

Nutritional and microbiological attributes of soybean*(Glycine max)* during fermentation with *Rhizopus oligosporus*

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Abstract
Soybeans *(Glycine max)* which belong to the family leguminosae constitute one of the oldest cultivated crops of the tropics and sub-tropical regions, and one of the world's most important sources of protein and oil. It is being used because of its good quality and functionality of its proteins, surplus availability and low cost. In this study, a mold *Rhizopus oligosporus* was used to ferment soybean, and the time course (12 h for 3 days) of changes in its characteristic was observed in order to investigate the period of maximum changes, and the consequent effect on nutritional benefits. Proximate composition, vitamins, mineral contents and microbiological characteristics were determined using standard procedures. Results obtained revealed that fermentation for 36 h produced optimal changes ($p < 0.05$) in terms of the contents of protein, ash, fibre, fat, moisture and carbohydrate. Low levels of moisture and carbohydrate contents of fermented soybean flour with increase in fermentation period were obtained while vitamins A, B_2 and niacin increased significantly ($p < 0.05$). Significant increases were also observed with respect to values of the mineral elements. Microbiological analyses indicated that no faecal coliforms were detected, although the populations of other groups of microorganisms increased significantly within the period of fermentation. Therefore, the study has demonstrated a desirable use of *Rhizopus oligosporus* in order to obtain fermented product of soybean which could be used to fortify starchy foods.
Keywords: Fermentation, soybean, *Rhizopus*, nutritional and microbiological attributes

1. Introduction

Reasearch has continued to focus on the need and how to augment the low level of protein in the diets of the vulnerable group of people in developing nations. In these developing nations where protein deficiencies are endemic and where animal-proteins are not available in adequate quantities, a well planned vegetarian diet, based on the concept of mutual supplementation seems to be a logical solution to the protein problem(Sanni *et al.,* 2005). The tremendous extra values previously ascribed to proteins of animal origin have been critically re-examined. It is now generally accepted that highly digestible plant proteins, which are heat treated to remove anti-nutritional factors and properly supplemented with essential amino acids where needed, can produce results equivalent or sometimes superior to those obtained with animal protein sources (Mensah and Sefa-Dedeh, 1991; Sanni *et al.,*2005). In Nigeria, and several other developing tropical countries soybean as a result of its rich and quality protein has been gaining enhanced utilization. In this regard, processed flour from the soybean is being used to supplement a variety of starchy food products for the purposes of enrichment and fortification (Otunola, *et al*., 1998; Oyelade *et al*., 2002; Sunny-Roberts *et al*., 2003).

Fermentation processes generally bring many benefits to mankind and this is being heavily relied upon in developing countries. It is known to play important role by providing food security, enhancing livelihoods and improving the nutritional and social well being of millions of people around the world, particularly the marginalized and vulnerable. FAO (1988) highlighted the world food insecurity problem as a result of undemocratic and inadequate distribution of, and access to resources rather than a problem of global food production.

Tempeh, a fermented soybeans product that originated in Asian countries has been widely reported to be far richer in protein, vitamins and minerals, and much lower in antinutritional and toxic constituents than the raw soybeans. Its consumption, which was initially limited to Indonesia and other Asian countries has therefore in recent time increased tremendously. This trend is all over the developed and parts of the developing world, especially in those countries where the cost of animal protein is increasingly becoming prohibitive (Steinkraus, 1996).

The main objective of this study was to determine the effect of fermentation using *Rhizopus oligosporus* on the nutritional and microbiological attributes of soybean flour. The nutritional attributes include proximate (protein, ash, fat, fibre, moisture and carbohydrate contents), vitamin contents (vitamin A, vitamin B_2 and niacin contents) and mineral contents (calcium, magnesium, potassium, iron and phosphorus) while the microbiological properties which include total viable bacteria, yeast, mould, lactic acid bacterial, coliforms and feacal coliform counts were investigated.

2.Materials and methods

2.1Material preparation

The soybean (*Glycine max*) and rice grain (*Oryzae sativa*) used for this study were obtained from a local market in Ogbomoso (Nigeria) while the inoculum (*Rhizopus oligosporus*) was obtained from the Indonesian Embassy, Victoria Island, Lagos (Nigeria). The chemicals that were used were of analytical grade and were obtained from reputable suppliers.

2.1.1 **Preparation of subculture for soybean fermentation**

The subculture of *R. oligosporus* was prepared by the procedure described by Olanipekun *et al.* (2009) as indicated in Figure 1.

