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Nutritional and Chemical Properties of Fermented Soybean-supplemented Cassava Flour

G. A. Thomas[1*] and C. O. O. Olaiya[1]

[1]*Nutritional and Industrial Biochemistry Laboratories, University of Ibadan, Ibadan, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Authors COOO and GAT designed the study. Author GAT performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GAT managed the analyses of the study. Authors GAT and COOO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Cassava is the most important tropical root crop; its starchy roots are a major source of dietary energy for more than 700 million people. To combat the poor protein content of cassava and help with the malnutrition faced by the millions of people who consume cassava, the bulk of which are in developing countries, protein supplementation using soybeans is used to offset this deficiency. Even with the increasing amount of research on the nutritional benefits of soybean fermentation, there is a dearth of information on how using fermented soybeans as a protein supplement can help improve nutritional and physiochemical characteristics of soybean-supplemented cassava flour. This study investigated the effect of varying fermentation duration on the nutritive value of fermented soybean-supplemented cassava flour. The cassava flour was fortified with soybean flour in the ratio-90:10, and was grouped according to the duration of fermentation with cassava only and cassava: soybean flour both without fermentation serving as the negative and positive controls respectively. The results obtained showed varying effects on mineral content, with potassium, showing consistent increases with increasing fermentation, whilst zinc and phosphorous has its highest values after 4 days of fermentation, whilst Iron showed significant reduction across the

**Corresponding author: E-mail: gbengaayodeji@ymail.com;*

treatment groups. Notable improvements in crude protein, ash content and crude lipid levels were observed with fermentation, with other proximate parameters showing no significant difference when compared to both controls (*P*=.05). There were significant reductions in the pH and Titratable acidity values of the treatment groups.
From this study, fermentation has been proved to be a useful tool that can be used to improve the nutritional quality and safety profile of protein supplemented cassava flour as raw materials for the development of food products.

Keywords: Fermented soybeans; cassava; proximate analysis; food product development.

1. INTRODUCTION

Manihot esculenta (commonly called cassava) is a perennial woody shrub native to South America and introduced into Africa in the Congo basin by the Portuguese around 1558. It is the third largest source of food carbohydrates in the tropics, after rice and maize and is also a major staple food in the developing world, providing a basic diet for over half a billion people. It is one of the most drought-tolerant crops, capable of growing on marginal soils with limited labour requirements. Nigeria is the world's largest producer of cassava with an annual output that is over 45 million metric tonnes, while Thailand is the largest exporter of dried cassava [1]. Cassava is a perennial plant that grows best under tropical, moist, fertile, and well-drained soils. Completely grown plant reaches to a height of about 2-4 metres. After about 8-10 months of plantation; long, globular roots or tubers grow in a radial pattern downwards deep into the soil from the bottom end of stem up to the depth of 4 meters and feature grey-brown, rough, woody textured skin. Each tuber weighs from half a kilogram to several kilograms depending upon the cultivar. Its interior flesh features white/yellowish, starch rich sweet-flavoured meat, that should be eaten only after proper preparation due to the presence of anti-nutritional factors and toxins, the most important of which is cyanide which can cause acute cyanide intoxication, goitre, and even ataxia or partial paralysis if ingested [2]. It is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates [3]. In Sub-Saharan Africa, cassava is mainly a subsistence crop grown for food by small-scale farmers who sell the surplus. Roots can be harvested between 6 months and 3 years after planting. Apart from food, cassava has other uses and its derivatives are used in many types of industrial production generating diverse products such as confectionery, sweeteners, glues, plywood, textiles, paper, biodegradable products, monosodium glutamate, and drugs. Cassava chips and pellets are also used in animal feed and alcohol production.

