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Effect of Fermentation and Extrusion on the Nutrient and Anti-nutrient Composition of Soy Beans (*Glycine max*, L) and Acha (*Digitaria exilis* Stapf)

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Authors' contributions

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

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ABSTRACT

Aim: Effect of Fermentation and Extrusion on nutrient and anti-nutrient composition of Acha and deffated Soybeans.
Study Design: Four batches of Acha and defatted soy beans flour blends were varied at different ratio (100:0, 70:30 and 50:50). A batch was preconditioned and extruded. Another was fermented for 72 h. The third batch was fermented and extruded. The fourth batch was not fermented or extruded.
Place and Duration of Study: Department of Microbiology and Fishery Department Federal University of Akure, Ondo State between October 2015 and July 2016.

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Methodology: Microbial analysis was carried out using potato dextrose agar, nutrient agar and De man Rogosa agar. pH, temperature and total titratable acidity analysis were carried out. Proximate, mineral composition, nutrient, anti-nutrient, of the blends was also carried out using standard methods.

Results: Fifteen organisms were isolated (Eight bacteria, five moulds and two yeasts) which include *Lactococcus* sp, *Lactobacillus bulgaricus, Enterococcus, Staphylococcus aureus, Streptococcus thermophillus, Proteus mirabilis, Lactobacillus brevis, Pedicoccus* sp*, Rhizopous stonlonifer, Mucor mucedor, Aspergillus flavours, Penicillium freguentans, Fusarium merismoides, Saccharomyces cerevisiae* and *Candida utilis* from all the batches of the sample after fermentation. The pH and titratable acidity (TTA) significantly varied during fermentation. The proximate composition showed an increase in nutritive value of the fermented extruded samples when compared with raw samples. There was significant increase in the mineral composition of the fermented samples when compared with the raw samples. Fermentation and extrution significantly reduced the anti-nutrient content of the samples. No significant difference was recorded in the fiber content of the raw flour blends and extrudates. This study revealed that fermentation and extrusion of acha and soybeans improved the nutritional quality of the samples and reduced the anti- nutrient content, also it can be used in weaning children in developing countries especially Nigeria.

Keywords: Fermentation; extrusion; acha; soybean.

1. INTRODUCTION

Complementary foods are foods that are gradually introduced to infants. They are used for gradual withdrawal of the infant from the mother's milk, and for preparing the infant for total adaptation to the regular food of the family [23]. Complementary foods should therefore be low bulk density, high nutrient content and be microbiologically safe.

Foods are complex systems consisting of various components such as proteins, fats, carbohydrates, minerals etc which influence the behavior of foods in a system. Functional properties relate the ability of food to interact in a system in order to impart desirable properties to the food. Though the study of food functionality had remained complicated functionality of individual food ingredients in simple systems are useful to predict control and eventually impart desirable characteristics to real food systems [11].

Extrusion cooking technology has been described as a process in which raw materials are heated and worked upon mechanically while passing through compression screws and is forced through a die or other restrictions [9].

In the developing world, fermentation is one of the oldest technologies used for food processing and preservation. Fermentation reduces anti-nutrient properties of foods. It can be described as a desirable process of biochemical modification of primary food products brought about by microorganisms and their enzymes [13]. Fermentation and extrusion improve the nutritional value of weaning foods by reducing the water-binding capacity of cereal flour. This allows the fortified to have a free-flowing consistency even with high proportion of flour. Extrusion has been reported as an effective processing treatment to improve the nutritional quality of cereals [3].

Acha (*Digitaria exillis*), a cereal grain, in the family of poaceae and commonly referred to as folio or hungry rice is grown in areas with at least 800 mm of rainfall such as the mountain Fouta Dyallon region of Guinea and dry Savanna zone of Mali and upper Volra [34]. It is rich in calcium, magnesium, iron and copper than most cereals but poorer in potassium, sodium, lead and manganese. With the exception of methionine, the essential amino acid content of acha is lower than that for most grains while the leucine, methionine and cysteine values are slightly high [35].

The use of acha differs, its grains may be boiled like rice; flour from acha may be fortified with other cereals flour especially for the production of infant food. Acha have a high water absorption capacity a property that could be linked to appreciable amount of pentosan which could be utilized in baked goods at a 2-3% level [36]. Pentasan consist primarily of polymeric pentosan sugars, rather than hexose sugar of starch which have been found to be a very important regulator of water absorption and distribution in dough. Acha is traditionally recommended for children, old people and for people suffering from diabetes

or stomach diseases due to its easy digestibility [37,38].