2.1.2 Preparation of fermented soybean flour

This was done according to the method of Olanipekun *et al.* (2009) and shown in Figure 2. The unfermented soybean (0 h fermentation) served as the control.

2.2 Analyses

The proximate composition (crude protein, ash, fat, fibre, moisture and carbohydrate contents), vitamin and mineral contents were determined using standard methods (AOAC, 2000). All the determinations were made in triplicates.

The microbiological properties were carried out by using standardised procedures (Adegoke, 2004). Ten grammes (10g) of fermented soybean flour was added to 100 ml of sterile distilled water and mixed thoroughly using Stuat scientific auto vortex mixer. 1ml of the mixture was serially diluted for estimating the number of microorganisms. Nutrient agar (NA) was used for total viable bacteria count, while potato dextrose agar (PDA) was used for yeast and mould counts. Mac Conkey agar was used for coliform count and Eosin Methylene Blue (EMB) for faecal coliform count, while de-Man Rogosa and Sharpe agar (MRS) was used for lactic acid bacteria count.

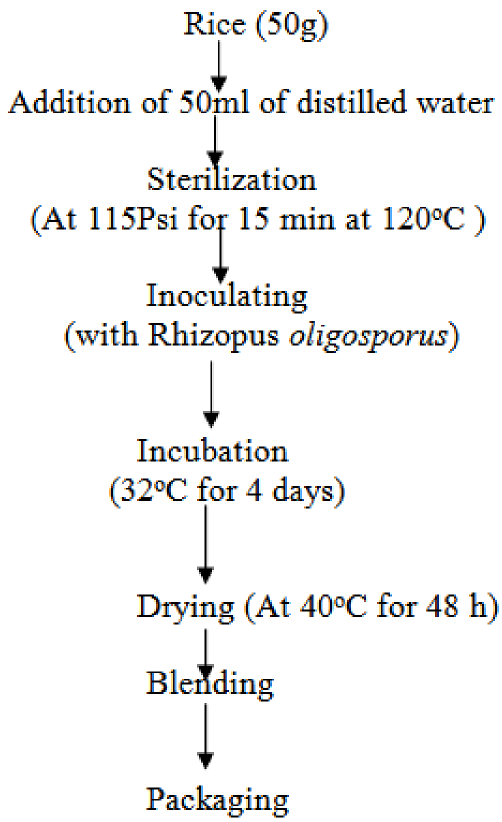

Figure 1: Flow chart for the preparation of subculture for soybean fermentation
Source: Olanipekun *et al.* (2009)

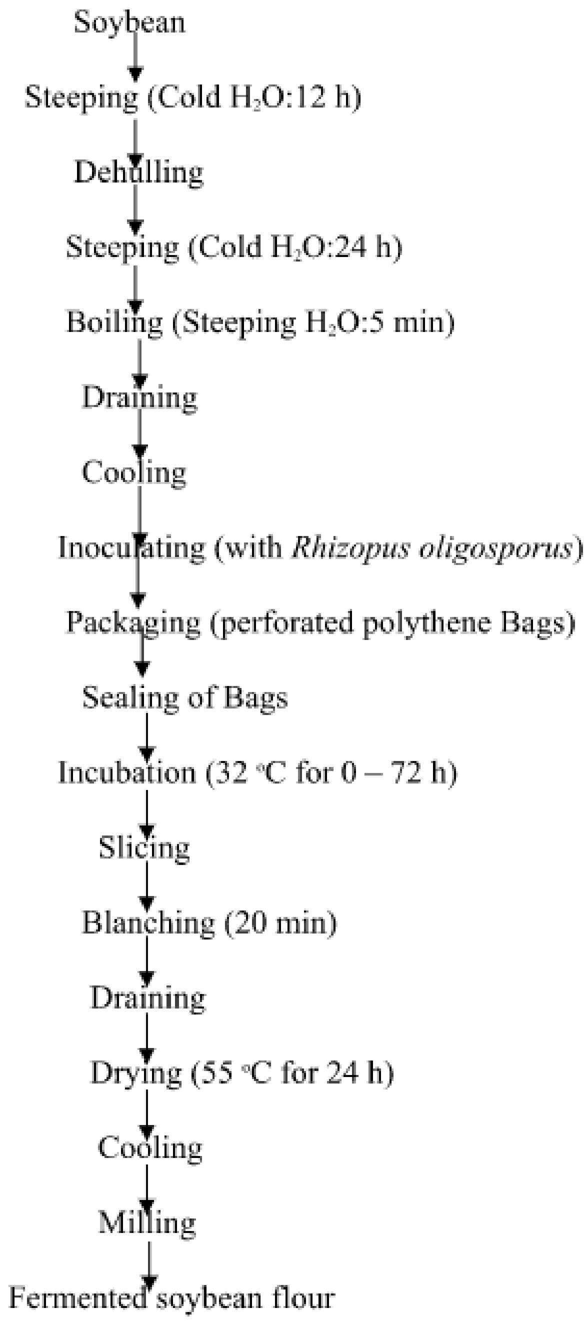

Figure 2: Flowchart for the production of fermented soybean flour
Source: Olanipekun *et al*. (2009)