Cassava roots are very rich in starch and contain significant amounts of calcium (50 mg/100 g), phosphorus (40 mg/100 g) and vitamin C (25 mg/100 g). However, they are poor in protein and other nutrients with nutritional composition differing according to the variety and age of the harvested crop, soil conditions, climate, and other environmental factors during cultivation [4]. To help supplement the low protein content of cassava, soybeans have been incorporated into cassava food products especially in sub-Saharan Africa due to its availability and its high rank amongst vegetable protein sources. Soybeans are an exceptional source of essential nutrients, like iron, manganese, phosphorus, vitamin K, magnesium, zinc and potassium and several B vitamins, including folate. This process of supplementing the cassava flour with soybeans increases the low concentration of micronutrients and protein in one of the most commonly consumed staple foods thereby helping to alleviate some nutritional deficiencies faced by people whose diets consist mainly of cassava. Supplementation is particularly relevant in these times when price increases for non-staple foods further curtail dietary diversity and food security amongst the poor [5]. In principle, this strategy allows the population to grow and consume the same foods they are accustomed to eating while improving the quality of their food. Although concentrations of micronutrients in many of these biofortified crops will remain relatively low, staple foods are eaten in such large quantities in many at-risk populations that, over time, the micronutrients consumed in this manner can enhance micronutrient status and prevent nutritional deficiencies. Numerous studies have shown the nutraceutical benefits of fermented soybeans to include cancer prevention [6], coronary heart disease prevention due to its anti-atherogenic properties, improving glucose control and reducing insulin resistance [7] as well as an

improvement in the nutritional profile of fermented soybeans when compared to unfermented soybeans due to increased protein content, elimination of trypsin inhibitors, and a reduction of peptide size in soybeans allowing for better amino acid assimilation [8]. This study aims to look at the possible effects of using fermented soybeans to fortify cassava flour by checking the nutritional content and some physicochemical parameters of the fermented soybean-supplemented cassava flour.

2. MATERIALS AND METHODS

2.1 Sample Source

Fresh cassava tubers were purchased from a farm in Ibadan, Nigeria while soybeans were purchased from Bodija market, Ibadan, Nigeria. The samples were identified and authenticated at the Botany Department, University of Ibadan, Nigeria.

2.2 Sample Preparation

2.2.1 Cassava flour

About 15 kg of the cassava tubers were harvested and sorted according into five groups. They were peeled using stainless steel knives with the woody tips removed, they were cut into chunks and seeped in water and left to naturally ferment for two to eight days at room temperature. The fermented tuber chunks were then crushed /pulped by hand and dewatered with a press, after which they are sun dried for two days. The dried pieces were milled with an attrition mill to obtain the flour and then sieved with a 450-microns mesh to remove the fibre.

2.2.2 Soybean flour

The soybeans were picked, sorted and cleaned before seeping in water and allowed to naturally ferment for two to eight days at room temperature. The fermented beans were then removed from the fermenter and air-dried. The dried fermented beans were then milled and sieved with a 450 microns mesh to obtain the soybean flour.

2.3 Study Design

The cassava flour was fortified with soybean flour in the ratio-90:10, and were grouped according to the duration of fermentation.

Sample A: Cassava Flour Only (No fermentation) (Control)
Sample B: Cassava Flour + Soy Bean Flour (No fermentation) (Positive Control)
Sample C: Cassava Flour + Soy Bean Flour (2 Days fermentation)
Sample D: Cassava Flour + Soy Bean Flour (4 Days fermentation)
Sample E: Cassava Flour + Soy Bean Flour (6 Days fermentation)
Sample F: Cassava Flour + Soy Bean Flour (8 Days fermentation)

2.4 Determination of Physical Parameters

2.4.1 Determination of pH

Ten grams of the cassava flour samples were mixed with 100 ml of distilled water, and the resulting mixture allowed to stand for 15 minutes, shaken at 5 minutes interval and filtered. The pH of the filtrate was then measured using a pH meter (PHS-3C, Yoke Instruments, China).

