Physiochemical Properties of Soy and Kinema Flours and Their Application in Formulation of High Protein Biscuits

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Abstract

Kinema was prepared by natural fermentation of soybeans. Tentatively identified Bacillus strains were isolated from the traditional product and used for making kinema in the laboratory. Kinema prepared by both methods was dried and ground to fine flour. Full fat and low fat soy flours were also prepared. Chemical analysis showed little change in protein and total fat content during fermentation, whereas free fatty acids increased substantially. Crude fiber and total sugar contents were reduced with a simultaneous increase in reducing sugars. Whereas water and oil retention capacity, gelation, foaming capacity and urease activity of kinema flours were lower than soy flours, emulsifying activity, bulk density, viscosity and particle size were higher for kinema flours. Nitrogen solubility index was high in kinema flours at 64.3% and 66.0% for traditional and pure culture kinema products, respectively. Kinema flours were significantly (P<0.05) darker, more red and yellow than soy flours. Soy and kinema flours were incorporated at 15% level into biscuits formulation. Protein content of all products was more than 17%. There was little difference in the proximate composition of the four types of biscuits prepared except for biscuits supplemented with low-fat soy flour. Incorporation of kinema contributed red and yellow colour to the corresponding biscuits. Kinema supplementation resulted in decreased hardness but increased weight and spread ratio in fortified biscuits. Evaluation of sensory characteristics showed greater acceptance of kinema-supplemented biscuits in comparison to full fat soy flour-supplemented biscuits.

Keywords: Bacillus fermentation, Chemical composition, Functional properties, Hardness, Sensory analysis

Introduction

Soybean [Glycine max (L.) Merrill] is one of the nutritionally richest natural vegetable foods known due to its high protein and oil content. However, it has little direct use because of high satiety value caused by high oil content, poor digestibility, green beany taste, long cooking time and persistent bitterness. Fermentation has been proven to be one of the best methods of improving the flavour, texture and nutritional quality of soybean (Snyder & Kwon 1987). Kinema is one of such traditional fermented soy foods prepared in the Eastern hills of Nepal, Darjeeling and Sikkim in India. It has a pungent smell of ammonia, slimy texture and short shelf life, in many ways similar to ‘natto’ of Japan and ‘thua-nao’ of Thailand (Sarkar & Tamang 1994). Traditionally kinema is prepared by washing, soaking, boiling till beans are softened, cracking, wrapping the beans with banana or fern leaves and fermenting in a bamboo basket for 1 to 2 days. Sarkar et al. (1994) and Sarkar and Tamang (1994) have investigated the effect of process variables, proximate composition and microflora of kinema and have reported that Bacillus subtilis is the most dominant fermenting organism for kinema production.

It has a greater amount of essential fatty acids, better protein quality and higher digestibility than boiled soybean (Sarkar et al. 1996, Sarkar et al. 1997a, Sarkar et al. 1997b). Natto, a Japanese counterpart of kinema, is nutritious and easily digestible with many important physiological roles in the body (Liu 1997). In Japan, dry natto powder has been successfully incorporated in biscuits, crackers and soup mix (Watanabe 1969). Fresh kinema is produced and consumed only in certain localities where its taste is favored and rarely used in any other forms. However, kinema flour has the potential to be used as a possible protein enrichment medium for cereal based-foods. The behaviour of

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kinema flours in food systems depends on their functional properties. Therefore, to be useful in food applications, data on functional, chemical and sensory properties of kinema flour are needed. The first objective of this study was to prepare kinema by traditional as well as pure culture method, study the chemical and functional properties of the dry powder, and to compare them with full fat soyflour (FFSF) and low fat soyflour (LFSF).

