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*Full Length Research Paper*

# Physicochemical and microbiological study on tunjanee – a traditionally fermented Sudanese food from groundnut (*Arachis hypogaea*) seed cake

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**Tunjanee is traditionally prepared by fermenting groundnut (*Arachis hypogaea* L.) seed cake for 21 days. Physicochemical and microbiological properties of the seed cake and tunjanee were analyzed. Tunjanee fermentation resulted in significant changes in major nutrients of the seed cake. Mineral content, total polyphenols, phytic acid and in vitro protein digestibility of the cake were not affected. Mobilization of amino acids content was found. The significant increase in total acidity and fat acidity coupled with a decrease in pH suggests microbial hydrolysis of the major nutrients. *Bacillus* and *Lactobacillus* spp. are the main active organisms in tunjanee. The number of total bacteria and yeast increased significantly within 21 days of fermentation. *Staphylococcus aureus* was absent. Coliforms and faecal coliforms were detected in groundnut cake; however, they increased significantly in tunjanee. A significant lower viable cells count of total bacteria and coliforms after addition of combo salt was observed.**

**Keywords:** Arachis hypogaeae; fermentation; tunjanee; in vitro protein digestibility; physicochemical and microbiological properties

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## INTRODUCTION

Protein-energy malnutrition is the most common deficiency disease in the world, especially in developing countries. This kind of malnutrition is related mainly to inadequate quantity and low quality of food and therefore more food proteins are needed. Accordingly, the world attitude is oriented to develop low cost protein foods of plant origin, especially for low-income groups in developing countries (Desphande, et al., 2000; Nnam, 2001). Plant proteins provide nearly 65% of the world supply of proteins for humans with 10 – 15% from legumes or vegetables (Mahe *et al.*, 1994). Importance of plant proteins in the average diet varies from the least developed regions (where animal proteins are scarce and poverty precludes the consumption of meat) to the highly developed regions (where animal production is particularly abundant). Nonetheless there is now an expanding consumption of protein foods of legume and vegetable origin throughout the world. Protein quality in leguminous seeds does not however reach the same level as in animal products. Unbalanced amino acids, low digestibility of protein and the presence of antinutritional factors are the reasons behind the low quality of legume proteins. Processing methods, such as soaking, cooking or fermentation, can improve the quality of legume proteins (Alonso *et al*., 2000; Habiba, 2001).

Groundnut (*Arachis hypogaea* L.) seed is an important food source of protein and its oil is one of the major oils in the human diet. Groundnut seed contains 40–50% oil, 22 to 32% protein and considerable amounts of minerals (Savage and Keenan, 1994). In recent years several cereals and legumes based foods using groundnuts as protein supplements have been developed to alleviate protein-calories malnutrition problem. Groundnut seed in the form of flour, protein isolates and meal in a mixed product have been found to be very desirable from a

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sensory quality point of view. The seed cake, defatted meal, contains 46-60% protein and used in food and feed formulae (Omafuvbe, 2006; Asibuo *et al*., 2008).

In Africa, oil seeds such as the African locust bean, melon seed, castor oil seed, and soybean are usually naturally fermented to produce condiments (Oboh *et al*., 2008). Groundnut, however, is fermented to produce a tempe-like product and a youghurt- like drink (Bhavanishankar *et al*., 1987; Lee and Beuchat, 1991). Beside that peoples of Darfur (Sudan) prepared a meat-like tunjanee product by naturally fermenting a solid paste of groundnut seed cake for a period of about 3 weeks, combo salt (mineral extract of ashed sorghum or millet stalks) is added a day before the end of fermentation, sun dried and then ready for use as an ingredient of stew. In rural Sudan meat and milk are the common foods for the communities. Lately, due to shortage in these food products the villagers have developed milk and meat substitutes primarily from plant materials, which they manipulate by fermentation to give flavors and textures more or less simulating those of proteolytic meat and sour milk (Dirar, 1994; Steinkraus, 1996). However, the strong flavors characterize the traditional food fermentation in which *Bacillus spp. and Lactobacillus spp.* dominate (Dakwa *et al*., 2005; Omafuvbe, 2006; Ouoba, 2009). In general, a mixed culture originating from the native microflora of the raw materials is in action in most of the indigenous food fermentations. Hence, starter culture fermentation is of first preference in the industrial scale because quality of finished products can be maintained (Sahlin, 1999). Therefore, in order to develop controlled fermentation and improve on the quality of a product, the traditional process must be well understood scientifically. In line with this concept, investigations on kinds of organisms dominate tunjanee fermentation as well as the biochemical changes encountered in groundnut seed cake is claimed for future control of the fermentation process.

## MATERIALS AND METHODS

### MATERIALS

Groundnut (*Arachis hypogaea* L.) seed cake was purchased from the local market in Nyala; south Darfur state, Sudan. The seed cake was cleaned, and part of the cake was divided into two batches. One batch was kept in a deep freezer for microbiological tests .The other batch was milled to pass 0.4 mm sieve, kept in a tightly sealed container and stored at $4^{o}$C until chemical analysis. The remaining part was reserved for the subsequent processing steps to produce tunjanee.

### METHODS

#### Tunjanee fermentation

Tunjanee was prepared following the method adopted by the indigenous people of Darfur. About 1 kg of groundnut seed cake was put in a glass container. Distilled water was added to the cake, and the mixture was then well kneaded to prepare a firm paste. The paste was pressed by hand to minimize the amount of air, and then the container was covered tightly and left to ferment at room temperature (~$30^{o}$C) for 21 days. Fermented samples were withdrawn at intervals of 3 days. Part of the fermented samples was reserved for microbiological tests (the tests were done within 72 h). The rest of the ferments was then dried at $70^{o}$C, milled (0.4mm sieve) and kept in containers at $4^{o}$C until chemical analysis.