2.3 Statistical analysis
Statistical analyses were carried out using SAS software. Data were subjected to analysis of variance and Duncan's multiple range test was used for comparison of means and the significance level at $P \leq 0.05$ (Duncan, 1955; Poste *et al.,* 1991).

3. Results and discussion
3.1 Proximate composition of fermented soybean flour
Data obtained on the proximate composition of soybean flour during fermentation with *Rhizopus oligosporus* are as illustrated in Table 1. The level of protein content increased from 39.40% in the unfermented soybean flour to 48.28% after 72 h of fermentation. The increases were more rapid within the first 0 - 30 h ($P<0.05$) of fermentation. Thereafter there were no significant increases between 30 - 72 h of fermentation ($P<0.05$) as

shown in Table 1. The results obtained in this study for protein agrees with the trend obtained during the fermentation of oil bean *(Pentaclethra macrophylla)* seed with *Bacillus subtilis*, *Staphylococcus spp.*, *Micrococcus spp.* and *Corynebacterium spp*., as reported by Achinewhu (1983). This increase could result from slight protein synthesis proliferation of the micro organism used and a synthesis of enzyme proteins or from a rearrangement of the composition following the degradation of other constituents (Totora *et al.,* 2002). Similar results were also reported by the researchers such as Adebowale and Maliki (2011) ; Makanjuola and Ajayi (2012).
The fat content decreased from 20.37% in the unfermented soybean flour to 17.76% after 72 h of fermentation. This decreased trend was more significant at p<0.05 within the first 0-30 h of fermentation. Thereafter, the decreases became less noticed for rest of the fermentation period. The decrease in fat content could have been due to the action of lipase produced by *Rhizopus oligosporus* as fermentation period progressed in agreement with the report on fermentation of oil bean by Achinewhu (1983). Decrease in fat content of soybean and condiment produced from fermented legumes were most likely to be due to their utilization by the growing microorganism (Ganiyu, 2006; Babalola and Giwa, 2012).

Table 1: **Proximate composition of fermented soybean flour**

Sample	Period of fermentation (h)	Protein content	Fat content	Fibre content	Ash content	Moisture content	Carbohydrate content
A	0	39.40_d	20.37_a	5.15_b	3.73_b	5.51_a	25.85_a
B	12	43.46_c	19.77_a	5.03_b	4.05_{ab}	5.46_{ab}	22.54_b
C	24	47.83_a	18.77_b	4.92_b	4.27_a	5.45_{ab}	20.00_c
D	30	48.03_a	17.87_c	4.57_c	4.39_a	5.35_a	19.99_c
E	36	48.15_a	17.82_c	5.87_a	4.09_{ab}	5.10_c	19.09_d
F	48	48.24_a	17.79_c	5.91_a	4.11_{ab}	5.09_c	18.96_d
G	60	48.24_a	17.79_c	5.93_a	4.18_{ab}	5.06_c	18.82_d
H	72	48.28_a	17.76_c	5.94_a	4.21_{ab}	5.05_c	18.76_d

Values are means of three determinations.
Values with the same subscript in the same column are not significantly different (P<0.05).