Soybean, *Glycine max*, belongs to the family Leguminosae, which refers to the fruits of the flowering plants, legumes. Historical and geographical evidence indicates that the soybean originated from East Asia, specifically Northern China. Soybean has been cultivated and incorporated as food and medicine into the daily lives of the Chinese for the past 5,000 years.

Based on current statistics, 150 million metric tons of soybeans are produced. The major producers of soybean are currently t he Unites States, Brazil, China, India and Argentina. Demands of soybeans have been significantly increasing due to its high lysine content and other amino acids essential for human health which is limiting in most cereals [39].

Soybean seeds differs in shape (spherical to long ovals), colour (yellow, blue, green, dark brown, purplish black, or black) and sizes. The seed coat of a mature soy seed is extremely hard and water resistant so that germ that is encased within the soy seed is protected. Soybean seeds are extremely high in protein content, carbohydrate, oil, and ash. Soy protein is a heat-stable protein, thus allowing soy seeds to undergo high temperature cooking and fermentation, without destroying the entire chemical composition of the soybean. The soluble carbohydrates in soybeans are made up of various saccharine: disaccharide sucrose, trisaccharide raffinose, and tetrasaccharide stachyose. These soluble carbohydrates can easily be broken by microbes down during fermentation to create a distinct flavor, odour and texture in soy products. Other valuable components that are found in soybean are phospholipids, vitamins, minerals, and isoflavones [40].

Nutritional benefits of Acha and Soybeans flour are limited without fermentation and Extrution. The use of only one method of processing may not impact desired level of improvement of staple foods. Therefore, food processing technologies such as extrusion cooking coupled with fermentation can improve the nutritional quality of food. To maximize the nutritional benefits of Acha and soybeans flour, there is need to determine the effects of fermentation and extrusion on these staple foods. The objective of this research is to determine the effect of fermentation and extrusion on the and nutrient and anti-nutrient composition of soy beans (*Glycine max*, l) and acha (*Digitaria exilis* stapf).

2. METHODOLOGY

2.1 Collection of Samples

Soybeans were obtained from International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State. While Acha was purchased from Gwagwalada Market in Federal Capital Territory, Abuja. Nigeria. The samples were transported to the laboratory in clean low density polyethylene bags.

2.2 Processing of Acha Flour

Dried Acha grains were sorted and cleaned by removal of stones and other foreign materials. The sorted grains were then fed into an attrition mill. The milled flour was then sieved into fine flour.

2.3 Processing of Defatted Soybean Flour

Soybeans seed were cleaned by sorting out dirt's, stones and all other foreign materials. The clean Soybeans seeds were coarsely milled to separate the coat from the cotyledon. The dehulled seeds were fed into attrition mill and then sieved into fine flour. The fi ne flour was defatted using cold extraction with n-hexane from a 21% fat content to 15.17%. The deffated flour was then air-dried and the clumps were broken and sieve into fine flour through 60 mesh screen.

2.4 Formation of Acha and Defatted Soybean Flour Blends

The flour samples from Acha and Soyean were mixed at three [3] levels of combinations as follows:

Acha flour (g)	Defatted soybeans flour (g)
A 100	0
B 70	30
C 50	50

2.5 Fermentation and Extrusion of Flour Blends

A batch of the flour blend was fermented using semi- solid state fermentation for 72 hours. 50 ml

of sterile distilled water was added to 100 g of Acha, 70 ml of sterile distilled water was added to 70 g of Acha and 30 g of Soybeans while 90 ml of sterile distilled water was added to 50 g of Acha and 50 g of Soybeans samples in 3 different cleaned containers and properly sealed. The fermentation process was terminated by oven drying at 60℃ for 24 hours.

Two batches of samples were subjected to extrusion cooking. The first batch was fermented while the second was unfermented blends. The blends were hydrated and preconditioned by adding 50 ml of sterilized water to 100 g of the samples and manually mixed in a sterile bowl to ensure even distribution of water.

The samples were extruded using a Brabender 20DN single screw laboratory extruder (Brabender OHG, Duisburg, Germany). The samples were extruded at 100℃, 20 revolution per minute and feeding rate of 30 kg/h. All the extrudates were air dried for 12 hours after kept in properly labeled air tight containers. The control which consists of the raw blends which were neither fermented nor extruded was kept in air tight containers.