#### Proximate analysis

Lipids, ash, total carbohydrates, and total nitrogen (micro-Kjeldahl) were determined according to AOAC methods (1995). Protein was calculated as N% x 6.25. Moisture content was determined by drying a sample at 105 °C overnight (AOAC, 1995), and then dry matter was calculated. Crude fiber content was determined according to the acid/alkali digestion method of Southgate (1976).

#### Total minerals

Minerals were determined in samples' extracts prepared by the dry-ashing method as described by Pearson (1981). Ferrous, Copper, Zinc znd Manganeses were determined according to the analytical method of atomic absorption spectroscopy (Perkin- Elmer 1100 V, Waltham, MA, USA). Phosphorus was determined by the ammonium molybdate/ammonium vandate method and Calcium and Magnesium by titration (Chapman and Pratt, 1961).

#### Tannin and phytic acid content

Vanillin–HCl in methanol reagent method was used to assay tannin (Price *et al.*, 1977). Catechin was used as reference standard. Phytic acid was determined by the method of Wheeler and Ferrel (1971). Ferric nitrate was

used as reference standard. A ratio of iron to phosphorus of 4:6 was assumed.

### *In vitro* protein digestibility

*In vitro* protein digestibility of the samples was measured by the pepsin digestion method described by Monjula and John (1991). The digestible protein was analyzed for nitrogen using the micro-Kjeldahl procedure (AOAC, 1995) and expressed as a percent of the total nitrogen.

### Amino acids analysis

Amino acids composition of samples was measured on hydrolysates using amino acids analyzer (Sykam- S433, Eresing, Germany). Sample hydrolysates were prepared following the method of Moore and Stein (1963). Two hundred milligrams of sample were taken in hydrolysis tube. Then 5 mL 6N HCl were added to the sample into the tube, evacuated, tightly closed and incubated for 24 h at 110°C. After incubation period, the solution was filtered and 200 mL of the filtrate were evaporated to dryness at 140°C for an hour. The dried hydrolysate was diluted with 1 mL of 0.12N, pH 2.2 citrate buffer, the same as the amino acid standards (amino acid standards H; Pierce Inc., Rockford; IL, USA). Aliquot of 150 µL of the sample hydrolysate was injected in a cation separation column at 130°C. Ninhydrin solution and an eluent buffer (the buffer system contained solvent A, pH 3.45 and solvent B, pH 10.85) were delivered simultaneously into a high temperature reactor coil (16 m length) at a flow rate of 0.7 mL $min^{-1}$. The buffer/ninhydrin mixture was heated in the reactor at 130°C for 2 min to accelerate chemical reaction of amino acids with ninhydrin. The products of the reaction mixture were detected at wavelengths of 570 nm and 440 nm on a dual channel photometer. The amino acids composition was calculated from the areas of standards obtained from the integrator and expressed as percentages.

### Microbiological tests

A quantity of 10 g of sample was placed in 90 mL of sterile 0.1% peptone water and shaken to prepare 0.10 dilution. A decimal dilution series was then prepared in 0.9% NaCl saline solution. Aliquots (1.0 mL) were inoculated onto the surface of agar media; using a spread agar pour-plate procedure; for a count of total viable bacteria, *Staphylococcus aureus*, and yeasts and moulds. The following agar media were employed: plate count agar incubated both aerobically and anaerobically; Baird Parker agar selective for *Staphylococcus aureus*; and malt extract agar containing 100 µg/mL chloramphenicol, selective for yeasts and moulds. After inoculation, media were incubated at 37°C (malt extract agar was also incubated at 28°C) and examined after 24–48 h. Colonies on the agar plate were counted (colony-forming units, cfu/g) and a proportional subsampling procedure was used to select colonies of bacteria for identification. Gram staining, spore staining, presence of active enzymes and growth in air tests were employed to identify the genera of the bacteria. Total coliforms were determined by inoculating tubes containing Lauryl tryptose (LT) broth with an aliquot of 1.0 mL each from a suitable dilution and incubating at 35°C for 48 h. Tubes producing acid and gas were used for further testing. Aliquots from acid- and gas-positive tubes were inoculated into brilliant green bile lactose broth and then one set of tubes was incubated at 35°C for 48 h and the other at 44.5°C for 24 h. For further confirmation, aliquots were taken from tubes giving a positive reaction at 44.5°C and streaked onto eosin methylene blue (EMB) agar and incubated at $35^{0}$C for 24 h, and then examined for colonies types. Then colonies from each EMB plates were cultured onto plate count agar slant (PCAS) and incubated at $35^{0}$C for 24 h. Growth on PCAS were subjected to morphological and biochemical tests (Indole, methyl red, Koser citrate and gelatin tests). Positive test colonies were then counted as MPN/g (Harrigan and McCane 1976).

### Statistical analyses

Statistical analysis of data obtained from triplicate samples was performed using a one-way ANOVA and the software of SAS Institute, version 6.3 (SAS 1997). Significant differences among the means were determined by Duncan multiple-range test (at $P \leq 0.05$).

## RESULTS AND DISCUSSION

### Proximate composition and mineral composition

The effect of tunjanee preparation on the proximate composition and mineral composition of groundnut seed cake is shown in Table 1. The groundnut seed cake contains 58.68, 7.12, 7.22, 9.00 and 17.98% protein, oil, ash, crude fiber and carbohydrates, respectively. These results are in accordance with those found by Bhatt *et al*. (2005) and Babiker *et al*. (2009) in some groundnut seed cakes. Fermentation resulted in deviation of some nutrients from the raw seed cake. The differences in oil, ash, crude fiber and carbohydrates contents found after fermentation are probably due to changes in dry matter composition due to microbial activity (Table 5 & 6). Addition of combo salt one day before the end of fermentation resulted in significantly higher oil and carbohydrates and lower crude fiber compared to the non-treated salt sample.