The fibre content of the fermented soybean flour initially remained fairly constant within the first 30 h of fermentation. Thereafter, it slightly decreased for the rest of fermentation period. Fibres are known to help in the lowering of serum chloesterol, control of blood sugar, increase in stool bulk which may prevent colon cancer and other several digestive disorders (NSRL, 2002). A number of feed formulations can also be expected to include the use of fermented soybean flour as part of livestock ration due to the capability of enhanced digestibility (FFTC, 2002; NSRL, 2002) This will be in relation to increased fibre value particularly when the fermentation period increased between 30 - 72 h.
Also, the general low moisture contents of the fermented soybean flour further reduced slightly from 5.51% to 5.10% between 0 and 36 h of fermentation. There were no significant differences at P<0.05 in moisture contents from 36 to 72 h of fermentation. The significantly low level of moisture contents in all the samples has the potential to cause reduction in microbial spoilage. The ash content increased significantly (P<0.05) from 3.73% to 4.27% within the first 24 h of fermentation. Subsequently, no significant differences were observed (P<0.05) from 24 to 72 h. Increase in the ash content of the fermented soybean was in accordance with the reports of Sefa-Dedeh *et al.* (2000) and Adebowale and Maliki (2011) on cowpea fortification for the production of traditional foods and fermented pigeon pea. Ash content increment leads to increase in the mineral contents.
The carbohydrate content decreased with increases in the period of fermentation (P<0.05). The reduction in carbohydrate content of fermented soybean flour could be attributed to the possible bioconversion of the substrate by enzymes from *Rhizopus oligosporus* into other substances, especially protein and fats, in addition to the portion used as carbon and energy source by the organism. Similar decreasing trend in carbohydrate content have been reported to occur during fermentation in some starchy substrates such as yam, cassava and sweet potato (Ogundana *et al., 1*992; Akindahunsi *et al.,* 1991; Oboh *et al*., 2002 and Atum , 2003).

Vitamins and mineral contents
Tables 2 and 3 showed the influence of fermentation time on the vitamins and mineral levels of soybean flour. It was observed that increased fermentation time increased the mineral and vitamin levels. Vitamins A, B_2 and Niacin were found to increase significantly at P<0.05 from 0.95 to 1.50, 0.05 to 0.12 and 0.01 to 0.04 mg/100g,

respectively, within the period of fermentation. Steinkraus (1992) had earlier reported that vitamin content of fermented soybean was higher than that of the starting unfermented soybeans in certain cases and lower in others. Riboflavin specifically doubled while niacin increased seven times as fermentation period increased. Minerals such as calcium, magnessium, potassium, iron and phosphorus were also observed to follow increasing trend which was significant at P<0.05. As the fermentation increased from 0 - 72 h, the aforementioned minerals ranged between 17.84 to 21.26, 4.10 to 7.28, 2.20 to 4.73, 0.77 to 1.09 and 3.75 to 5.74 mg/100g, respectively. These constitute essential minerals for the growth, proper formation of bones and other metabolic activities of the body. The fermentation of oil bean as reported by Achinewhu (1983) also resulted in increase in vitamin and mineral contents.

Table 2: **Vitamin contents of fermented soybean flour**

Sample	Period of fermentation (h)	Vitamin A (mg/100g)	Vitamin B_2 (mg/100g)	Niacin (mg/100g)
A	0	0.933_{d}	0.057_{d}	0.013_{d}
B	12	1.033_{d}	0.063_{cd}	0.017_{d}
C	24	1.067_{d}	0.073_{cd}	0.017_{d}
D	30	1.133_{cd}	0.080_{bcd}	0.020_{cd}
E	36	1.200_{bcd}	0.093_{abc}	0.023_{bc}
F	48	1.367_{abc}	0.107_{ab}	0.030_{ab}
G	60	1.467_{ab}	0.120_{a}	0.030_{ab}
H	72	1.500_{a}	0.123_{a}	0.043_{a}

Values are means of three determinations.
Values with the same subscript in the same column are not significantly different (P<0.05)

Table 3: Mineral contents of fermented soybean flour

Sample	Period of fermentation (h)	Calcium (mg/100g)	Magnessium (mg/100g	Potassium (mg/100g	Iron (mg/100g
A	0	17.843_{g}	4.100_{g}	2.203_{h}	0.770_{f}
B	12	18.233_{f}	5.120_{f}	3.170_{g}	0.857_{e}
C	24	18.493_{e}	5.383_{e}	3.213_{f}	0.867_{e}
D	30	19.417_{d}	5.643_{d}	3.250_{e}	0.930_{d}
E	36	19.513_{cd}	5.647_{d}	3.720_{d}	1.007_{c}
F	48	19.637_{c}	5.940_{c}	3.930_{c}	1.047_{b}
G	60	20.393_{b}	6.380_{b}	4.137_{b}	1.063_{ab}
H	72	21.260_{a}	7.277_{a}	4.730_{a}	1.087_{a}

Values are means of three determinations.
Values with the same subscript in the same column are not significantly different (P<0.05)

Microbiological analysis

The results of the total viable counts of the samples are shown in Table 4. The unfermented sample had the least growth while sample fermented for 72 h had the highest growth. The growth was significant at P<0.05. This could be due to prolonged fermentation which increased the presence of lactic acid bacteria as reported by Sunny-Roberts *et al.* (2003).

