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Effect of Fermentation Methods on Chemical and Microbial Properties of Mung Bean (*Vigna radiata*) Flour

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ABSTRACT

Mung flours were fermented using spontaneous and backslopping methods for 72 h and microbial analysis over a period of 72 h fermentation was carried out. The samples were subjected to biochemical test, anti-nutrient and selected mineral and vitamin contents evaluation using standard methods. There was a gradual decline in pH from an initial value of 6.24 in unfermented flour to a pH of 3.68 in spontaneous fermentation and 3.87 in backslopping fermentation after 72 h. The total titratable acidity increased from 0.0085% to 0.0105% in fermented sample. The level of the increase did not differ significantly ($p > 0.05$) for the methods. Flours from back-slopping fermentation showed higher increase in protein and ash (17.59% and 18.25% respectively) than spontaneous fermentation with 16.70 % and 6.35% respectively. Similar increases were observed for calcium and iron. The decrease in zinc content was higher in spontaneous fermentation method than in back-slopping methods. Back-slopping fermentation sample had higher Vitamin A, lower phytate and tannin contents than spontaneous fermented sample. Samples from spontaneous fermentation showed significantly ($p < 0.05$) higher increases in microbial load ($1.27 \times 10^5 – 4.08 \times 10^5$ CFU/ml) than that from the back-slopping fermentation method ($0.4 \times 10^5 – 2.9 \times 10^5$ CFU /ml) within the same time interval. Back-slopping method improves the nutritional properties than spontaneous methods and could be encouraged at community levels.

Keywords: Mungbean, spontaneous, backslopping, chemical composition, fermentation.

Introduction

Legumes are probably the second most important source of food next to cereal grains and are consumed worldwide as a major source of protein especially in developing countries. Thus, legumes are good supplements in areas where the staple food is high in carbohydrate but low in protein. Mung beans (*Vigna radiata*) (*olaludi*) is one of the lesser known and underutilized legume in Nigeria. It has high nutritional potentials and has been recently introduced in Nigeria (Mensah and Olukoya, 2007).

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The high lysine content of mung beans makes it a good complementary food for rice-based diets, in which lysine is usually the first limiting amino acid (Chen *et al*., 1987). Mung bean has excellent digestibility and freedom from flatulence; and could be used in creating value-added products for infants, recuperating patient and aged people. Traditionally mung beans can be eaten alone or combined with rice or vegetable to make soup. However, anti-nutritional factors limit the food applications of mung beans as in other legumes. Application of fermentation in processing of some cereals and legumes used in complementary food had shown to reduce their anti-nutrients and increase the safety of those products. Today a variety of foods are produced globally using fermentation technology at household, small, medium and in large commercial levels of productions. Through fermentation process unique flavours are created, textural/rheological properties are modified and developed. According to Lawal *et al.* (2009) fermentation improves food digestibility and nutritional quality. In Nigeria for instance, the popular traditional food 'ogi' is made from fermented maize (*Zea mays*), sorghum (*Sorghum biclolor*) or millet (*Pennisetum typhoides*) and some legumes such as cowpea, bambara groundnut and soya bean has been fermented for complementary food. However the effect of fermentation on mung beans has not been extensively studied. Fermented foods are associated with "good bacteria" referred to as probiotics. Probiotics are beneficial bacteria in that they favourably alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection (Helland *et al.*, 2004). Raw foods themselves may harbour pathogenic micro-organisms. Monitoring the presence of pathogenic bacteria during fermentation process becomes important so as to prevent the problem associated with them.

Fermentation can be achieved by either of the methods. Solid state fermentation, submerged culture fermentation, spontaneous fermentation and back-slopping fermentation methods. Spontaneous fermentation widely employed in developing countries have the disadvantages of low yields of product, variable product quality and in some cases the safety concerns associated with proliferation of microorganism during processing. Use of starter cultures (generally produced using a back-slopping fermentation) could be an alternative method. The objective of the study was to compare the effects of spontaneous and back-slopping fermentation methods on the properties of mung bean flour.

Materials and Methods

Collection and processing of mung bean seeds

Mung bean seeds (*Vigna radiata*) were purchased from Ogige Market Nsukka, Nigeria. Two kilogram (2 kg) of the mung bean seeds was cleaned by winnowing and hand-sorting. The seeds were soaked in 10 litres of water for 12 hours, wet dehulled and oven dried at 65^0C in a Gallenkamp oven (Model 1H-150 England), milled into flour using Bental attrition mill (Model 200 L090) and sieved with sieve (mesh size 200 μm).