**Table 1.** Chemical composition* of peanut seed cake and tunjanee

| Sample | Protein (%) | Oil (%) | Ash (%) | Crude fiber (%) | Carbohy-drates (%) | Ca (%) | Mg (%) | P (%) | Fe (mg/100g) | Cu (mg/100g) | Zn (mg/100g) | Mn (mg/100g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed cake | 58.68$^{a}$ (0.83) | 7.12$^{c}$ (0.11) | 7.22$^{b}$ (0.07) | 9.00$^{c}$ (1.00) | 17.98$^{a}$ (0.15) | 0.350$^{a}$ (0.02) | 1.93$^{a}$ (0.07) | 0.550$^{b}$ (0.01) | 39.63$^{a}$ (2.03) | 2.00$^{a}$ (0.10) | 7.15$^{b}$ (0.45) | 4.24$^{a}$ (0.28) |
| Tunjanee 21DF | 59.00$^{a}$ (1.42) | 8.20$^{a}$ (0.10) | 8.12$^{a}$ (0.23) | 11.10$^{a}$ (1.39) | 13.51$^{b}$ (2.27) | 0.347$^{a}$ (0.02) | 1.83$^{a}$ (0.04) | 0.576$^{a}$ (0.01) | 39.31$^{a}$ (1.13) | 2.06$^{a}$ (0.12) | 7.26$^{a}$ (0.65) | 4.18$^{a}$ (0.24) |
| #21DF | 59.13$^{a}$ (1.31) | 7.96$^{b}$ (0.27) | 8.06$^{a}$ (0.12) | 10.25$^{b}$ (0.65) | 14.60$^{b}$ (1.55) | 0.350$^{a}$ (0.04) | 1.82$^{a}$ (0.05) | 0.578$^{a}$ (0.01) | 39.92$^{a}$ (3.73) | 2.08$^{a}$ (0.19) | 8.32$^{a}$ (0.41) | 4.17$^{a}$ (0.28) |

Means are calculated from three replicate samples. Figures in parentheses are standard deviations. Within a column, means followed by different letters are significantly different at $P \leq 0.05$. DF, Days of fermentation. Calculations based on dry matter. #Combo salt is added one day before the end of fermentation period.

Table 1 also listed contents of some macro- and micro-elements of groundnut seed cake and tunjanee. Results revealed that the Ca, Mg, and P contents of groundnut seed cake were 0.35, 1.93 and 0.55%, respectively. Bhatt *et al*. (2005) and Babiker *et a*l. (2009) reported comparable values for Ca and P in groundnut seed cake. Tunjanee fermentation did not affect the inherent contents of macro- and micro-elements studied

**Table 2.** Total acidity (mg/100g), fat acidity (mg/100g) and pH of peanut seed cake and tunjanee*

| Sample | Total acidity | Fat acidity | pH |
|---|---|---|---|
| Peanut seed cake | 71.2$^{d}$ (0.09) | 41.5$^{d}$ (1.27) | 6.72$^{a}$ (0.02) |
| Tunjanee | | | |
| 3DF | 2110.7$^{c}$ (35.80) | 154.2$^{c}$ (5.30) | 4.98$^{f}$ (0.01) |
| 6DF | 2285.2$^{b}$ (107.10) | 148.6$^{c}$ (1.22) | 5.18$^{c}$ (0.02) |
| 9DF | 2037.3$^{c}$ (65.73) | 148.7$^{c}$ (0.29) | 5.12$^{d}$ (0.02) |
| 12DF | 2088.0$^{c}$ (107.84) | 150.0$^{c}$ (3.84) | 5.08$^{de}$ (0.07) |
| 15DF | 2331.5$^{b}$ (38.60) | 150.9$^{c}$ (1.82) | 5.05$^{e}$ (0.01) |
| 18DF | 2492.5$^{a}$ (15.15) | 181.9$^{b}$ (2.71) | 5.05$^{e}$ (0.01) |
| 21DF | 2583.8$^{a}$ (85.60) | 205.2$^{a}$ (4.65) | 4.97$^{f}$ (0.06) |
| #21DF | 2300.8$^{b}$ (129.22) | 205.1$^{a}$ (7.17) | 5.77$^{b}$ (0.03) |

Means are calculated from three replicate samples. Figures in parentheses are standard deviations. Within a column, means followed by different letters are significantly different at $P \leq 0.05$. DF, Days of fermentation. Calculations based on dry matter. #Combo salt is added one day before the end of fermentation period

**Total acidity, fat acidity and PH of groundnut seed cake and tunjanee**

Table 2 shows the total titratable acidity (TA), fat acidity (FA) and pH of the groundnut seed cake and tunjanee preparation products. Results indicated that the TA, FA and pH of the seed cake were71.2 mg/100 g, 41.5 mg/100 g and 6.72, respectively. After 5 days of fermentation, a significant ($P \leq 0.05$) increase in TA (2110.7 mg/100 g) and FA (154.2 mg/100 g) and a decrease in pH (4.98) was observed. Further significant ($P \leq 0.05$) increase, in a gradual manner, in TA and FA was found until the 21$^{th}$ day of fermentation period (2583.8 and 205.2 mg/100 g, respectively). Similar results were found for tunjanee treated with combo salt. Moreover, addition of combo salt one day before the end of fermentation resulted in a significant ($P \leq 0.05$) higher pH compared to non-treated tunjanee (5.77 and 4.97, respectively. The increase in the viable cells count of the bacteria (Table 5) during fermentation may be responsible for the increase in the acid production as a result of degradation of carbohydrates and fats. A faster acidification and a lower pH in oil seed-based traditionally fermented foods has been reported by other workers (El Faki *et al*., 1991; Yagoub *et al*., 2004; Mohammed and Yagoub, 2007).

**Phytic Acid and total polyphenols**

Results indicate that the PA and the TPP contents of the groundnut seed cake were 276.1 and 751.9 mg/100g, respectively (Table 3). The level of the TPP in the seed cake is comparable to the value obtained by (Salunkhe *et al*., 1992). Fermentation regime to produce tunjanee (without and with addition of combo salt) did not change significantly ($P \geq 0.05$) the contents of the phytic acid and polyphenols of the substrate.