Efforts to increase the availability of protein in human diets have encouraged the use of high-protein plant materials, particularly soybean, as ingredients in a variety of foods. Soybean in different forums such as, flours, grits, concentrate and isolate, have partially replaced wheat flour in some baked products (Tsen et al. 1982). Fortification of wheat flour with soy proteins increases protein quality by improving amino acid profiles (Stark et al. 1975); however, accompanying adverse changes in physical qualities such as dough character and baking properties have sometimes occurred. McWatters (1978) reported that biscuits containing 10% soyflour did not differ significantly in diameter, height, spread ratio and texture values from biscuits containing 100% wheat flour. The major problems with soy-enriched biscuits is the strong and unattractive flavour that soybean imparts (Manly 1996). The second objective of this study was to prepare and compare the chemical, physical and sensory properties of kinema-supplemented biscuits in relation to soy-fortified biscuits.

**Methodology**

**Preparation of soy and kinema flours**

Seeds of an indigenous Nepalese cultivar of soybean [Glycine max (L.) cultivar ‘local yellow’] were dehulled by heating the bean at 80°C for 2 h in a cabinet drier (Reico, QID5, India), cooling, and milling in a hammer mill (Asoka 16, India) with a wider milling gap and finally winnowing. Dehulled beans were soaked for about 1 h, drained, boiled for 45 min, and dried in a cabinet drier to moisture content of about 7%. Dried beans were ground in a hammer mill to produce full fat soyflour (FFSF). Low fat soyflour (LFSF) was prepared by expressing oil from dehulled beans by using a locally made mechanical oil expeller and grinding the dried cake in a hammer mill.

Whereas traditionally kinema is prepared by using crushed whole soybeans, dehulled soybeans were used for this study because the seed coat could not be ground to a smooth flour thus affecting the texture and appearance of soy and kinema flour. Dehulled soybeans were cleaned, washed, soaked for 3 h, steamed for 2 h, drained and cracked lightly using a wooden mortar and pestle. They were then placed in a bamboo basket lined with banana leaves. The basket was covered with banana leaves and then a jute bag. It was left beside an earth oven to ferment for about 2 days. The fermented soybeans (fresh kinema) were dried in a cabinet drier at 65-70°C for 10 to 12 h to a moisture content of about 6%, and ground to smooth flour, denoted as traditional kinema flour (TKF).

**Isolation and screening of kinema producing organisms**

Microorganisms responsible for kinema fermentation were isolated from traditional kinema and screened according to Pelczar and Reid (1972). The microorganisms appearing after 24-36 h incubation at 37°C on the nutrient agar (HiMedia M087, India) plate and nutrient broth (HiMedia 088, India) were examined for their characteristics such as gram staining, spore staining, size and shape, colony and broth characteristics, and pH change (Pelczar & Reid 1972). The organisms were also tested for their fermentability by preparing kinema with soaked and steamed (sterilized) soybeans in a 250 mL flask plugged with cotton wool. Those strains that had brought desirable changes in soybeans within 24 h of incubation at 37°C were considered to be kinema producing organisms. Kinema prepared by these strains was evaluated on a rating scale for sensory qualities by a panel of six trained panelists including food scientists and technologists using a 30-point score card specially prepared for the purpose. The strain that produced kinema with the best eating quality was selected for this study (BS1, BS2...BS5). The tentatively identified microorganism was inoculated into nutrient agar and kept for 24 h at 37°C. It was then transferred to nutrient broth and incubated under similar conditions. One mL of broth containing the microorganism was then inoculated to a soaked and sterile beans (10 g) in a 250 mL conical flask, covered with cotton and fermented as before. Then kinema was prepared in the laboratory using the seed culture under controlled conditions following the method described above to yield pure culture kinema flour (PKF).

**Cookie formulation and preparation**

One lot of unbleached soft wheat cookie flour (Mahalaxmi Flour Mill, Duhabi, Nepal) was used. Other ingredients for development of the cookie
formulations were commercially available in Nepal such as wheat flour, granulated sugar, shortening, salt, baking soda, glucose syrup and water. A soft dough biscuit (cookie) was prepared by replacing commercial cookie flour with LFSF of various percentages, keeping other ingredients constant, in a laboratory baking oven (Remi, India). The formulation that used 15% LFSF flour was chosen because biscuits containing more than 15% LFSF flour were tough with a strong beany odor.