2.4.2 Determination of titratable acidity

Ten grams of the cassava flour, was weighed into a conical flask and dissolved in 10 ml of distilled water and subsequently filtered with Whatman No.1 filter paper. The filtrate was titrated against 0.1 M NaOH using 1% phenolphthalein as an indicator.

2.5 Proximate Composition Analysis

2.5.1 Determination of ash content

Ash Content determination was carried out according to the method described by AOAC [9]. Five grams of each of the samples was weighed into a crucible, the samples were then placed on a Bunsen flame to char it; and transferred into a preheated muffle furnace at 600℃ and heated for 2 hours. The sample was then removed from the muffle furnace, cooled in a desiccator to room temperature with the resultant ash reweighed immediately. The weight of the residual ash was then calculated as:

$$Ash\ (\%) = \frac{[weight\ of\ crucible + ash](g) - weight\ of\ crucible\ (g)}{Sample\ weight\ (g)} \times 100$$

2.5.2 Determination of moisture content

Moisture content was determined according to the method of AOAC [9]. Two grams of each of the samples was weighed into a dried weighed

crucible and put into a moisture extraction oven at 75℃ for 20 hours and then at 105℃ for the next 4 hours with the sample allowed to cool in a desiccator, the process was repeated until constant weight was achieved. The moisture content of the sample was calculated as:

$$Moisture\ content\ (\%) = \frac{W_2 - W_3}{W_1} \times 100$$

Where, W_1 = weight of sample, W_2 = weight of undried sample + crucible, and W_3 = weight of dried sample + crucible.

2.5.3 Determination of crude fibre

Crude Fibre was determined by the method described by AOAC [9]. One gram of each sample was weighed into a 500-ml conical flask with 100 ml of digestion reagent poured into it. The conical flask was boiled and refluxed for 45minutes and the flask was removed from the heater and cooled. After the sample, had been cooled, it was filtered through a Whatman No. 1 filter paper. The residue on the filter paper was washed six times with hot water and once with methylated spirit. The filter paper was opened and the residue (fibre) was transferred to a silica dish with a spatula and ashed at 600℃ overnight in a muffle furnace, then cooled in a desiccator and weighed.

$$Crude\ Fibre\ (\%) = \frac{dry\ weight\ of\ residue\ before\ ashing - weight\ of\ residue\ after\ ashing}{weight\ of\ the\ sample} \times 100$$

2.5.4 Protein determination (Kjeldhal method)

The kjeldahl method described by AOAC [9] was used. Two grams of each sample was weighed into a Kjeldhal flask with five grams of sodium sulphate and one gram of copper sulphate also added. 25 ml of concentrated sulphuric acid with glass beads were also introduced into the Kjeldhal flask in order to prevent bumping during heating. The solution was then heated in a fume cupboard and shaken gently until the solution became green. The solution was then cooled and reheated until the green colour disappeared, then it was allowed to cool and subsequently transferred into a 250-ml volumetric flask followed by several washings. It was then distilled using a Markham distillation apparatus.

2.5.4.1 Method of distillation

The Markham apparatus was steamed through for 15 minutes and 5 ml of the digest was added into the apparatus and washed down with distilled water followed by 5 ml of 60% NaOH solution to prevent the loss of ammonium, the mixture was then distilled and the distillate collected into a receiving flask containing 4% boric acid solution with 3 drops of methyl red indicator. The solution in the receiving flask was titrated against N⁄100 (0.01N) hydrochloric acid and the crude protein values were calculated with the following formulas:

$$\text{Crude Protein } (\%) = 6.25^{*} \times \ \%\text{N } (^{*}.\text{Correction factor})$$

$$\text{N } (\%) = \frac{(\text{S} - \text{B}) \times \text{N} \times 0.014 \times \text{D} \times 100}{\text{Wt. of the sample} \times \text{V}}$$

Where, S = Sample titration reading, B = Blank titration reading, N = Normality of HCl, D = Dilution of sample after digestion, V = Volume taken for distillation, 0.014 = Milli equivalent weight of Nitrogen.