Production of fermented flour

Processed mung bean flour was divided into 3 portions. Two hundred grams (200 g) of the flour was left unfermented and it was packed in polyethylene bags and stored at 4^0C. Three hundred grams (300 g) of the flour was fermented using the spontaneous fermentation method for 72 h. Another 300 g portion of mung bean flour was fermented using the back-slopping fermentation method where 10% of the previous day slurry was added as a starter culture to the next day fermentation (Nout *et al.*, 1989). Sample (fermented slurry) was taken at 24 h interval from each fermenting medium for microbial analysis for a period of 72 h, the remaining slurry at the end of 72 h was oven dried at 65^oC in a Gallenkamp oven (Model 1H-150 England), the cake was milled into flour using Bental attrition mill (Model 200 L090) and sieved with sieve (mesh size 200 μm) to get fermented flours.

Microbial analysis

Microbial analysis was carried out on the fermented slurry using the pour plate method (Prescott *et al.*, 2005) and biochemical tests was carried out by enumeration and purification methods.

pH and titratable acidity determination

pH and titratable acidity of the mung bean samples were determined using the method described by Pearson (1976).

Proximate composition determination

Mung bean flour samples were dried and analysed for moisture, crude protein, ash, fat and crude fibre contents according to the standard method of (AOAC, 2010), carbohydrate content of the samples was determined by difference as follows: % Carbohydrate = 100 – (% moisture + % protein + % fat + % Crude fibre + % ash).

Tannin, phytate and saponin determination

The Folin-Denis spectrophotometric method was used to determine the tannin and saponin content of the mung bean samples as described by Pearson (1976) while phytate content was analyzed using the method described by Oberlease (1962).

Minerals and vitamin A content determination

The flours were analysed for calcium, iron and zinc contents using the methods as described by AOAC (2010) and vitamin A was determined according to the rapid method of Car-price described by Pearson (1976).

Statistical analysis

Determinations were carried out in triplicates and the results obtained from the proximate, antinutrients, minerals and vitamin A analysis were subjected to statistical analysis using one way analysis of variance (ANOVA) and the least significant differences were calculated by Duncan multiple range test using SPSS version 16.00 software. Significance was accepted at $p < 0.05$ levels.

Results and Discussion

Effect of fermentation on microbial load of spontaneous and back-slopping Fermented mung bean slurry

Table 1 shows the total viable count, coliform count and mould count during different fermentation methods. The result indicated that there was higher microbial load in spontaneous fermented sample. The microbial load (4.08×10^5 CFU/ml) for spontaneous fermentation samples were higher than that of the sample fermented by back-slopping (4×10^5 CFU/ml). The inoculation of 10% slurry in back-slopping fermentation could have resulted to reduction in microbial load as compared to spontaneous fermentation where fermentation was carried out for 72 h at a stretch and the slurry contains the entire microorganism. No coliform or mould was detected in the fermented sample. The absence of coliform was of significance as the presence of pathogens in food cannot be over emphasized as they have been reported to be a good source of diarrhoea and gastrointestinal disturbance to both adults and children (Owheureghe *et al.*, 1993).

Biochemical characteristics of the bacteria isolated during spontaneous and back-slopping fermentation methods

Table 2 shows the biochemical characteristics of the bacteria isolated during spontaneous and back-slopping fermentation. The suspected bacteria isolated during spontaneous and back-slopping fermentations were both *Lactobacilli* spp. The absence of pathogens during fermentation could be probably as a result of the presence of bacteriocins and acids produced from lactic acid bacteria during the process of fermentation which inhibit the growth of other microorganism. This observation agrees with the work of Olukoya *et al.*, (1994) in his production of *Ogi* called "dogik" where a lactobacillus strain used as a starter culture inhibited the growth of *Vibrio cholera*, *Shigella flexneri*, *Salmonellae typhimurium* and *Escherichia coli* after 36 hours of fermentation. Fermentation leads

Table 1: Microbial load of mung bean slurry fermented by spontaneous and backslopping methods

Fermentation Methods	Fermentation Time/h	Total Viable count (CFU/ml)	Coliform Count (CFU/ml)	Mould Count (CFU/ml)
Spontaneous fermentation	72	8.93×10^5	-	-
Backslopping fermentation	72	8.58×10^5	-	-

- means No growth

to production of acids and probable bacteriocin that prevent growth of microorganism hence increasing shelf life of fermented products (Kalui *et al.*, 2009). This is a very valuable attribute especially in rural areas where advanced food preservation technologies such as refrigeration are not affordable and considering the fact that people are beginning to appreciate more of naturally preserved than chemically preserved.

Effect of fermentation methods on the physico-chemical composition of mung bean flour

The result of pH revealed that there was a gradual fall in pH from an initial value of 6.24 in unfermented flour to a pH of 3.68 in spontaneous fermentation and 3.87 in back-slopping fermentation after 72 h of fermentation. There was a significant difference ($p < 0.05$) in the pH of spontaneous fermented sample and back-slopping fermented sample. The pH is a measure of the degree of acidity or alkalinity of a product. This range of pH is low enough to inhibit the growth of pathogenic microorganism since the minimum pH for the growth of some pathogenic organism such as *E. coli* is pH 4.4, *Salmonella* and *Shigella* spp. (4.5)(Mensah *et al.*, 1990).