***In vitro* protein digestibility**

Table 3 shows that the in vitro protein digestibility (IVPD) of the groundnut seed cake was 60.23 %. Fermentation of the cake for 3 days insignificantly affects the original value of the IVPD. But thereafter, the IVPD starts to decrease significantly ($P \leq 0.05$) as the fermentation process prolonged to the day 18 (44.93%). At day 21, the IVPD of tunjanee increased significantly ($P \leq 0.05$) reaching 57.97 and 56.23% for samples without and with combo salt, respectively. Nature of the protein bodies and the aggregation of the hydrophopic protein fractions may be the factors mostly responsible for the decrease in protein (Oria *et al*., 1995; Fageer and El Tinay, 2004).

**Table 3.** Phytic acid (mg/100g), total polyhenols (mg/100g) and in vitro protein digestibility (%) of peanut seed cake and tunjanee

| Sample | Phytic acid | Total polyphenols | In vitro protein digestibility |
|---|---|---|---|
| Seed cake | 276.1$^{a}$ (6.3) | 751.9$^{ab}$ (6.92) | 60.23$^{ab}$ (0.68) |
| Tunjanee | | | |
| 3DF | 275.1$^{a}$ (3.1) | 739.9$^{ab}$ (9.45) | 59.67$^{ab}$ (0.67) |
| 6DF | 283.6$^{a}$ (4.98) | 737.5$^{b}$ (9.76) | 56.23$^{c}$ (0.67) |
| 9DF | 279.3$^{a}$ (7.85) | 744.2$^{ab}$ (6.93) | 56.43$^{c}$ (1.58) |
| 12DF | 285.5$^{a}$ (12.70) | 747.5$^{ab}$ (10.52) | 50.28$^{d}$ (1.98) |
| 15DF | 274.8$^{a}$ (5.17) | 752.9$^{ab}$ (7.10) | 50.17$^{d}$ (1.04) |
| 18DF | 274.7$^{a}$ (6.13) | 754.7$^{a}$ (9.24) | 44.93$^{e}$ (2.10) |
| 21DF | 275.0$^{a}$ (3.00) | 749.5$^{ab}$ (7.31) | 57.97$^{bc}$ (0.95) |
| #21DF | 275.3$^{a}$ (6.43) | 752.2$^{ab}$ (7.10) | 56.23$^{c}$ (0.27) |

Means are calculated from three replicate samples. Figures in parentheses are standard deviations. Within a column, means followed by different letters are significantly different at $P \leq 0.05$. DF, Days of fermentation. Calculations based on dry matter. #Combo salt is added one day before the end of fermentation period

**Amino acids composition of Groundnut seed cake and tunjanee**

Amino acids content of groundnut seed cake and tunjanee are illustrated in Table 4. Arginine, tyrosine plus phenylalanine, aspartic acid and glutamic acid were the major amino acids (15.09, 11.81, 9.50 and 9.19%, respectively) in the seed cake. The essential amino acids valine, leucine and isoleucine are comparable to those of the FAO reference pattern (FAO/WHO, 1975). Threonine and lysine (2.94 and 3.85 g/100 g, respectively) are the limiting essential amino acids in the cake. Threonine was reported to be the first limiting amino acid in groundnut seed (Venkatachalam[‡] and Sathe, 2006). Fermentation of the seed cake for 3 days decreased significantly ($P \leq 0.05$) threonine, aspartic acid, alanine, leucine, isoleucine, phenylalanine plus tyrosine and arginine while serine, glycine, valine, lysine and histidine increased significantly ($P \leq 0.05$). The fermenting tunjanee (i.e. 21-old ferment with and without combo salt) contains a significantly ($P \leq 0.05$) lower aspartic acid, threonine, serine, glutamic acid, phenylalanine plus tyrosine and histidine compared to their levels in the seed cake; while the rest of the amino acids are significantly ($P \leq 0.05$) higher than those in the cake. Compared to FAO protein pattern; valine, leucine and isoleucine are found in abundant but lysine remains the limiting amino acid in tunjanee. In respect to ammonia, the inherent content in the seed cake (10.05 g/100 g protein) increased significantly ($P \leq 0.05$) to 11.74 g/100 g protein after 3 days of fermentation. Further significant ($P \leq 0.05$) increase in the amount of ammonia to day 12 (14.06 g/100 g protein) was observed. In the end, the content of ammonia was 13.45 and 14.60 g/100 g protein for tunjanee without and with combo salt, respectively. The release of ammonia suggests the enhanced proteolytic activity and utilization of the free amino acids by the fermenting bacteria (Table 5 & 6). Production of ammonia is reported to be a common feature of fermentation of vegetable proteins (Omafuvbe, 2006). Transamination reaction that may occur during fermentation could also be responsible for the changes observed in amino acids profile of the groundnut seed cake.

**Fermenting microorganisms in tunjanee**

Viable cells count of bacteria in the groundnut seed cake was 6.11 Log cfu/g. In the first 3 days of tunjanee fermentation; the cells count of bacteria increased significantly ($P \leq 0.05$) to 10.11 Log cfu/g and thereafter decreased significantly ($P \leq 0.05$) to 8.90 Log cfu/g at the 9$^{th}$ day (Table 5). However, *Bacillus spp.* dominates the first 9 days of fermentation (Table 6). In day 12; the bacterial count increased significantly ($P \leq 0.05$) to 10.04 Log cfu/g and then decreased significantly ($P \leq 0.05$) in day 15 and 18. Viable cells count of bacteria in the 21-old ferments (without and with combo salt) was significantly higher compared to that in the seed cake (Table 5). Table 6 gives clues that *Propionibacterium* and *Lactobacillus* species are the major bacteria present in the medium since day 12 to the end of the fermentation period. Moreover, addition of combo salt to the medium one day before the end of fermentation period favors growth of