2.5.5 Determination of crude fat

Crude fat levels were determined using the soxhlet extraction method described by AOAC [9]. About 300 cm^3 of petroleum ether was placed into the boiling flask, 2 grams of each sample was added into labelled weighed thimbles and plugged lightly with cotton wool. The thimble with its content was then placed into the extractor, the ether in the boiling flask is then heated. The Soxhlet apparatus was then allowed to reflux for about 6 hours, afterwards the thimble was removed. With most of the petroleum ether having been distilled from the flask into the extractor, the flask was then removed and dried at 105-110℃ for 1 hour and transferred from the oven into desiccators and allowed to cool, then weighed.

$$Crude\ Fat\ (\%) = \frac{W_2 - W_1}{W_3} \times 100$$

Where, W_1 = weight of the empty extraction flask, W_2 = weight of the flask and oil extracted, and W_3 = weight of the sample.

2.5.6 Determination of total carbohydrates

The total carbohydrate content (Nitrogen free extract) for of each sample was determined by subtracting the sum of percentages of the other proximate analysis parameters from 100.

Calculation:

Carbohydrate (%) ═ 100- (crude fibre + moisture + ash content + crude fat + crude protein).

2.6 Determination of Minerals

One gram of each sample was added into a 100-ml beaker, 5 ml of nitric acid and 2 ml of $HClO_4$ was added into the beaker. The beaker was covered and digested by heating to a final volume of 3-5 ml. A light/pale yellow clear solution was obtained which was made up to 25 ml with distilled water in a standard volumetric flask. Readings were then taken using an Atomic Absorption spectrophotometer to measure the mineral content. (Unicam model 929).

2.7 Statistical Analysis

Analyses was carried out in triplicates and all data obtained were subjected to One-way Analysis of Variance (ANOVA) followed by Sidak's multiple comparisons test using GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. The level of significance used was *P*=.05.

3. RESULTS

3.1 Chemical Properties

The proximate values of the samples are shown in Table 1, the cassava flour samples that were supplemented with fermented soybeans all showed significant (*P*=.05) increases in their crude protein levels, with values ranging from 4.9% to 9.5%, the ash content of the flour samples that were supplemented with soybeans fermented for two, four and six days showed no significant change when compared with the positive control, while the cassava flour supplemented with soybeans fermented for eight days had an average value of 3.003±0.035 indicating a significant increase. There was no significant (*P*=.05) difference in the crude fibre (4.02-4.167%), dry matter (92.79-93.44%) and moisture content (6.56-7.21%) values of the samples. The crude fat values of the samples ranged from 2.43-2.95%, with sample F showing the most significant (*P*=.05) increase when compared to the control with an average value of 2.95±0.05. For the total carbohydrates, the cassava flour samples supplemented with fermented soybeans showed a significant (*P*=.05) decrease when compared to the controls.

The pH and titratable acidity of the samples are shown in Table 2, The pH values range from 3.03-5.22 with the samples supplemented with fermented soybeans showing a significant reduction across the treatment groups when compared to the control. The titratable acidity of the samples (0.020-0.088 %Lactic acid) also follow the same trend.

3.2 Mineral Content

The mineral composition of the samples is shown in Figs. 1, 2 and 3. The calcium and Iron values are presented in Fig. 1. Iron was the least abundant mineral in the flour samples with values ranging from 42.75-80.75 mg/kg. Calcium level was highest in the sample supplemented with soybeans fermented for two days with the other samples not showing a significant difference when compared to the soybean-supplemented cassava flour. Fig. 2 shows the amount of potassium and magnesium in the samples. The minerals, phosphorus and zinc are presented in Fig. 3, there was no significant change in the amount of Phosphorus in the flour samples when compared to the controls, Zinc was highest in cassava flour supplemented with soybeans that has been fermented for four days.