Total titratable acidity of mung bean flour is shown in Table 3. There was a gradual increase in total titratable acidity from an initial value of 0.0085% in unfermented flour to a value of 0.0105%. Titratable acidity of samples fermented by both methods increased during fermentation but the level of the increase did not differ ($p > 0.05$) from each other. Acid production has been reported to be responsible for product stability and flavour development.

Table 2: Biochemical characteristics of bacteria isolate during spontaneous and back-slopping fermentation

Test	Spontaneous fermentation		Back-slopping fermentation	
	First isolate	Second isolate	First isolate	Second isolate
Gram staining	+	+	+	-
Shape of cells	Rods (in chains)	Rods (in chains)	Rods (in chains)	Rods (in chains)
Motility	-	-	-	-
Catalase	-	-	-	-
Oxidase	-	-	-	-
Spores	-	-	-	-
Citrate	-	-	-	-
Sugar fermentation				
Glucose	+	+	+	+
Lactose	+	+	+	+
Mannitol	+	+	+	+
Sucrose	+	+	+	+
Suspected organism	*Lactobacilli* spp	*Lactobacilli* spp	*Lactobacilli* spp	*Lactobacilli* spp

- = Negative   + = Positive

The moisture content of unfermented mung bean flour is 11.95% and fermented samples B and C 10.06% and 10.26% respectively (Table 3). The moisture content of the fermented flours were significantly different ($p < 0.05$) from each other. The lower moisture content of all the samples tested showed that they will have better keeping quality. Moisture content in excess of 14% in flours has greater danger of bacteria action and mould growth which produce undesirable changes (Ihekoronye and Ngoddy, 1985)

Table 3: Physicochemical composition of mung bean flour fermented by spontaneous and back-slopping methods

Nutrient	Sample A	Sample B	Sample C
pH	$6.24^{a} + 0.002$	$3.68^{b} + 0.01$	$3.87^{c} + 0.01$
Titratable acidity (%)	$0.009^{a} + 0.07$	$0.011^{a} + 0.07$	$0.011^{a} + 0.07$
Moisture (%)	$11.95^{a} + 0.07$	$10.06^{b} + 0.06$	$10.26^{c} + 0.06$
Crude protein (%)	$30.08^{a} + 0.13$	$32.77^{b} + 0.03$	$33.02^{b} + 0.03$
Crude fat (%)	$1.90^{a} + 0.01$	$1.27^{b} + 0.11$	$1.16^{b} + 0.02$
Crude fibre (%)	$3.01^{a} +0.08$	$4.50^{b} + 0.14$	$4.05^{c} + 0.06$
Ash (%)	$2.52^{a} + 0.02$	$2.68^{c} + 0.28$	$2.98^{b} + 0.04$
Carbohydrate (%)	$52.54^{a} + 0.12$	$48.72^{b} + 0.66$	$48.53^{c} + 0.09$

Results are means of triplicate determination; values carrying different superscript in the same row are significantly different ($p < 0.05$); A = Unfermented mungbean flour (control); B = Mung bean flour fermented by spontaneous fermentation method; C = Mung bean flour fermented by back-slopping fermentation method.

There were significant ($p < 0.05$) difference in the protein contents of unfermented and fermented mung bean sample. The crude protein of unfermented flour (30.08%) was comparable to the value observed by other researchers. Oburuoga and Anyika (2012) reported protein content of 31.31% in dehulled mung bean flour. Fermentation of mung bean flour increased the protein content of samples which was more in back-slopping fermentation sample than spontaneous, however there was no significant different ($p > 0.05$) between the protein content of the fermented samples.

The fat content (1.9%) of the unfermented flour differed significantly ($p < 0.05$) from that of fermented samples (B and C) with value of 1.27% and 1.16% respectively. There was no significant difference ($p > 0.05$) between the fat content of the fermented samples (B and C). The decrease in fat content observed could be attributed to the activities of lipolytic enzymes during fermentation (Uvere *et al.*, 2010).

The crude fibre content of sample A was 3.01% which fell within the range of 0.57 to 5.01% reported by Adel *et al.* (1980). Fermentation increased the fibre content of samples to 4.50% and 4.05% for spontaneous and back-slopping samples respectively. High fibre content makes mung beans a good digestive food.

There were significant difference ($p < 0.05$) in the ash content among the three samples and the values ranged from 2.52% for sample A, 2.68% for sample B to 2.98% for sample C. Fermentation was found to slightly increase the ash content which was observed to be higher in back-slopping method than in the spontaneous method. The high ash content could also be due to loss of dry mater caused by the activities of enzymes and microorganism during fermentation (Uvere *et al.*, 2010).