**Table 4.** Effect of tunjanee fermentation on the amino acids composition (g/100 g protein)* of peanut seed cake.

| Amino acid | Tunjanee fermentation (Days) | | | | | | | | | FAO/WHO** |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zero | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 21# | |
| Aspartic acid | 9.50$^{a}$ (0.09) | 8.98$^{b}$ (0.11) | 9.13$^{b}$ (0.11) | 9.04$^{b}$ (0.07) | 9.02$^{b}$ (0.04) | 8.99$^{b}$ (0.11) | 8.32$^{d}$ (0.07) | 8.26$^{d}$ (0.12) | 8.73$^{c}$ (0.03) | |
| Threonine | 2.94$^{a}$ (0,03) | 2.80$^{b}$ (0.02) | 2.88$^{b}$ (0.02) | 2.80$^{b}$ (0.06) | 2.76$^{d}$ (0.03) | 2.75$^{a}$ (0.03) | 2.77$^{cd}$ (0.03) | 2.77$^{cd}$ (0.01) | 2.82$^{bc}$ (0.03) | 4.00 |
| Serine | 2.62$^{b}$ (0.04) | 2.77$^{a}$ (0.02) | 2.73$^{a}$ (0.02) | 2.61$^{b}$ (0.03) | 2.59$^{b}$ (0.02) | 2.44$^{cd}$ (0,02) | 2.40 (0.02) | 2.45$^{c}$ (0.02) | 2.48$^{c}$ (0.04) | |
| Glutamic acid | 9.19$^{ab}$ (0.10) | 9.12$^{b}$ (0.07) | 9.29$^{ab}$ (0.02) | 9.15$^{b}$ (0.04) | 9.34$^{a}$ (0,03) | 8.54$^{c}$ (0,06) | 7.55$^{e}$ (0.06) | 7.64$^{e}$ (0.06) | 7.99$^{d}$ (0.19) | |
| Glycine | 4.79$^{a}$ (0.04) | 4.93$^{a}$ (0.03) | 4.90$^{a}$ (0.02) | 4.91$^{a}$ (0.03) | 4.89$^{a}$ (0.04) | 5.09$^{a}$ (0.08) | 4.87$^{a}$ (0.03) | 4.97$^{a}$ (0.03) | 4.94$^{a}$ (0.03) | |
| Alanine | 5.89$^{b}$ (0.04) | 5.76$^{c}$ (0.06) | 5.65$^{cd}$ (0.10) | 5.72$^{cd}$ (0.04) | 5.63$^{e}$ (0.05) | 6.14$^{a}$ (0.12) | 6.21$^{a}$ (0.04) | 6.19$^{a}$ (0.03) | 6.11$^{a}$ (0.04) | |
| Valine | 6.29$^{cd}$ (0.03) | 6.25$^{d}$ (0.04) | 6.05$^{e}$ (0.09) | 6.20$^{d}$ (0.10) | 6.20$^{d}$ (0.08) | 6.37$^{c}$ (0.04) | 6.50$^{b}$ (0.04) | 6.51$^{ab}$ (0.04) | 6.61$^{a}$ (0.03) | 5.00 |
| Methionine | 0.60$^{a}$ (0.04) | 0.63$^{a}$ (0.03) | 0.59$^{a}$ (0.03) | 0.58$^{a}$ (0.02) | 0.60$^{a}$ (0.02) | 0.59$^{a}$ (0.04) | 0.62$^{a}$ (0.03) | 0.58$^{a}$ (0.03) | 0.61$^{a}$ (0.02) | |
| Isoleucine | 5.12$^{a}$ (0.11) | 4.89$^{bc}$ (0.23) | 4.85$^{c}$ (0.02) | 4.86$^{c}$ (0.02) | 4.85$^{c}$ (0.02) | 4.87$^{c}$ (0.03) | 4.91$^{bc}$ (0.02) | 5.07$^{ab}$ (0.08) | 5.15$^{a}$ (0.14) | 4.00 |
| Leucine | 8.63$^{c}$ (0.03) | 8.51$^{d}$ (0.05) | 8.48$^{d}$ (0.05) | 8.49$^{d}$ (0.02) | 8.49$^{d}$ (0.02) | 8.75 (0.04) | 8.83$^{a}$ (0.02) | 8.79$^{ab}$ (0.04) | 8.77$^{ab}$ (0.03) | 7.00 |
| Phenyl alanine + Tyrosine | 11.81$^{a}$ (0.04) | 11.32$^{bc}$ (0.03) | 11.24$^{bc}$ (0.03) | 12.01$^{a}$ (0.06) | 11.12$^{c}$ (0.11) | 10.66$^{d}$ (0.06) | 10.61$^{d}$ (0.03) | 10.50$^{d}$ (0.04) | 10.57$^{d}$ (0.08) | 6.00 |
| Histidine | 3.64$^{e}$ (0/02) | 3.73$^{d}$ (0/04) | 3.60$^{e}$ (0.03) | 3.53$^{f}$ (0.02) | 3.90$^{c}$ (0.03) | 4.18$^{a}$ (0.03) | 4.09$^{b}$ (0.07) | 3.50$^{f}$ (0.02) | 3.49$^{f}$ (0.03) | |
| Lysine | 3.85$^{b}$ (0.02) | 3.97$^{a}$ (0.05) | 3.95$^{a}$ (0.02) | 4.04$^{a}$ (0.07) | 3.99$^{a}$ (0/04) | 3.99$^{a}$ (0.04) | 4.03$^{a}$ (0.07) | 3.95$^{a}$ (0.05) | 3.97$^{a}$ (0.03) | 5.50 |
| Arginine | 15.09$^{b}$ (0.10) | 14.66$^{c}$ (0.09) | 14.66$^{c}$ (0.09) | 13.89$^{g}$ (0.20) | 14.18$^{g}$ (0.05) | 14.15$^{g}$ (0.05) | 15.35$^{a}$ (0.12) | 14.60$^{c}$ (0.37) | 13.29$^{e}$ (0.09) | |
| Ammonia | 10.05$^{h}$ (0.08) | 11.74$^{g}$ (0.05) | 12. 11$^{f}$ (0.09) | 13.19$^{d}$ (0.08) | 14.06$^{b}$ (0.12) | 12.78$^{e}$ (0.06) | 13.04$^{d}$ (0.12) | 13.45$^{c}$ (0.13) | 14.60$^{a}$ (0.10) | |

*Means are calculated from three replicate samples. Within a row, means followed by different letters are significantly different at $P \leq 0.05$. #Combo salt was added a day before the end of fermentation period. **FAO reference protein pattern (FAO/WHO, 1975).