2.6 Determination of pH and Total Titratable Acidity (TTA)

Daily recording of the temperature and pH of the samples were taken throughout the period of the experiment. 10 g of each sample was suspended in 90 mls sterile distilled water and homogenized. The pH was ascertained using the pH meter metrom E520. The TTA analysis was done using [13] method. A 10 ml of the sample was pipetted into a beaker and 3 drops of Phenolphthalein indicator was added. Titration was done using 0.1M NaOH to a faint pink colour for at least one minute compared against a white background. The acidity was calculated as follows:

> % acid = [mls NaOH used] x [0.1 N NaOH] x [milliequivalent factor] x [100] / grams of sample

2.7 Microbiological Analysis of the Samples

Bacteria and fungi were evaluated using nutrient agar (NA) and potato dextrose agar (PDA) respectively while De Man Rogosa sharpe agar was used to isolate lactic acid bacteria. Techniques were enumerated by using appropriate serial dilution and pour plate techniques. The bacterial culture was incubated at 37℃ for 18 to 24 hours, fungal plates were inverted and incubated at 24℃ for 48 to 72 hours. De Man Rogosasharpe agar plates were incubated at 32℃ for 18- 24 hours anaerobically. The organisms were characterized based on biochemical and morphological observations according to the methods of Cowan and Steel [25]. Fungi isolates were identified according to [26].

2.8 Determination of Proximate Analysis

All samples were analyzed for Moisture, Ash, Fat, Protein, Crude fiber and Carbohydrate determined by difference according to the method described by [24].

2.9 Determination of Mineral Composition

The mineral composition of the raw flour blends and fermented blends were determined using the methods described by [24]. A 1 g of each sample was ashed in muffle furnace at 550℃ for 120 minutes. The ashed sample and dishes were removed and transferred into the desiccator to cool after which the samples were dissolved with 10 ml of 20% nitric acid (HNO3). The content was filtered into a clean small plastic bottle using number 43 whatman filter paper. Distilled water was later added to dilute the solution up 50ml. Atomic absorption spectrophotometer was used in determining the concentration of the metals: iron (Fe), zinc (Zn) and calcium (Ca) while flame photometry method was used for Sodium (Na) and potassium (K)

2.10 Tannin Determination

A 200 mg of finely ground sample was weighed into a 50 ml sample bottle. 10 ml of 70% aqueous acetone was added and properly covered. The bottles were put in an ice bath shaker for 2 hours at 30℃. Each solution was then centrifuged and the supernatant stored in ice. A 0.2 ml of each solution was pipetted into test tubes and 0.8 ml of distilled water was added. Standard tannic acid solutions were prepared from a 0.5 mg/ml stock and the solution made up to 1 ml with distilled water. A 0.5 ml folin reagent was added to both sample and standard followed by 2.5 ml of 20% Na2CO3. The solutions were then vortexed and allowed to incubate for 40 minutes at room temperature after which absorbance was read at 725 nm

against a reagent blank concentration of the samples from a standard tannic acid curve [27].

2.11 Determination of Phytate

Phytate was determined according to the method of [31]. A 4 g of the Sample was soaked in 100 ml of 2% HCl for 3 hrs and then filtered through a No 1 Whatman filter paper. A 25 ml ml was taken out of the filtrate and placed inside a conical flask and 5 ml of 0.3% of ammonium thiocyanate solution was added as indicator. After which 53.5 of distill water was added to give it the proper acidity and this was titrated against 0.00566 g pe r milliliter of standard iron (iii) chloride solution that contain about 0.00195 g of iron per milliliter until a brownish yellow colouration persist for 5 min.

2.12 Determination of Saponin

The spectrophotometric method of [32] was used for Saponin determination. A 2 g of the finely grinded sample was weighed into a 250ml beaker and 100 ml of Isobutyl alcohol or (But-2-ol) was added. Shaker was used to shake the mixture for 5 hours to ensure uniform mixing. The mixture was now filtered with No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate (MgCo3). The mixture obtained again was filtered through No 1 Whatman filter paper to obtain a clean colourless solution. 1 ml of the c olourless solution was taken into 50 ml volumetric flask using pipette, 2 ml of 5% iron (iii) chloride (FeCl3) solution was made up to the mark with distill water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm.

