standard biscuit.

3.6. Evaluation of Sensory Quality

The result of the sensory analysis based upon pure culture kinema flour, traditional kinema flour supplemented biscuits and wheat biscuits were shown in Figure 2. The crust color of biscuit prepared from pure culture kinema flour was significantly ($P \leq 0.05$) favored than that of traditional kinema flour and wheat flour. The characteristic golden brown color of both categories of biscuits was due to their high sensory scores. In addition to this, kinema

consists of a significant amount of soluble free amino acids which react with sugar fractions and can facilitate the nonenzymatic browning or Maillard reaction, resulting in the characteristic color (Sarkar, Jones, Craven, Somerset, & Palmer, 1997b). Palatability characteristic corresponds to the composite sensation of texture and flavor. The palatability of traditional kinema flour fortified biscuit was greater in score than its counterpart and wheat biscuit. A mild typical kinema aroma was reported in kinema flour supplemented biscuit. Sensory analysis showed that pure culture kinema flour fortified biscuit was significantly ($P \leq 0.05$) crisper than the other two varieties. The textural analysis of traditional kinema flour supplemented biscuit showed a higher score than its counterpart but less than wheat biscuit which proves its hard structure. The overall acceptability of pure culture kinema flour supplemented biscuit showed a significantly ($P \leq 0.05$) higher value as compared with traditional one and wheat category.

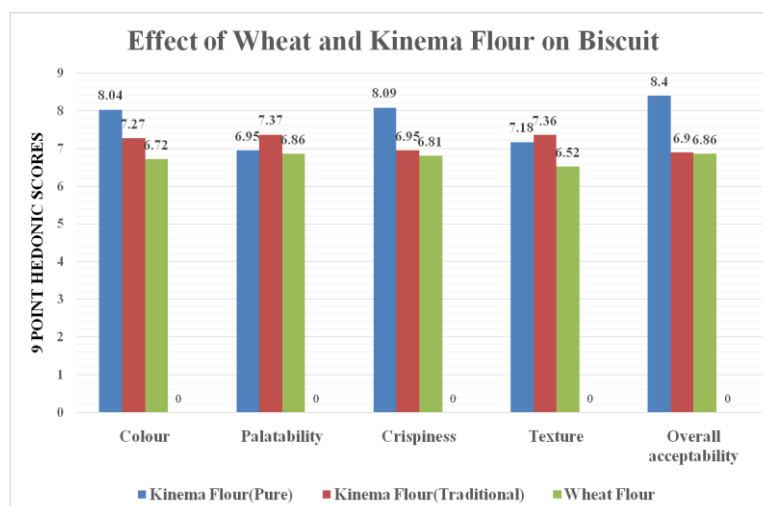


Figure 2. Sensory evaluation of wheat and kinema supplemented biscuit.

4. CONCLUSIONS

Biscuits prepared from pure culture kinema fermentation were qualitatively superior in overall acceptability than the traditional method. Fermentation carried out by *Bacillus subtilis* significantly enhances the amount of crude fat and protein content in both kinema flour supplemented biscuits varieties derived from pure culture and traditional methods. Protein content was doubled as compared with the wheat biscuit. Kinema biscuits were darker than the biscuit prepared with wheat flour. Therefore, kinema flour supplementation with wheat flour for biscuit preparation is an efficient formulation by considering its organoleptic and nutritional importance in terms of enhancement of crude fat, crude protein, and crude ash i.e. mineral content in comparison to only wheat flour (control) based biscuit.

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