*Bacillus spp*. in the end product (Table 6). Omafuvbe (2006) observed an increase in growth of *Bacillus subtilis* on addition of salt to the medium during fermentation of soy-dawdawa. *Bacilus spp*. was isolated from some traditional fermented foods as the major fermenting bacteria (Ogbadu and Okagbue, 1988; Omafuvbe *et al*., 2000; Dakwa *et al*., 2005; Yagoub and Mohammed, 2008). Viable cells of *Staphylococcus spp*. were not detected in the fermenting medium throughout the whole course of fermentation (Table 5).

Results also revealed presence of yeast and moulds in groundnut seed cake with viable cells count of 5.26 Log cfu/g. A significant ($P \leq 0.05$) increase in growth of yeasts and moulds within 3 days of fermentation (6.38 Log cfu/g) was observed. Then the cells count of yeasts and moulds decreased significantly ($P \leq 0.05$) to 6.18 Log cfu/g at the 9$^{th}$ day and thereafter the cells count remained stable to the end of the fermentation period (Table 5). An increase in acidity (Table 2) and/or reduction in potential oxygen during fermentation process may have provided suitable conditions for the growth of yeasts and moulds.

**Coliform bacteria in tunjanee**

Viable cells count and species of coliform and fecal coliform bacteria in groundnut seed cake and fermenting tunjanee are presented in Table 5 and 6. The most probable number (MPN) method for viable cells count of coliforms was employed. Viable cells count indicates MPN indices of 0.3 per gram for total coliforms and fecal *E. coli* in groundnut seed cake (Table 5). After 3 days of fermentation, total and fecal coliform loads of groundnut seed cake increased significantly ($P \leq 0.05$) to count 2.7 MPN/g. The coliforms indices remained unchange to day 12 (2.8 MPN/g for both total and fecal coliforms). At the 15$^{th}$ day of fermentation; the load of coliforms and the fecal coliforms declined significantly ($P \leq 0.05$) to 2.1 MPN/g and further declined to 1.1 MPN/g at the end fermentation. The gradual decrease in loads of coliforms and fecal coliforms may be a result of the activity of the lactic acid bacteria (Table 5). Probiotic bacteria in fermented foods showed inhibitory effect against pathogens (Shah and Dave 2002; Avery, et al., 2005;

**Table 5.** Changes in total count of viable microbes of peanut seed cake during tunjanee fermentation.

| Microorganism | Fermenting tunjanee (Days) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 21# |
| Total bacteria (Log cfu/g) | 6.11$^{e}$ (0.08) | 10.11$^{a}$ (0.19) | 9.11$^{bc}$ (0.26) | 8.90$^{c}$ (0.04) | 10.04$^{a}$ (0.11) | 9.18$^{b}$ (0.07) | 7.95$^{d}$ (0.02) | 10.20$^{a}$ (0.09) | 8.90$^{c}$ (0.03) |
| Yeast and mould (Log cfu/g) | 5.26$^{d}$ (0.08) | 6.38$^{a}$ (0.26) | 6.18$^{c}$ (0.08) | 6.18$^{c}$ (0.08) | 6.23$^{bc}$ (0.09) | 6.26$^{abc}$ (0.07) | 6.24$^{abc}$ (0.04) | 6.26$^{abc}$ (0.07) | 6.18$^{c}$ (0.10) |
| *Staphylococcus Aureus* | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Coliforms (MPN/g) | 0.30$^{d}$ (0.01) | 2.70$^{a}$ (0.04) | ND | 2.80$^{a}$ (0.07) | 2.80$^{a}$ (0.10) | 2.10$^{b}$ (0.10) | 1.10$^{c}$ (0.02) | 1.10$^{c}$ (0.02) | 0.30$^{d}$ (0.02) |
| Fecal coliforms (MPN/g) | 0.30$^{d}$ (0.03) | 2.70$^{a}$ (0.10) | ND | 2.80$^{a}$ (0.19) | 2.80$^{a}$ (0.05) | 2.10$^{b}$ (0.04) | 1.10$^{c}$ (0.04) | 1.10$^{c}$ (0.03) | 0.30$^{d}$ (0.03) |
| pH | 6.72$^{a}$ (0.02) | 4.98$^{f}$ (0.01) | 5.18$^{c}$ (0.02) | 5.12$^{d}$ (0.02) | 5.08$^{de}$ (0.01) | 5.05$^{e}$ (0.01) | 5.05$^{e}$ (0.4) | 4.97$^{f}$ (0.01) | 5.77$^{b}$ (0.03) |

Means are calculated from three replicate samples. Within a row, means followed by different letters are significantly different at $P = 0.05$. #Combo salt was added a day before the end of fermentation period.

**Table 6.** Fermenting bacteria and coliforms found in tunjanee during processing

| **Microorganism** | **Fermenting tunjanee (Days)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **0** | **3** | **6** | **9** | **12** | **15** | **18** | **21** | **21#** |
| *Bacillus spp.* | + | + | + | + | - | - | - | - | + |
| *Propionibacterium spp.* | - | - | - | - | + | - | + | - | - |
| *Lactobacillus spp.* | - | - | - | - | - | + | - | + | - |
| *Citrobacter spp.* | - | - | - | - | + | - | - | + | + |
| *Enterobacter spp.* | - | - | - | - | - | + | - | - | - |
| *Klebsiella spp.* | - | - | - | - | - | - | + | - | - |

(+) present; ( -) absent. #Combo salt was added a day before the end of fermentation period.