2.13 Determination of Trypsin Inhibitor

Trypsin inhibitor was extracted by mixing 1 g of the sample with 50 ml of 0.01N NaOH at pH of between 8.4-10.00 and allowing the mixture to stand for 3 h, while stirring at intervals. A 2 ml of diluted extract was then dispensed into test tubes to which 2 ml of cold trypsin solution (4 mg in 200 ml of 0.001 M HCl) was added, and the tubes were placed in water bath at 370C, 5 ml of Benzoyl-DL-arginine-P-nitro anilide hydrochloride (BAPNA) (40 mg was dissolved in 1 ml of dimethyl sulfoxide and diluted to 100 ml with tris buffer 0.05 M, pH 8 and diluted to 100 ml with tris buffer 0.05 M, pH 8.2, pre warmed to 370C) was added as substrate to each tubes. After 10 min the reaction was terminated by adding 30% acetic acid and the content of each tube was thoroughly mixed. Thereafter the content of each tube was centrifuged at 3000 rpm and the absorbance of the filtrate was measured at 410 nm against reagent blank. The reference was prepared in the same as the sample except that 2 ml of distilled water was added in place of extract Smith et al. [30].

2.14 Statistical Analysis

All data obtained were carried out in triplicates and subjected to descriptive statistics, analysis of variance (ANOVA) and Duncan Multiple Range Test and the level of significance was set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 Microbial Growth during Fermentation of Acha and Defatted Soybeans Blend

Fifteen organisms were isolated from the fermentation of Acha and defatted Soybeans blend which were confirmed and identified by biochemical test shown on Table 4-5. Eight bacteria: *Lactococcus sp, Lactobacillus bulgaricus, Lactobacillus brevis, Enterococcus* sp*., Staphylococcus aureus, Streptococcus thermophillus, Proteus mirabilis and Pedicoccus* sp. Five mould were identified as*: Rhizopous Stonlonifer, Mucor mucedor, Aspergillus flavus, Penicillium freguentans, Fusarium merismoides,* two yeasts were identified as*: Saccharomyces cerevisiae and Candida utilis*

3.2 Changes in Bacteria Population during Fermentation of Acha and Defatted Soybeans Blend

Fig. 1 shows the changes in the bacteria population of the blends during fermentation for 72 hours. The aerobic bacteria po pulation of the entire blend varied from 0 hour to 72 hours. The initial aerobic count of sample A (Acha 100%) at 0 hour was 2.92 x 105 CFU/g, and increa sed to 3.45 x 105 CFU/g at 24 hours and 4.70 x 105 CFU/g at 48 hours which decreased at 72hours to 3.10 x 105 CFU/g. Flour blend B (Acha 70%, Soybeans 30%) was recorded to have aerobic count of 3.63 x 105 CFU/g at 0 hour, at 24 hours 3.90 x 105 CFU/g then at 48 hours increased to 4.60 x 105 CFU/g while at 72 hours 4.70 x 105 CFU/g. The aerobic count for flour blend C (Acha 50%, Soybeans 50%) at 0

hour 2.00 x 105 CFU/g, then increased at 24 hours 3.20 x 105 CFU/g and at 48 hours 4.20 x 105 CFU/g decrease was observed at 72 hours 2.8 0 x 105 CFU/g. The highest aerobic bacteria count was observed from flour blend A at 48 hours while the lowest count was observed in the flour blend C at 0 hour.

3.3 Changes in Lactic Acid Bacteria Population during Fermentation of Acha and Defatted Soybean Flour Blend

Fig. 2 shows the lactic acid bacteria population of the blends during fermentation for 72 hours. However, the lactic acid bacteria (LAB) count was observed to have increased during fermentation of the flour blends. Flour Blend A has 0 growth at 0 hour at 24 hours 2.0 x 10^5 CFU/g count was observed, 2.98 x 10^5 CFU/g at 48 hours and 3.17 x 10^5 CFU/g at 72 hours. An increase was observed in flour blend B from 1.50 x 10^5 CFU/g at 0 hour to 2.70 x 10^5 CFU/g , 2.98 x 10^5 CFU/g and 3.17 x 10^5 CFU/g at 24,48 and 72 hours. An increase was also observed in flour blend C, at 0 hours 2.10 x 10^5 CFU/g , 2.98 x 10^5 CFU/g , 3.00 x 10^5 CFU/g and 3.99 x 10^5 CFU/g at 24 hours, 48 hours and 72 hours respectively. However the highest LAB count was observed in flour blend C at 72 hours while the least count was observed in flour blend A at 0 hour.