Biscuits were prepared in a medium scale biscuit factory in Dharan, Nepal. The dough was prepared by creaming together the shortening (400 g), sugar (600 g), sugar syrup (120 g), skimmed milk powder (40 g), glycerol-mono-stearate (20 g), soy lecithin (4 g) and water for 5 min in a high speed mixer (Hamilton Beach, UK). The mixture of flour (2000 g), soy or kinema flours (300 g), baking soda (12 g), ammonium bicarbonate (32 g), salt (20 g), flavouring agents and enough water were added to the creamed mixture to form soft dough. The final mixing was done by hand till a desirable consistency was obtained. The finished dough was shaped in a cookie moulder into rectangular pieces and baked on a moving flat steel band. The average oven temperature was 154±8°C (Rama Engineering, India) and baking was done for 4 min. A trial run with the same dough was carried out before the actual baking was done. The biscuits were cooled to room temperature, weighed and packed in air-tight plastic bags. Biscuits from the same batch were then analyzed for physical, chemical and sensory parameters.

Chemical analysis
All the flour samples, in triplicate (n=3), were analyzed for moisture content, crude oil (Soxtec system HT 2), total and reducing sugars and pH value (Systronics type 335 pH meter) (Egan et al. 1981). The crude fiber content was estimated according to the method recommended by Rangana (1986) and total ash by AOAC Method 923.03 (1995). The crude protein as N x 6.25 was determined by the Kjeldahl method (AOAC, Method 920.87, 1995). The total carbohydrate was calculated by the difference method. Energy values (KCal) were calculated applying the factors, 4, 9 and 4 for each gram of protein, lipid and carbohydrate, respectively (Greenfield & Southgate 1992).

Functional properties
Bulk density and wettability were determined according to Ozekie and Bello (1988). Emulsifying activity index (EAI) was determined according to Mutilangi et al. (1996). Gelation properties, foam capacity and foam stability were determined according to Coffman and Garcia (1977). Water and oil retention capacities were measured according to Beuchat (1977). The colour characteristics of soy and kinema flours were measured by a Colour Difference Meter (Model TC-PIIIA, Tokyo Denshoku, Co., Ltd) using different colour parameters such as 'L' (Lightness), 'a' (Redness) and ‘b’ (Yellowness) (Francis 1983). Protein solubility profile was determined at different levels of pH according to Sathe et al. (1982) and Franzén and Kinsella (1976). Protein in the supernatant was quantified using the biuret reagent (Umbreit et al. 1972) in a spectrophotometer (Model Pye Unicam PU 8685, USA). Means of triplicates were calculated and expressed as mg protein per mL of water. The solubility profile was obtained by plotting protein solubility versus pH where the solubility at pH 12 was 100%.

Nitrogen solubility index (NSI) was measured following the procedure: Five g of sample was dispersed in 200 mL distilled water. Dispersion was stirred continuously with a magnetic stirrer for 2 h at room temperature. The resulting mixture was then centrifuged at 1500 rpm for 10 min. The supernatant was analyzed for total nitrogen by a Kjeldahl method (AOAC, Method 920.87, 1995). NSI was calculated as follows:

\[ \text{NSI} = \frac{\% \text{ soluble nitrogen in water}}{\% \text{ total nitrogen of sample}} \times 100 \]

Physical parameters of biscuits
Weight (g), length (cm) and height (cm) were measured as averages of five replicates for each sample and the spread ratio was calculated as the diameter (length) to thickness ratio. The surface colour characteristics of biscuit samples were also measured by the color difference meter.