4. DISCUSSION

Cassava tubers are a very important source of nutrition especially for people in the tropics / developing countries and as a result of its importance, a large amount of work has gone into determining its nutritional composition which led to the revelation of the tuber's poor protein content, leading to the recommendation that cassava meals can be supplemented with protein-rich sources, one of the more recommended protein sources for cassava supplementation is soybeans, due to its high protein content, low cost and widespread availability. Soybean fermentation has been shown to improve the nutritional values of the legume [10], and as a result, this study was designed to investigate the nutritional and chemical values of fermented soybean-supplemented cassava flour.

Table 1. Effect of fermentation on proximate composition

	Crude protein (%)	ASH (%)	FAT (%)	Crude fibre (%)	Dry matter (%)	Moisture (%)	CHO (%)
A	4.667 ±0.126	2.967± 0.065	2.533±0.252	4.07±0.0361	93.2 ±0.02	6.8±0.02	78.967±0.388
B	4.867 ±0.029	1.557±0.021	2.917±0.076	4.167±0.115	93.137±0.135	6.897±0.091	80 .59±0.21
C	4.933±0.058	1.48±0.026[a]	2.433±0.153[b]	4.02±0.026	92.953±0.136	7.047±0.136	80.087±0.257[ab]
D	5.95±0.05[ab]	1.367±0.029[a]	2.733±0.058	4.117±0.076	93±0.1	7±0.1	78.8±0.05[b]
E	9.467±0.029[ab]	1.533±0.058[a]	2.783±0.029	4.12±0.026	92.79±0.215	7.21±0.215	74.88 7±0.228[ab]
F	6.333±0.058[ab]	3.003±0.035[b]	2.950±0.05[a]	4.033±0.042	93.437±0.319	6.563±0.319	77.117±0.335[ab]

A= Cassava Flour Only, B= Cassava Flour + Soy Bean Flour (No fermentation), C= Cassava Flour + Soy Bean Flour (2 days fermentation), D= Cassava Flour + Soy Bean Flour (4 days fermentation), E= Cassava Flour + Soy Bean Flour (6 days fermentation), F= Cassava Flour + Soy Bean Flour (8 days fermentation)

[a]significant difference at negative control (P=.05), [b]significant difference at positive control respectively (P=.05)