3.4 Changes in Fungi Population during Fermentation of Acha and Defatted Soybeans Blend

Fig. 3 shows the Fungi population of the blends during fermentation for 72 hours. From the results, an increase from 1.40 x 10^5 CFU/g at 0 hour to 2.30 x 10^5 CFU/g at 24 hours followed by an increase of 2.58 x 10^5 CFU/g at 48 hours and 3.10 x 10^5 CFU/g at 72 hours. Flour Blend B, recorded a very sharp increase from 1.70 x 10^5 CFU/g at 0 hour to 2.90 x 10^5 CFU/g at 24 hours and 3.18 x 10^5 CFU/g at 48 hours followed by an increase to 3.96 x 10^5 CFU/g at 72 hours. The flour blend C increase 2.09 x 10^5 CFU/g at 0 hour to 4.29 x 10^5 CFU/g at 24 hours, 4.50 x 10^5 CFU/g at 48 hours and 4.69 x 10^5 CFU/g at 72 hours. The highest fungal count was observed in flour blend C at 72 hours while the lowest fungi count was observed in flour blend A at 0 hour.

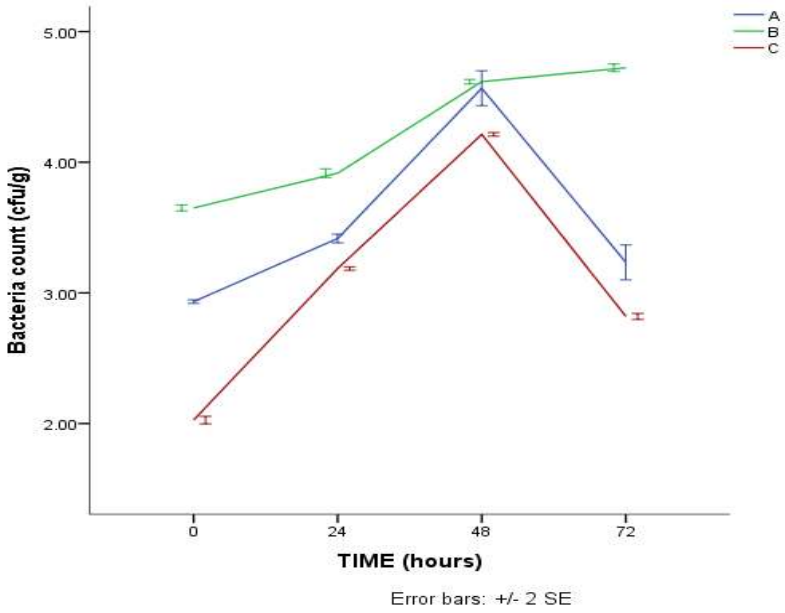

Fig. 1. Changes in bacteria count during the fermentation of Acha and soya bean blends

Keys: 0 =0 hour, A= Acha 100%
24=24 hours, B= Acha 70%, Soyabeans 30%
48=48 hours, C= Acha 50%, Soyabeans 50%
72=72hours

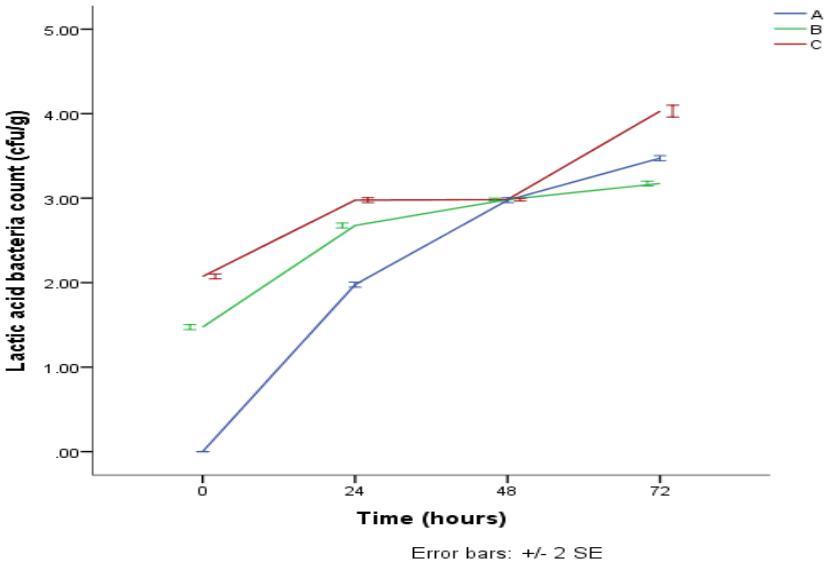

Fig. 2. Changes in lactic acid bacteria count during the fermentation of Acha and soya bean blends