Force/deformation curves of biscuit samples were obtained a week after baking, using an Instron Universal Testing Machine (Model 1140; 500 kg
Data for chemical analyses of the four flours are shown in Table 1. Comparisons with FFSF showed that there was a slight increase of 4.9% and 2.2% protein content for TKF and PKF, respectively. Nikkuni et al. (1995) reported a 7.8% increase in protein content during kinema production. Sarkar and Tamang (1995) also reported a significant increase in total nitrogen content in kinema. Hayashi (1959) reported an increase in protein content of natto probably as a result of the enzymatic activity of microorganisms that can use atmospheric nitrogen. While oil, ash and energy value remain unchanged (except LFSF), crude fiber markedly reduced. Bacillus produces many carbohydrate-splitting enzymes, which may have hydrolyzed the polysaccharides lowering the crude fiber level (Priest 1989). The kinema production process reduced total sugar level with a simultaneous increase in reducing sugar level. Sarkar et al. (1997a) reported that heating and Bacillus fermentation is accompanied by degradation of oligosaccharides e.g., raffinose and stachyose, thus eliminating a potential cause of flatulence for consumers.

**Table 1. Chemical characteristics of soy and kinema flours**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>FFSF</th>
<th>LFSF</th>
<th>TKF</th>
<th>PKF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>6.6±0.1</td>
<td>8.2±0.1</td>
<td>7.2±0.1</td>
<td>6.8±0.1</td>
</tr>
<tr>
<td>Protein (%)*</td>
<td>44.1±1.0</td>
<td>51.7±1.2</td>
<td>46.2±1.1</td>
<td>45.1±1.0</td>
</tr>
<tr>
<td>Lipid (%)*</td>
<td>21.8±1.4</td>
<td>6.7±0.3</td>
<td>22.2±2.5</td>
<td>23.0±1.7</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>4.7±0.1</td>
<td>5.4±0.1</td>
<td>4.8±0.1</td>
<td>4.5±1.1</td>
</tr>
<tr>
<td>Total carbohydrate (%)</td>
<td>29.8±2.1</td>
<td>30.2±2.7</td>
<td>26.3±1.6</td>
<td>27.3±2.1</td>
</tr>
<tr>
<td>Crude fiber (%)*</td>
<td>6.5±0.5</td>
<td>7.5±0.2</td>
<td>4.2±0.5</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>Total sugar (%)*</td>
<td>21.0±1.1</td>
<td>23.8±1.2</td>
<td>14.5±1.1</td>
<td>16.5±1.1</td>
</tr>
<tr>
<td>Reducing sugar (%)*</td>
<td>3.1±0.1</td>
<td>3.5±0.2</td>
<td>5.1±0.2</td>
<td>4.7±0.2</td>
</tr>
<tr>
<td>Free fatty acid (%)*</td>
<td>0.7±0.0</td>
<td>ND</td>
<td>3.1±0.0</td>
<td>6.2±0.1</td>
</tr>
<tr>
<td>pH</td>
<td>6.9±0.0</td>
<td>6.9±0.0</td>
<td>6.6±0.0</td>
<td>6.5±0.0</td>
</tr>
<tr>
<td>Energy (KCal/100 g)</td>
<td>458±2.9</td>
<td>366±1.6</td>
<td>444±1.9</td>
<td>454±2.1</td>
</tr>
<tr>
<td>Urease activity</td>
<td>0.02±0.0</td>
<td>0.02±0.0</td>
<td>0.02±0.0</td>
<td>0.01±0.0</td>
</tr>
</tbody>
</table>

*Calculations based on moisture free basis

The pH values of the fermented flours were lower than the soyflours (Table 1). In contrast, Sarkar and Tamang (1995) reported that fresh kinema had a pH of about 8.4. Heating (drying) remove ammonia and other volatiles present in kinema which may have pH lowering effect in these foods. The free fatty acids
value of both TKF and PKF increased markedly, 4.6 and 9 times, respectively. Sarkar et al. (1996) reported a 33-fold increase in free fatty acids value of fresh kinema as compared to raw soybean, suggesting release of lipase during the fermentation process. The urease activity test indicated that all the samples, except LFSF, were properly cooked to inactivate the trypsin inhibitor and other biologically active enzymes. The soybeans used for LFSF were only mildly heated, before oil extraction, while other samples were moist heat-treated.