Tharmaraj and Shah, 2009). Addition of combo salt to tunjanee one day before the end of fermentation decreased significantly ($P \leq 0.05$) the load to 0.3 MPN/g for total and fecal coliforms (Table 5). In respect to coliform species, *Citrobacter species* were found in the fermenting medium the whole first 12 days and disappeared thereafter (Table 6). *Enterobacter* and *Klebsiella species* appears at the 15$^{tth}$ and 18$^{tth}$ days of fermentation, respectively. But *Citrobacter species* re-dominate the last days of fermentation (Table 6). Coliforms and fecal coliforms were reported by Roy *et al*. (2007) in some legume-based traditional fermented foods in India. The presence of coliform bacteria in food may indicate fecal contamination, presence of potential pathogens, food spoilage, and unsanitary food processing conditions. The high amount of acids produced in the fermenting tunjanee (Table 2) may retard growth of coliform bacteria to the minimal. In most of the fermented foods, especially in lactic acid bacterially fermented ones, the inhibition of growth of bacterial pathogens is common and can often ensure safety where levels of contamination are low (Adams and Nicolaides, 1997; Chiang *et al*., 2000; Roy, *et al*., 2007).

## CONCLUSION

The present study indicates that the fermenting tunjanee has nutrient content similar to that of the raw material. Fermentation did not affect significantly the levels of polyphenols and phytic acid. In vitro protein digestibility also is unaffected. Amino acids composition of groundnut seed cake did not improve after fermentation. *Bacillus* spp. and *Lactobacillus* spp. are the dominant microorganisms in tunjanee. Yeast and moulds are higher in the fermenting tunjanee. Slight contamination by coliform bacteria was found.

### REFERENCES

Adams MR, Nicolaides L (1997). Review of the sensitivity of diverent foodborne pathogens to fermentation. *Food Control,* 8: 227–239.

Alonso R, Aguirre A, Marzo F (2000). Effects of extrusion and traditional processing methods on antinutrients and *in vitro* digestibility of protein and starch in faba and kidney beans. *Food Chemistry*, 68: 159–165.

AOAC (1995). Official methods of Analysis of the Association of Official Analytical Chemists, W. Horwitz (Ed.), 16 ed. Association of Official Analytical Chemists, Washington, D.C

Asibuo JY, Akromah R, Adu-Dapaah HK, Safo-Kantanka O (2008). Evaluation of nutritional quality of groundnut (*Arachis hypogaea* L.) from Ghana (2008)b.. Afri. J. food, Agric. Nutri. Devel. 8: 133-150.

Avery SM, Walters ID, Hutchison MI (2005). Fate of *Escherichia coli* O157 and detection of stx phage during fermentation of maize, an animal feedstuff. *Letters of Applied Microbiology*, 40: 99 – 105.

Babiker MS, Kijora CC, Abbas SA, Danier J (2009). Nutrient composition of main poultry feed ingredients used in Sudan and their variations from local standard tables values*. Inte. J. Poultry Sci.* 8: 355-358.

Bhatt RS, Shama SR, Singh U, Kumar D, Risam KS (2005). Effect of substituting groundnut cake with different levels of rice bran on growth and wool production of German angora rabbits. *World Rabbit Science*, 13: 179 - 187

Bhavanishankar TN, Rajashekaran T, Sreenivasa Murthym V (1987). Tempeh-like product by groundnut fermentation. *Food Mocrobiology*, 4: 121-125.

Chapman HD, Pratt FP (1961). Ammonium molybdate–ammonium vandate method for determination of phosphorus. In: H.D. Chapman & F.P. Pratt (Eds). *Methods of Analysis for Soils, Plants and Water*. (169–170). Berkeley: California Univ. Public. Division of Agric. Sci.

Chiang BL, Sheih YB, Wang LH, Lino CK, Gill HS (2000). Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (*Bifidobacterium lactis* HN019) optimization and definition of cellular immune responses. *Eur. J. Clinical Nutri.* 54: 849-855.

Clarke E, Wiseman J (2000). Developments in plant breeding for improved nutritional quality of soya beans II. Anti-nutritional factors. *J. Agric. Sci.* 134: 125-136.

Dakwa S, Dawson S, Diako E, Annan NT, Amoa Awuo WF (2005). Effect of boiling and roasting on the fermentation of soybeans into dawadawa (soy-dawadawa*). International Journal of Food Microbiology*, 104: 69 – 82.

Desphande SS, Salunkhe DK, Oyewole OB, Azam-Ali S, Battcock M, Bressani R (2000). Fermented Grain Legumes, Seeds and Nuts. A Global Perspective. *FAO Agricultural Services Bulletin* 142: FAO: Rome, Italy,; pp. 1-53.

Dirar HA (1993). Meat substitute of plant origin. In: H.A. Dirar (Ed.). *The Indigenous fermented Foods of the Sudan*. (412-467.). Univ. Press. Cambridge, Great Britain

Dirar HA (1994). Kawal, meat substitute from *Cassia obtusifolia* leaves. *Economic Botany*, 38: 342-249.

El Faki AE, Dirar HA, Collins MA, Harper DB (1991). Biochemical and microbiological investigation of Sigda - A Sudanese fermented food derived from sesame seed oil cake. *J. Sci. Food and Agric.* 57: 351-361.

Fageer ASM, El Tinay AH (2004). Effect of genotype malt pretreatment and cooking on in vitro protein digestibility and protein fractions of corn. *Food Chemistry*, 84: 613-619.

FAO/ WHO (1973). Energy and protein requirements. Report of a joint FAO/WHO Ad Hoc Expert Committee. Geneva: World Health Organization Technical Report, Series 522.

Habiba RA (2001). Changes in anti-nutrients, protein solubility, digestibility and HCl-extractibility of ash and phosphorus in vegetable peas as affected by cooking methods. *Food Chemistry*, 77: 187–192.