Keys: 0 =0 hour, A= Acha 100%
24=24 hours, B= Acha 70%, Soyabeans 30%
48=48 hours, C= Acha50%, Soyabeans 50%
72=72hours

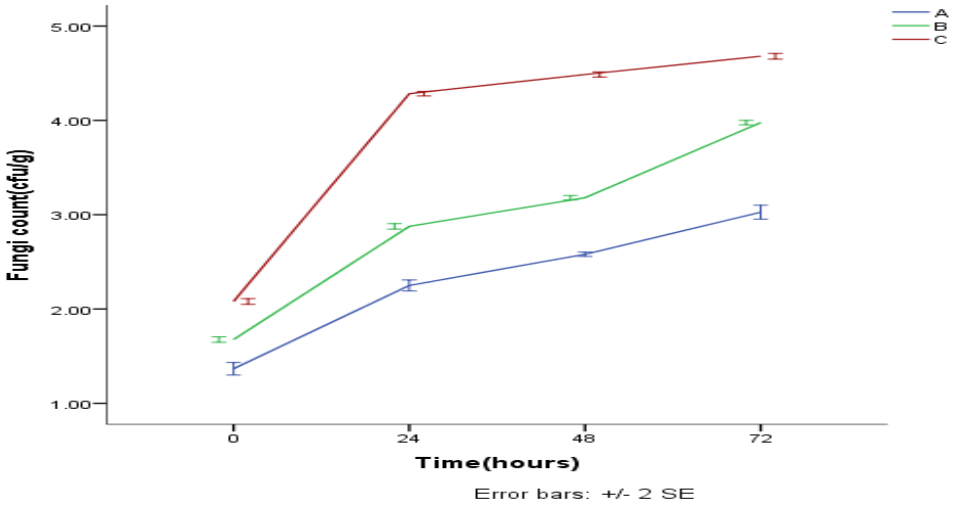

Fig. 3. Changes in Fungi Count during the Fermentation of Acha and Soya bean blends

Keys: 0 =0 hour, A= Acha 100%
24=24 hours, B= Acha 70%, defatted Soyabeans 30%
48=48 hours, C= Acha50%, defatted Soyabeans 50%
72=72 hours

3.5 Bacteria Occurrence during Fermentation of Acha and Defatted Soybeans Flour Blends

Results of the bacteria isolated during fermentation are shown on Table 6 that *Lactobacillus bulgaricus* and *Lactobacillus brevis* were isolated from all blends A,B and C at 24,48 and 72 hours, while *Lactococcus* sp. was isolated from B and C at 0 and 24 hours. *Enterococcus* sp. was isolated from only flour blend C at 24 hour. *Staphylococcus aureus* was

isolated from A and C flour blend at 0 and 24 hours. *Streptococcus thermophillus* was isolated from flour blend A abd B at 48 and 72 hours. *Proteus mirabilis* was isolated form B and C flour blends at 0 and 2 4 hours. *Pediococcus* sp was isolated from A and C flour blends at 48 and 72 hours. *Lactobacillus* sp. and *Lactococcus* sp. were the dominant microorganisms isolated from all flour blends at 48 and 72 hours.

3.6 Fungi Occurrence during Fermentation of Acha and Defatted Soybean Flour Blends

Results of fungi isolated during fermentation of Acha and defatted Soybeans flour blends are shown on Table 7. There was no m uch growth in the flour blend A and B at 0 hour. *Aspergillus flavus* was dominant at 0, 24 and 48 hours in all blends A,B and C. *Rhizopous stolonifer* was isolated from flour blend B and C at 0,24,48 and 72 hours. *Mucor mucedo* was isolated from flour blend B and C at 48 and 72 hours. *Penicillium frequentans* was isolated from B and C at 48 and 72 hours. *Fusarum merismoiedes* was isolated from flour blends B and C at 48 a nd 72 hours. *Saccharomyces cerevisiae* was isolated from all the flour blends A,B and C at 24,48 and 72 hours. *Candida utilis* was isolated from flour blends B and C at 48 and 72 hours. However *Aspergillus flavus* and *Saccharomyces cerevisiae* were the dominant microorganisms isolated from all flour blends at 24,48 and 72 hours.