The carbohydrate content ranged from 52.42% for sample B, 48.72% for sample C to 48.53% for sample A. The decrease in carbohydrate content of fermented flours (obtained from spontaneous and back-slopping fermentations) could be attributed to the use of carbohydrate as a source of energy by microorganism.

Effect of fermentation methods on the calcium, iron, zinc and vitamin A contents of mung bean flour

Table 4 shows the calcium, iron, zinc and vitamin A content of the mung bean flour. The calcium content ranged from 21.50 mg/100 g for unfermented mung bean flour (sample A) to 49.30 mg/100 g for mung bean flour fermented by back-slopping method (sample C). There was a significance different ($p < 0.05$) among the calcium content of the sample. The iron content in sample A (43.45 mg/100 g) was lower than that in sample B. (53.00 mg/100 g) and sample C (56.50 mg/100 g). The higher increase in iron content of sample C could be attributed to the effect of fermentation on legumes. Fermentation was found to be effective in increasing mineral bioavailability (Kiplamai and Tuitock, 2010). Iron and zinc content in sample B was lower when compared to C and this could be as a result of the higher microbial load in sample B and these microorganisms may have utilized the minerals during fermentation. Fermentation results in a lower proportion of dry matter in the sample and the concentrations of some minerals appear to increase when measured on a dry weight basis (Adams, 1990).

Table 4: Selected mineral (mg/100 g) vitamin (μgRE/100g) and antinutrient (mg/100 g) contents of the mung bean flour

Constituents	Sample A	Sample B	Sample C
Calcium	$21.500^{a} \pm 0.01$	$23.40^{b} \pm 0.04$	$49.30^{c} \pm 0.12$
Iron	$43.45^{a} \pm 0.50$	$53.30^{b} \pm 0.01$	$56.50^{c} \pm 0.14$
Zinc	$3.80^{a} \pm 0.03$	$3.05^{b} \pm 0.02$	$3.60^{a} \pm 0.03$
Vitamin A	$265^{a} \pm 0.12$	$105.00^{b} \pm 0.18$	$106.67^{b} \pm 0.06$
Tannin	$1.13^{a} \pm 0.04$	$0.81^{b} \pm 0.13$	$0.63^{b} \pm 0.05$
Phytate	$0.40^{a} \pm 0.01$	$0.17^{b} \pm 0.02$	$0.11^{b} \pm 0.01$
Saponin	$0.18^{a} \pm 0.11$	$0.09^{b} \pm 0.14$	$0.03^{c} \pm 0.04$

Results are means of triplicate determination; values carrying different superscript in the same row are significantly different ($p < 0.05$); A = (control); B =Mung bean flour fermented by spontaneous method; C = Mung bean flour fermented by back-slopping fermentation method.

The vitamin A content of the fermented samples 105 and 106.67 μg RE/100 g for samples B and C respectively) was lower than in the control (265 μg RE/100 g). Fermentation has been reported to result in reduction of vitamin A. Fermented whole onion plant retained 97% of vitamin A activity; while fermented egg plant only retained 34% of the vitamin A activity (Speek *et al.*, 1988). This could be due to the combined effect of hydrolysis of β carotene and improved moisture loss resulting from fermentation.

Effect of fermentation methods on the anti-nutrient content of mung bean flour

Table 4 shows the tannin, phytate and saponin contents of mung bean flour fermented by spontaneous and back-slopping methods. There was a significant ($p < 0.05$) difference in the tannin contents between the control (Sample A with value of 1.13 mg/100 g) and fermented flours (Sample B and C with value of 0.81 mg/100 g and 0.63 mg/100 g respectively). Sample B did not differed ($p > 0.05$) significantly from samples C. Fermentation reduces polyphenol contents in food. Ene-Obong (1995) reported that decrease in tannin contents could be achieved through soaking, dehulling, fermentation and germination. The decrease in tannin content in the fermented product could be attributed to the activity of enzymes associated with seeds.

The result of phytate content followed a similar trend as tannins. The phytate content of the fermented flours was low and this could be due to the effect of fermentation. There was no significant difference ($p > 0.05$) between the fermented samples (B and C) with the value of 0.17 mg/100 g and 0.11 mg/100 g respectively. The results can be compared with the findings of Azeke *et al.*, (2005) who reported significant reduction in the phytic acid content of African yam bean as a result of lactic acid fermentation by *L. plantarum*.

Conclusion

It is evident from this work that the two fermentation methods affect the properties of mung bean flour differently. Back-slopping fermentation improved the crude protein, ash, calcium, iron, zinc better than spontaneous method and also result to lower values of tannin, phytate and saponin hence back-slopping fermentation should be encouraged at community level.

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