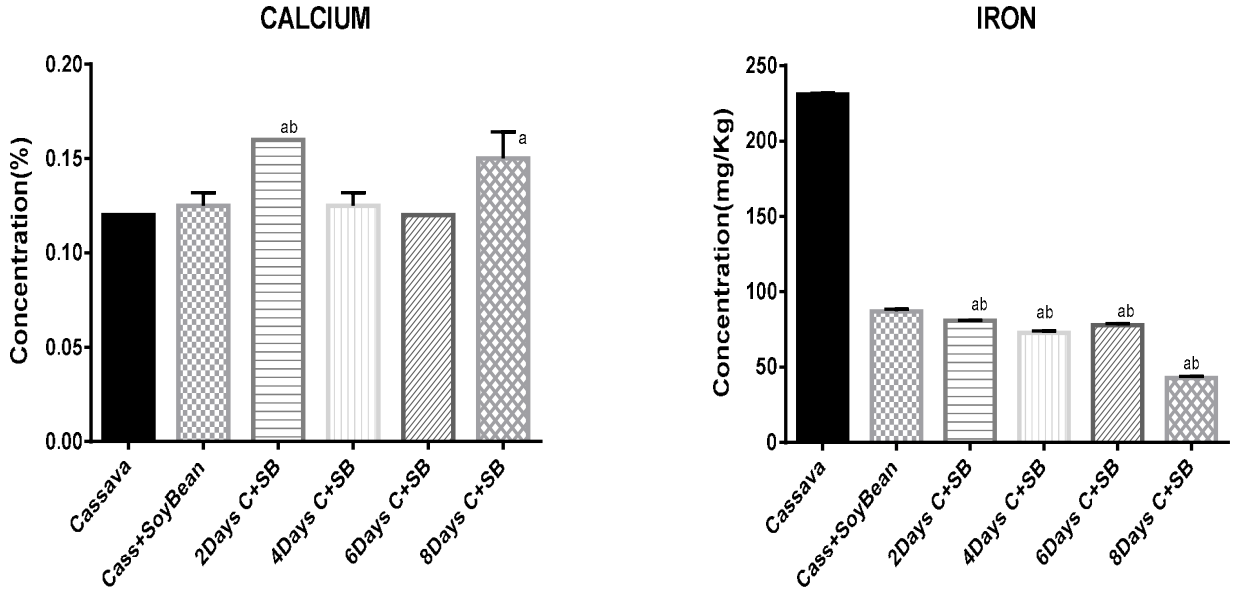

Fig. 1. The effect of varying fermentation duration on Calcium and Iron in Cassava-Soy supplemented flour

[a]significant difference at negative control (P=.05), [b]significant difference at positive control respectively (P=.05)

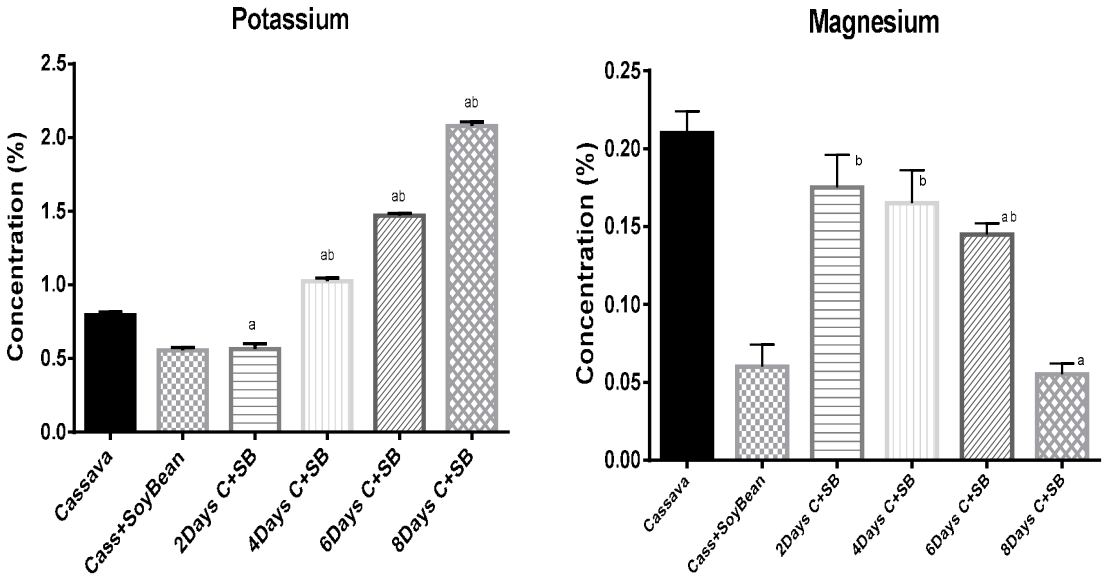

Fig. 2. The effect of varying fermentation duration on potassium and magnesium levels in Cassava-Soy supplemented flour

[a]significant difference at negative control (P=.05), [b]significant difference at positive control respectively (P=.05)