3.7 Changes in pH during Fermentation of Acha and Defatted Soybean flour Blend

Fig. 4 shows the changes in pH during the fermentation of Acha and defatted Soybean flour blends at room temperature. As the fermentation day progresses the blend experienced decrease in the pH value from 0 hour to 72 hours.

At day 0 (0 hour), the result recorded lowest pH in sample A (Acha 100%) at a value of 5.48, The highest pH at day 0 was recorded in sample C (Acha 50% Soybeans 50%) with a value of 6.67.At day 1 (24 hours) the lowest pH was 4.34 from sample A (Acha 100%) and the highest was from sample C (Acha 50% Soybeans 50%) 6.27. At day 2 (48 hour), the result recorded lowest pH in sample A (Acha 100%) at a value of 4.62, The highest pH at 48 hours was recorded in sample C (Acha 50% Soybeans 50%) with a value of 5.73, as at day 3 (72 hours) the lowe st value was 4.59 sample A (Acha 100%) while sample C (Acha 50% Soybeans 50%) had the highest value 6.05.

3.8 Changes in Temperature during Fermentation of Acha and defatted Soybean flour Blend

Fig. 5 shows the variation of temperature during the fermentation of Acha and defatted Soybean flour blend. The temperature value ranges from 29.3℃ to 31.5℃.as at 0 hour the temperature value was 31.5, at 24 hour the value was 29.5 by 48 hours, it increased to 30.5 and decreased to 29.5 at 72 hours.

3.9 Changes in Total Titratable Acidity during Fermentation of Acha and Defatted Soybean flour Blend

Fig. 6 shows the total titratable acidity value for the entire fermented Acha and defatted Soybeans flour blends.

The total titratable acidity (TTA) as at 0 hour for sample A (Acha 100%) was 0.2, at 24 hours 0.15,at 48 hours 0.25 and at 72 hours decrease to 0.15. For sample B (Acha 70% and Soybeans 30%) at 0 hour it was 0.4 reduced to 0.3 at 24 hours and increase to 0.15 at 48 hours and as well decrease to 0.5 at 72 hours. Sample C (Acha 50% Soybeans 50%) at 0 hour it was recorded to have 0.2 value increase to 0.4 at 24 hours and retain the same value 0.8 at 48 and 72 hours.

3.10 Proximate Composition of Acha and Defatted Soybean Flour Blends

The highest moisture content was recorded in Extruded unfermented Acha 100% (EUF1) with a value of 18.19±0.01. The lowest was recorded in Extruded unfermented Acha 70% Soybeans 30% (EUF2) with a value of 5.72±0.01.

The highest ash content was recorded in Extruded unfermented Acha 50% Soybean 50% (EUF3) with a value of 2.93±0.02. The lowest ash content was recorded in Extruded unfermented Acha70% / Soybean 30% (EUF2) with a value of 0.10±0.01.

The highest fat content was recorded in Fermented- unextruded Acha 50% Soybeans 50% (FUE3) with a value of 11.03. ±0.02 The lowest was recorded in Fermented unextruded Acha 100% (FUE1) with a value of 0.63±0.01.

Protein content had the highest value in Extruded Fermented Acha 50% Soybeans 50% (EF3) with a value of 30.87±0.02 while the sample with the lowest value was 16.52 ±0.01 Extruded Fermented Acha 100% (EFI).

The highest crude fibre content was recorded in Fermented unextruded Acha 50% Soybeans 50% (FUE3) with a value of 1.19±0.01 while the lowest value was recorded from Extruded fermented Acha 100% (EF1) 0.00±0.00.