No coliform bacteria were detected in any of the samples fermented within the period (0 - 72 h). This is probably due to low pH which did not support the survival and growth of pathogen (Hesseltine, 1965). This is probably caused by increase in the population of lactic acid bacteria within the period of fermentation. Also, antimicrobial substances produced by lactic acid bacteria inhibit coliform growth. All the samples fermented within the range of 0 - 72 h were completely free of faecal coliform contamination. This could be corroborated with the result obtained from microbiological analysis of fermented soybean (Babalola and Giwa, 2012). The results of the yeast in the samples fermented within the period (0 - 72 h) are as shown in Table 4. The population of yeasts increased from 1.21 to 2.95 x 10^5 cfu after 72 h of fermentation. The low pH which was due to increase in population of lactic acid bacteria favoured growth of yeast within the period of fermentation as also reported by Sunny-Roberts *et al*. (2003).

The populations of moulds in the samples are as indicated in Table 4. From the table, the population of moulds increased from 1.40 to 2.98 x 10^5 cfu after 72 h of fermentation. The reason is probably due to prolonged fermentation which increased the presence of viable spores of *Rhizopus oligosporus* as reported by Sunny-

Roberts et al. (2003). The mould growth was significant at P<0.05. Also, the populations of the lactic acid bacteria in the samples are as shown in Table 4. From the table, the population of lactic acid bacteria increased from 1.52 to 2.97 x 10^5 cfu after 72 h of fermentation. Their growth was favoured by the decrease in pH as fermentation progresses (Uzogara *et al.,* 1990). Lactic acid act as a flavouring agent and also as antimicrobial substances which inhibit pathogenic organisms. The growth was significant at P<0.05.

Table 4: Microbiological analysis of fermented soybean flours

Sample	Period of fermentation (h)	Yeast Count ($x10^5$) CFU	Mould Count ($x10^5$) CFU	Total Viable Bacteria Count ($x10^5$) CFU	Coliform Count (x 10^5) CFU	Faecal Coliform Count (x 10^5) CFU	Lactic Acid Bacteria Count (x 10^5) CFU
A	0	1.21_g	1.40_h	1.52_g	0	0	1.52_e
B	12	1.34_f	1.63_g	1.74_f	0	0	1.63_d
C	24	1.56_e	1.92_f	1.96_e	0	0	1.80_c
D	30	1.85_d	2.20_e	2.20_d	0	0	2.02_c
E	36	2.15_c	2.47_d	2.45_c	0	0	2.10_c
F	48	2.34_c	2.60_c	2.66_b	0	0	2.12_b
G	60	2.62_b	2.78_b	2.88_a	0	0	2.30_a
H	72	2.95_a	2.98_a	2.97_a	0	0	2.56_a

Values are means of three determinations.
Values with the same subscript in the same column are not significantly different (P<0.05)

Conclusions

This study revealed that the length of fermentation affected the nutritional profile of soybeans,*Glycine max*. Within the period of 0 - 72 h of which soybean was subjected to fermentation with *Rhizopus oligosporus*, it was observed that fermentation for 36 h produced optimal changes, after which no significant difference at $p<0.05$ were observed for the rest of fermentation period. The fermented soybean flours showed slight changes in moisture content, decreasing from 5.51 to 5.05% (wet basis) as fermentation period increased from 0 to 72 h. The protein, fibre and ash contents increased in values from 39.40 to 48.28, 5.51 to 5.94 and 3.73 to 4.21%, respectively, as fermentation time increased. However, the fat and carbohydrate contents decreased in values from 20.37 to 17.76 and 25.85 to 18.76%, respectively. Vitamin A, vitamin B_2 and niacin were found to increase from 0.95 to 1.50, 0.05 to 0.12 and 0.01 to 0.04 mg/100g. Minerals ranged between 17.84 to 21.26, 4.10 to 7.28, 2.20 to 4.73, 0.77 to 1.09 and 3.75 to 5.74 mg/100g, respectively for the calcium, magnesium, phosphorus, potassium and iron.

Recommendation

The focus of this study has been on the nutritional profiles of soybean flour as influenced by fermentation with *Rhizopus oligosporus* over a period of time. Due to the fact that some results obtained indicated significant improvements in the nutritional status. It is hereby recommended that fermented soybean flour should be popularised so as to improve the nutritional value of starchy diets and consequently reduce the level of malnutrition, especially in the developing countries. This can be achieved through appropriate awareness programme for women. There is also the need to make sub-culture for soybean fermentation which is *Rhizopus oligosporus* available commercially because of the attendant nutritional advantages it can offer.

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