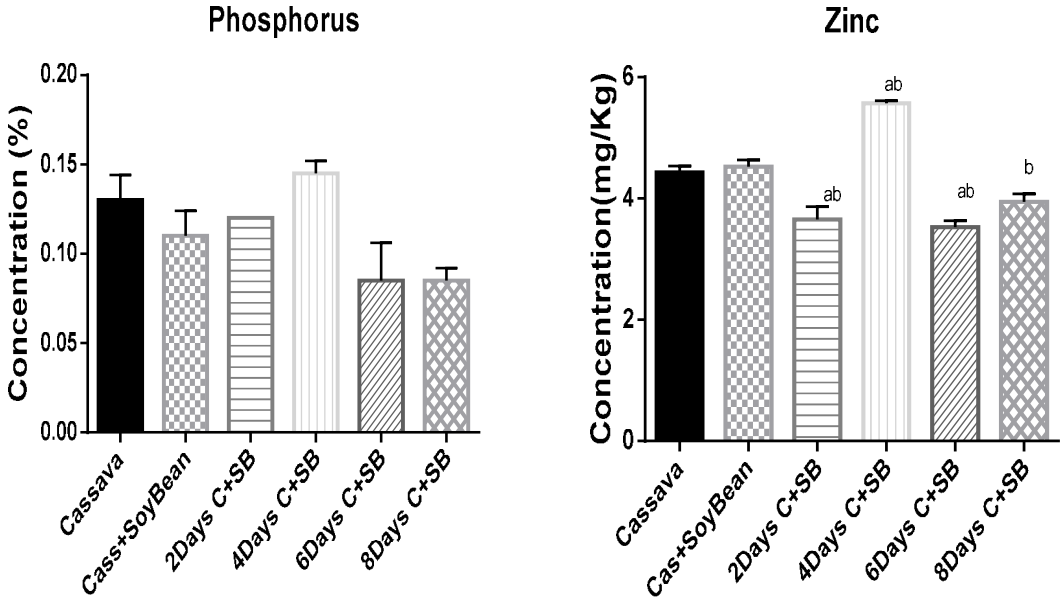

Fig. 3. The effect of varying fermentation duration on Phosphorus and Zinc in Casaava-Soy supplemented flour

[a]significant difference at negative control (P=.05), [b]significant difference at positive control respectively (P=.05)

Minerals are essential for good health and are present in all plants, which obtain them from their environment. The functions minerals serve in the body are diverse and include: serving as structural components in macromolecules, cofactors in enzymatic reactions, as osmotic solutes needed to maintain proper water potential, or as ionized species to provide charge balance in cellular compartments. There were varying levels amongst the minerals assayed for,

with some showing an increase or decrease with increasing fermentation time, and others showing no significant change in the mineral content. The reduction in mineral content upon the introduction of soybean flour can be attributed to the presence of antinutrients which bind to the minerals, making them unavailable for uptake. This phenomenon is quite pronounced for Iron. Asides from calcium and potassium which are higher with increasing fermentation time, the other minerals reduce with increasing fermentation time. This decrease can be ascribed to possible leaching of the soluble mineral elements into the fermenting medium or to the utilization of the minerals by the fermenting microflora for their biochemical activities. The increasing amount of calcium and potassium in the samples supplemented with fermented soybeans, can be ascribed to the reduction of the antinutritional factors (Phytate & Oxalate) binding them, this phenomenon is also corroborated by the study undergone by Sefa–Dedeh and Kluvitse [11].

Table 2. Effect of fermentation on pH and titratable acidity (TA)

Sample	pH	TA (% Lactic acid)
A	4.715±.007071	0.08799±.0003394
B	5.220±0.01414	0.04822±0
C	3.555±0.0778 [ab]	0.06675±0.0001131[ab]
D	3.025±0.03536 [ab]	0.07965±0.0002121[ab]
E	3.160±0.08485 [ab]	0.06198±0.00000282[ab]
F	4.360±0.05657[ab]	0.02041±0.001273[ab]

[a]significant difference at negative control (P=.05), [b]significant difference at positive control respectively (P=.05)

A= Cassava Flour Only, B= Cassava Flour + Soy Bean Flour (No fermentation), C= Cassava Flour + Soy Bean Flour (2 days fermentation), D= Cassava Flour + Soy Bean Flour (4 days fermentation), E= Cassava Flour + Soy Bean Flour (6 days fermentation), F= Cassava Flour + Soy Bean Flour (8 days fermentation)