Harrigan WF, McCane ME (1976). 'Laboratory methods in food and dairy microbiology.' 2nd edn. (Academic Press: London).

Lee C, Beuchat LR (1991). Functional and sensory properties of salad dressing containing fermented groundnut milk. *J. Food Sci.* 56L 1664–1667.

Mahe S, Gausseres N, Tome D (1994). Legume proteins for human requirements. *Grain Legumes* (AEP) **7**: 15–17.

Monjula S, John E (1991). Biochemical changes and *in vitro* protein digestibility of the endodermis of germinating (*Dolchos lablab*). *J. Sci. Food and Agric.* 55: 229–238.

Moore S, Stein WH (1963). Chromatographic amino acids determination by the use of automatic recording equipment. *Methods in Enzymology*, 6: 819–831.

Nnam NM (2001). Chemical, sensory and rheological properties of porridges from processed sorghum (*Sorghum bicolor*), bambara groundnut (*Vigna subterrananea* L. Verd) and sweet potato (*Ipomoea batatas*) flours. *Plant foods for human nutrition*, 56: 251-264.

Oboh G, Alabi KB, Akindahunsi AA (2008). Fermentation changes the nutritive value, polyphenol distribution, and antioxidant properties of *Parkia biglobosa* seeds (African Locust Beans). *Food Biotechnology,* 22: 363–376.

Ogbadu LJ, Okagbue RN (1988). Fermentation of African locust bean *Parkia biglobosa* seeds: Involvement of different species of Bacillus. *Food Microbiology*, 5: 195-199.

Omafuvbe BO (2006). Effect of salt on the fermentation of soybean (*Glycine max*) into daddawa using *Bacillus subtilis* as starter culture. *Afri. J. Biotechnol.* 5: 1001-1005.

Omafuvbe BO, Shonukan OO, Abiose SH (2000). Microbiological and biochemical changes in the traditional fermentation of soybean for soy-daddawa – Nig. food condiment*. Food Microbiology*, 17: 469 – 474.

Oria MP, Hamaker BR, Shull JM (1995). Resistance of sorghum alpha-, beta-, and gamma-Kafirins to pepsin digestion. *J. Agricul. Food Chem.* 43: 2148–2153.

Ouoba LI, Nyanga-Koumou CA, Parkouda C, Sawadogo H, Kobawila SC, Keleke S, Diawara B, Louembe D, Sutherland JP (2010). Genotypic diversity of lactic acid bacteria isolated from African traditional alkaline-fermented foods. *J. Applied Microbiol.* 108: 2019-2029.

Pearson ND (1981). In: H. Egon, R.S. Kirk & R. Sawyer (Eds). Pearson Chemical Analysis of Food, 8th edn. London, New York: Churchill Livingstone.

Price ML, Butler LG (1977). Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. *J. Agric. Food Chem.* 25: 1268–1273.

Roy A, Moktan B, Sarkar PK (2007). Microbiological quality of legume-based traditional fermented foods marketed in West Bengal, India. *Food Control*, 18: 1405-1411. Sahlin P (1999). Fermentation as a Method of Food Processing: Production of organic acids, pH development and microbial growth in fermenting cereals. Licentiate thesis. Division of Applied Nutrition and Food Chemistry, Center for Chemistry and Chemical Engineering, Lund Institute of Technology, Lund University.

Salunkhe DK, Chavan JK, Adsule RN, Kadam SS (1992). World oils: Chemistry, Technology and Utilization. New York, USA, Van Nostrand Reinhold.

SAS (1997) 'SAS/STAT user's guide, statistics release. (SAS Institute Inc.: Cary, NC)

Savage GP, Keenan JI (1994). The composition and nutritive value of groundnut kernels. In: J. Smart (Ed). *The Groundnut crop: Scientific basis for improvement*. (173-213). London, Chapman and Hall.

Shah NP, Dave R (2002). Antimicrobial substances including bacteriocins produced by lactic acid bacteria. *Bioscience Microflora*, 21: 217-223.

Southgate DA (1976). The analysis of dietary fiber. In: G.A Spiller, R.J Amen (Eds). Fiber in human nutrition. (73–103). Plenum Press: New York.

Steinkraus KH (1996). *Handbook of Indigenous Fermented Foods*. 2nd Edn., Marcel Dekker. New York, USA.

Tharmaraj N, Shah NP (2009). Antimicrobial effects of probiotics against selected pathogenic and spoilage bacteria in cheese-based dips. *Inte. Food Res. J.* 16: 261-276.

Venkatachalam M, Sathe SK (2006). Chemical composition of selected edible nut seeds. *J. Agric. Food Chem.* 54: 4705–4714.

Wheeler EI, Ferrel RE (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chemistry*, 48: 312-320.

Yagoub AA, Mohamed EB, Ahmed AHR, El Tinay AH (2004). Study on fururndu, a traditional Sudanese fermented roselle (*Hibiscus sabdariffa* L.) seed: Effect on in vitro protein digestibility, chemical composition and functional properties of the total proteins. *J. Agric. Food Chem.* 52: 6143- 6150

Yagoub AA, Mohammed MA (2008). Furundu, a meat substitute from fermented roselle (Hibiscus sabdariffa L.) seed: Investigation on amino acids composition, protein fractions, minerals content and HCl-extractability and microbial growth. *Pakistan J. Nutri.* 7: 352-358.

Yoshidaa H, Hirakawaa Y, Tomiyamaa Y, Nagamizua T, Mizushina Y (2005). Fatty acid distributions of triacylglycerols and phospholipids in groundnut seeds (*Arachis hypogaea* L.) following microwave treatment. *J. Food Composition and Analysis*, 18: 3–14.

Zheng Y, Wang Z, Xiaolong C (2000). α-Amylase productions by *Bacillus subtilis* with drugs in external loop airlift bioreactor. *J. Biochem. Engin.* 5: 115-121.