**Functional properties**

The data for functional properties of the four flours are shown in Table 2. The values for bulk density of the products were significantly (P≤0.05) different. The values obtained for these samples were higher than that reported for defatted soyflour 0.382 g/mL by Hassan and Abou-Arab (1993). The high-pressure extraction of oil may have a densification effect on LFSF. The formation of gummy materials on the surface of the beans due to Bacillus fermentation might have contributed greater inter-particle adhesion in kinema flour. The wettability of PKF was significantly (P≤0.05) higher than of the three other flours. Autoclaving might have affected the surface activity and porosity of particles, causing higher wettability.

<table>
<thead>
<tr>
<th>Properties</th>
<th>FFSF</th>
<th>LFSF</th>
<th>TKF</th>
<th>PKF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density (g/mL)</td>
<td>0.56±0.01</td>
<td>0.79±0.01</td>
<td>0.64±0.00</td>
<td>0.69±0.00</td>
</tr>
<tr>
<td>Water binding capacity (g/g)</td>
<td>3.51±0.05</td>
<td>3.52±0.00</td>
<td>2.77±0.10</td>
<td>2.51±0.01</td>
</tr>
<tr>
<td>Oil binding capacity (g/g)</td>
<td>2.42±0.03</td>
<td>1.95±0.02</td>
<td>2.00±0.03</td>
<td>1.86±0.06</td>
</tr>
<tr>
<td>Wettability (min)</td>
<td>0.38±0.08</td>
<td>0.37±0.07</td>
<td>0.35±0.10</td>
<td>1.11±0.02</td>
</tr>
<tr>
<td>Emulsifying activity index (m2/g)</td>
<td>4.71±0.05</td>
<td>5.66±0.09</td>
<td>8.20±0.03</td>
<td>8.46±0.38</td>
</tr>
<tr>
<td>Nitrogen solubility index (%)</td>
<td>17.07±1.00</td>
<td>29.19±0.89</td>
<td>64.32±1.07</td>
<td>66.02±0.95</td>
</tr>
<tr>
<td>pH</td>
<td>6.93±0.01</td>
<td>6.90±0.01</td>
<td>6.63±0.02</td>
<td>6.55±0.00</td>
</tr>
<tr>
<td>Foam volume increase (%)</td>
<td>8.07±0.67</td>
<td>22.00±0.70</td>
<td>7.97±0.30</td>
<td>6.06±0.32</td>
</tr>
<tr>
<td>LGC* (%w/v)</td>
<td>22.00</td>
<td>20.00</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>Ly</td>
<td>84.24±0.62</td>
<td>76.69±0.56</td>
<td>70.22±0.34</td>
<td>58.71±1.09</td>
</tr>
<tr>
<td>ay</td>
<td>0.54±0.14</td>
<td>2.32±0.09</td>
<td>9.026±0.41</td>
<td>13.34±1.84</td>
</tr>
<tr>
<td>by</td>
<td>26.50±1.15</td>
<td>27.44±2.18</td>
<td>32.72±0.26</td>
<td>35.95±0.87</td>
</tr>
<tr>
<td>Hue</td>
<td>88.84</td>
<td>86.16</td>
<td>77.20</td>
<td>69.66</td>
</tr>
</tbody>
</table>

Significance of LSD test of treatment means at P≤ 0.05 and standard deviation. Means with the same superscript within same row are not significantly different. 

*Means of six samples, otherwise means of triplicates.

*LGC, Least Gel Concentration and NG, no gelation.