Proximate analysis is a method for the quantitative analysis of the different macronutrients in food and was developed to duplicate animal digestion. Crude protein is the approximate amount of protein in foods and is calculated from the determined nitrogen content. Crude protein levels increased significantly in the cassava flour samples supplemented with fermented soybeans, which can be attributed to an increase in microbial mass during fermentation, causing extensive hydrolysis of protein molecules to amino acids and other simple peptides [12]. The increase could also be as a result of the enzymatic hydrolysis of some protein inhibitors during fermentation [13]. It may also be due to the higher amounts of structural proteins, which are an integral part of the microbial cell. The reduction in crude protein after 8days of fermentation possibly indicates a shift to protein metabolism by the fermenting microflora due to lower levels of carbohydrates. Ash is a measure of the total amount of minerals present within a food. The significant (*P*=.05) increase in ash content of the flour samples supplemented with soybeans that have been fermented for six-eight days is an indicator of antinutrient degradation. The introduction of soybean to cassava flour significantly increased the crude fat levels, Crude fat levels showed a significant (*P*=.05) increase with increasing fermentation time. The values ranged from 2.3-3%. This observation agrees with that of Onoja and Obizoba [14]. The increase could be as a result of extensive break down of large fat molecules to simpler fatty acid units due to the high activity of lipolytic enzymes. The increase in fat might also be from dead microflora or as a result of the fermenting microflora not using fats from soybeans as a source of energy [15]. The crude fibre content of the flour samples decreased with increasing fermentation duration during the study indicating the softening of the fibrous tissues during fermentation. The lower crude fibre values obtained could be due to the activities of microorganisms which are known for the bio-conversion of carbohydrates and lignocelluloses into protein agreeing with the findings of Hwei-Ming et al. [16] and Balagopalan [17]. Dry matter content relates to good cooking qualities and extended storage lives [18] indicating the long shelf life possessed by the flour samples. The dry matter remained relatively constant with increasing fermentation length which indicates the good storability potential of the flour samples. The reduction in carbohydrate content can be attributed to the microbial utilization of the soybean carbohydrate stores for their metabolism [19].

The pH of a flour suspension is important since some functional properties such as solubility, emulsifying activity and foaming properties are affected by it, the pH value is also an indicator of proteolytic activities and the ammonia release following the utilization of amino acids by microorganisms involved in the fermentation, this released ammonia is mainly responsible for the pungent smell that usually accompanies soybean fermentation and a large percentage of other vegetative protein fermentation [20]. High pH starches have been reported to have increased solubility because of increased hydrophilic

characters of the starch at these pH values [21]. pH values ranging from 5 and 7 have been reported to stimulate retrogradation and this is attributed to the absence of monovalent ions and cations that have been found to retard retrogradation [22]. The pH of the cassava-soy flour that has not undergone fermentation as shown in Table 2 indicates that it will be readily undergo retrogradation, the pH of cassava flour samples supplemented with fermented soybeans shows that they won't readily undergo retrogradation and as a result, will result in a longer-lasting flour. The pH is also a good quality indicator for cassava flour since flour with a pH of 4 or less will have a characteristic sour aroma and taste due to fermentation, which is not desirable for use in bakery products [23] making most of the treatment groups with the exception of the Cassava-Soy flour that has undergone eight days' fermentation not very suitable for use as bakery goods.

5. CONCLUSION

Fermentation was shown to have varying positive effects on the nutritive values of soybean-supplemented cassava flour, with obvious improvement in nutrient/mineral availability due to possible antinutrient reduction and/or fermenting microflora action. This study has proved fermentation to be a useful tool that can be used to improve the nutritional quality and safety profile of soybean-supplemented cassava flour, which can be used as a raw material for food products development, increasing the utilization of cassava flour and enhancing the production of a variety of food products. Further work can be done on ways to improve the fermentation processes for soybeans by eliminating the pungent smell that accompanies soybean fermentation which might discourage its wide adoption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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