While there was no gel formation by kinema flours, soyflours formed gel at 20% (Table 2). The values were lower than the least gelling concentration (LGC) of protein isolates at 8% (Liu 1997). The low gelling ability of these products might be due to coarser texture or the presence of large amounts of impurities as lipid, denatured protein and polysaccharides. The emulsifying activity indices of flour samples were significantly (P≤0.05) different, PKF was highest followed by TKF, LFSF and FFSF in decreasing order (Table 2). McWatters and Holmes (1979) reported that the emulsion capacity of soyflour is reduced when heating time is increased, a 22% reduction after 20 min heating. Liu (1997) stated that the emulsifying capacity of soy protein products increases with increasing protein solubility.

Data on the foaming capacity of soy and kinema flours indicated that LFSF had the highest foaming capacity (P≤0.05), well above that of PKF, with FFSF and TKF in between (Table 2). Lin et al. (1974) have reported a foaming capacity of 70% for soyflours. Yatsumatsu et al. (1972) reported the denaturation of protein during processing; and also the lipid materials in soy preparations are very detrimental to foaming because they destabilize
protein films. The stability of foam resulting from PKF was the least, total collapse occurred within 2 h (Fig. 1).

![Fig. 1. Foam stability of soy and kinema flours](image)

Water retention capacity is an index of the amount of water retained within a protein matrix or other system under certain conditions. Water retention by the kinema flours was significantly (P≤0.05) lower than by the soyflours (Table 2). The difference in values might have been caused by physical and chemical modification of the protein structure caused by heating and fermentation. Fleming et al. (1974) reported similar water holding capacities of 2.6 g/g, 2.75 g/g and 6.25 g/g of solids for soyflour, soy-concentrate, and soy-isolate, respectively. The oil retention capacity of all products was significantly (K0.05) different; while FFSF was found to have the highest, PKF had the lowest oil retention value. Lin et al. (1974) reported oil holding capacities of 1.84 g/g, 2.33 g/g, and 1.92 g/g of solids for soyflour, and commercial soy protein concentrates Isopro and Promosoy, respectively. The amount of oil bound to flours is markedly affected by the method of determination, the nature and amount of protein content, surface area, charge and topography, hydrophobicity, and the liquidity of oil (Kinsella et al. 1985).

The protein solubility profile showed minimum solubility for all four samples at pH 4.0-4.5, the apparent isoelectric pH (pI) range for soy protein (Wolf 1970) (Fig. 2). Compared to soyflours, kinema flours seemed to have less change in protein solubility with pH, especially in the range of 2-10. The nitrogen solubility index of kinema flours was significantly (P≤0.05) higher in comparison to soyflours (Table 2). Bacillus subtilis is known to produce a number of proteolytic enzymes that hydrolyze protein to smaller peptides and finally ammonia (Priest 1989). These chemicals are responsible for increase in water soluble nitrogen and flavor of kinema (Sarkar et al. 1994, Nikkuni et al. 1995).

![Fig. 2. Solubility profile of proteins at different pH: Means of six replicates; any two means for the given colour parameter not followed by the same letter are significantly different (P≤0.05).](image)

The lightness value ('L') for the products was significantly (P≤0.05) different, in decreasing order from FFSF to LFSF, TKF and PKF (Table 2). Kinema flours were significantly darker; redder ('a') and more yellow coloured ('b') than soyflours. The dark and brown colour of kinema flours, particularly PKF, was probably due to a greater degree of heat treatment during processing, resulting in a maillard type browning reaction.

**Chemical analysis of biscuits**

The proximate composition of biscuits prepared using wheat flour supplemented with any of the four different types of soyflour is given in Table 3. The higher moisture content of soy biscuits than kinema biscuits may be due to the greater water-binding properties of soyflours. The water binding capacities for FFSF, LFSF, TKF, and PKF 3.51, 3.52, 2.77, and 2.51 g water per g sample, respectively. The protein content of the supplemented biscuits was almost double that of conventional soft biscuits having about 8% protein (Egan et al. 1981, Singh et al. 2000). Biscuits from LFSF were found to have a higher protein value because of the high initial protein content of soyflour. The energy value of LFSF-containing biscuits was lower than other biscuits because lower amount of oil in LFSF. Acidity of extracted fat was about 0.5% for all samples, indicating no danger of rancidity in the biscuit (Manly 1996). The total ash and acid insoluble ash contents of supplemented biscuits were higher than those of ordinary wheat flour biscuits as reported by Singh et al. (2000). Correspondingly higher level of minerals in soybeans may be responsible for these increased values.
Table 3. Physical and chemical characteristics of soy and kinema supplemented biscuits

<table>
<thead>
<tr>
<th>Properties</th>
<th>FFSF</th>
<th>LFSF</th>
<th>TKF</th>
<th>PKF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>6.7±0.1</td>
<td>6.9±0.1</td>
<td>4.7±0.1</td>
<td>4.4±0.1</td>
</tr>
<tr>
<td>Protein (%)*</td>
<td>17.0±0.6</td>
<td>18.1±0.4</td>
<td>17.4±0.5</td>
<td>17.1±0.2</td>
</tr>
<tr>
<td>Lipid (%)*</td>
<td>11.6±0.4</td>
<td>8.3±0.2</td>
<td>12.5±0.2</td>
<td>13.0±0.6</td>
</tr>
<tr>
<td>Ash (%)*</td>
<td>1.7±0.0</td>
<td>1.5±0.0</td>
<td>1.5±0.0</td>
<td>1.8±0.0</td>
</tr>
<tr>
<td>Acid insoluble ash (%)*</td>
<td>0±0.01</td>
<td>0±0.01</td>
<td>0±0.00</td>
<td>0±0.00</td>
</tr>
<tr>
<td>Total carbohydrate (%)</td>
<td>67.6±3.2</td>
<td>70.1±2.4</td>
<td>66.6±1.7</td>
<td>66.1±2.6</td>
</tr>
<tr>
<td>Energy (KCal/100 g)</td>
<td>447±0.4</td>
<td>432±0.2</td>
<td>452±0.5</td>
<td>453±0.4</td>
</tr>
<tr>
<td>Acidity of extracted fat (%)*</td>
<td>0.5±0.0</td>
<td>0.6±0.0</td>
<td>0.4±0.0</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>Weight (g)Y</td>
<td>5.3±0.2</td>
<td>5.3±0.3</td>
<td>5.7±0.3</td>
<td>5.3±0.1</td>
</tr>
<tr>
<td>Thickness (mm) Y</td>
<td>5.1±0.1</td>
<td>4.3±0.1</td>
<td>5.0±0.0</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>Length (mm) Y</td>
<td>51.0±1.3</td>
<td>42.9±3.2</td>
<td>54.0±2.1</td>
<td>54.5±1.9</td>
</tr>
<tr>
<td>Spread ratio Y</td>
<td>10.0±0.4</td>
<td>10.0±0.1</td>
<td>10.8±0.2</td>
<td>10.9±0.3</td>
</tr>
<tr>
<td>Hardness (kg)</td>
<td>24.5±2.1</td>
<td>21.1±0.1</td>
<td>11.4±0.1</td>
<td>11.7±0.3</td>
</tr>
</tbody>
</table>

*Calculations based on moisture free basis. Data represent the three samples analyses (n=3) ± standard deviation, except Y which denotes means of 5 samples.

Significance of LSD test of treatment means is at P ≤ 0.05.
Means with the same superscript within same row are not significantly different.

Physical characteristics

The colour analysis of the biscuit samples is shown in Fig. 3. The LFSF-containing biscuits were significantly darker ('L'), redder ('a') and more yellow ('b') (except kinema-biscuits) and possessed lowest hue angle in comparison three other biscuits. Comparatively lower amounts of fat available to bind sugar in LFSF biscuits may have caused greater the exposure of sugar to heat, resulting in a browning reaction and darker product (Manly, 1996). Kinema containing biscuits were significantly (P≤0.05) darker ('L') (only TKF), redder ('a') and more yellow ('b') than those made from FFSF. The hue angle for all biscuits was almost same except LFSF biscuit. Incorporation of the pigmented fermented flours (slight red color) in the formulation was probably responsible for the darker brown kinema biscuits. In addition kinema contains higher levels of soluble, free amino acids, which react with sugars in a Maillard type browning reaction, intensifying the colour (Sarkar et al. 1997b). As the numerical values suggested, the difference in colour parameters was not big enough to cause a distinct difference in appearance and is probably of little consequence to consumers.

The weights of biscuits were not significantly (P≤0.05) different, except for the biscuit prepared from TKF, which was slightly heavier (Table 3). It was observed that spread ratio, an indicator of cookie density, was significantly (P≤0.05) increased with the addition of kinema flours. It has been established that cookie spread is strongly correlated to the water binding capacity (WBC) of the flour (Doescher et al. 1987). The higher WBC values for soyflours compared to fermented soyflours could have contributed the lower spread ratio and length in FFSF and LFSF biscuits.

The mechanical characteristics of the biscuits are important in determining the behaviors of biscuits in mouth and play important role in acceptance of the product. The peak force required to cause the total breakdown of the biscuits, rupture force or hardness, was significantly (P≤0.05) low for kinema-containing biscuits (Table 3). Szczesniak (1962) reported that brittleness is positively correlated with hardness but negatively with cohesiveness. The higher rupture force in soyflours-containing biscuits indicates a brittle product. Less fat in the LFSF may have resulted in brittleness in the corresponding biscuits as the amount of shortening also affects the texture of the biscuits (Manly 1996). Low hardness in kinema-containing biscuits indicates less brittle (crunchy) biscuits with greater internal cohesiveness and springiness (James et al. 1989). The physical texture of a biscuit is also related to its moisture content.
and functional properties of the raw materials used (Brewer et al. 1992). Kinema flours were found to have lower water binding capacity, oil binding capacity and poor gelation properties than soyflours (results not shown) that could have might have affected the formation of structural matrix and making products more fragile than others.

**Sensory evaluation**

Results of the sensory tests showed the crust color (appearance) of biscuits from PKF was significantly (P≤0.05) favored over that of biscuits from other sources, except TKF biscuits (Fig. 4). The uniformly golden brown colour with little streaks or spots in PKF-containing biscuits as compared to other biscuits was responsible for its high sensory score. There was no significant difference in palatability of biscuits among the treatments. The palatability of biscuits includes texture and flavour. A faint beany odour in soyflour-containing biscuits and a mild typical kinema aroma was reported in kinema-supplemented biscuits. The biscuits were expected to have a crunchy bite, hard but not fragile, and dry and easily broken texture. The sensory evaluation data indicated that kinema and LFSF-supplemented biscuits were significantly (P≤0.05) crisper than FFSF-containing biscuits. FFSF-containing biscuits were reported to be brittle, rather than a crunchy bite that is expected in most of the biscuits. Sensory evaluation for overall acceptance showed significantly (P≤0.05) higher values for biscuits supplemented with kinema flour as compared to soyflour-supplemented biscuits, particularly FFSF-containing biscuits.

**Conclusion**

Kinema prepared by pure culture fermentation was better in overall quality than traditionally prepared kinema. Bacillus fermentation reduced crude fiber content, increased free fatty acids whereas no change in protein, oil, ash, and energy value was observed. Protein quality was improved as indicated by the higher NSI. Water and oil retention capacity, gel forming ability and foaming capacity of kinema flours were lower (P≤0.05) but wettability and emulsifying ability were higher (P≤0.05) than in soyflours. The protein and ash content of fortified biscuits were more than double than the ordinary biscuits. Kinema biscuits were darker than the biscuits supplemented with full fat soy flour. Soyflour-containing biscuits were harder and tougher and brittle than kinema-containing biscuits. The level of acceptance was increased when kinema flour was incorporated in cookie formulation in place of soyflours.

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