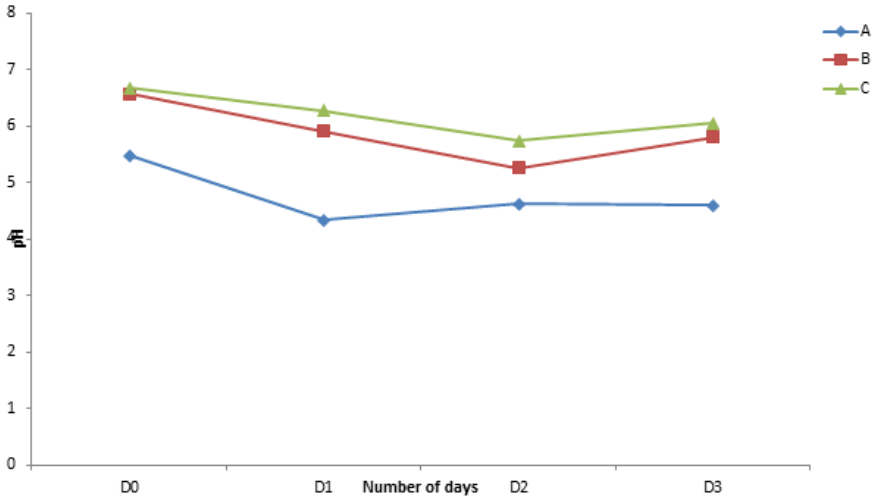

Fig. 4. pH Variation during fermentation of Acha and defatted soyabean blend.
Keys: D0 =0 hour A= Acha 100%
D1=24 hours B= Acha 70%, Soyabeans 30%
D2=48 hours C= Acha50%, Soyabeans 50%

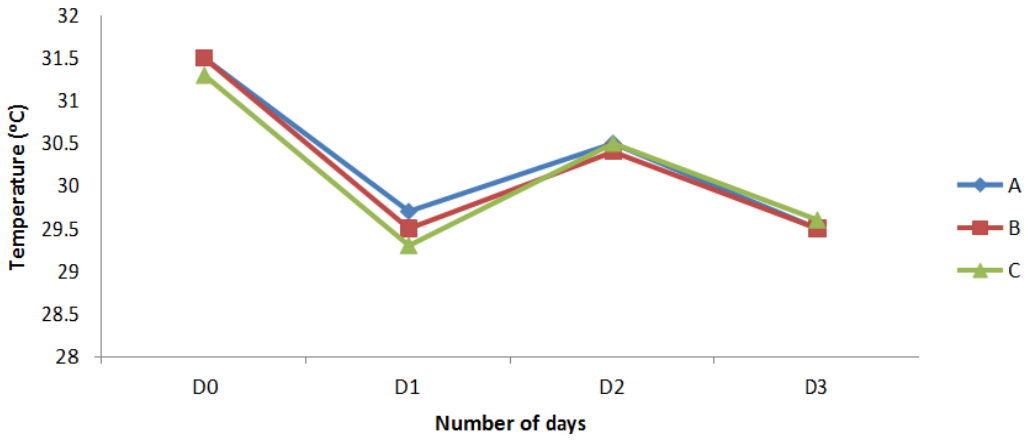

Fig. 5. Temperature during fermentation of Acha and defatted soybean flour blends
Keys: D0 =0 hour A= Acha 100%
D1=24 hours B= Acha 70%, Soyabeans 30%
D2=48 hours C= Acha50%, Soyabeans 50%
D3=72hours

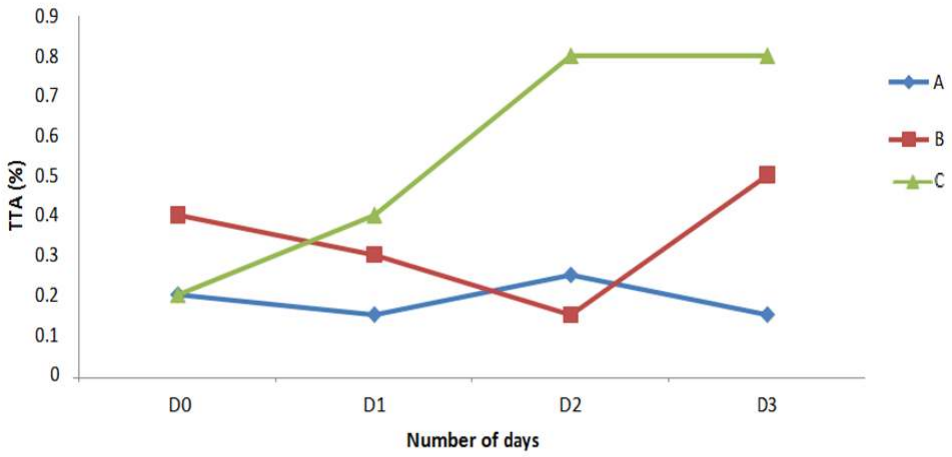

Fig. 6. Total titratable acidity variation during the fermentation of soybeans and acha blend

Keys: D0 =0 hour A= Acha 100%
D1=24 hours B= Acha 70%, Soyabeans 30%
D2=48 hours C= Acha50%, Soyabeans 50%
D3=72hour

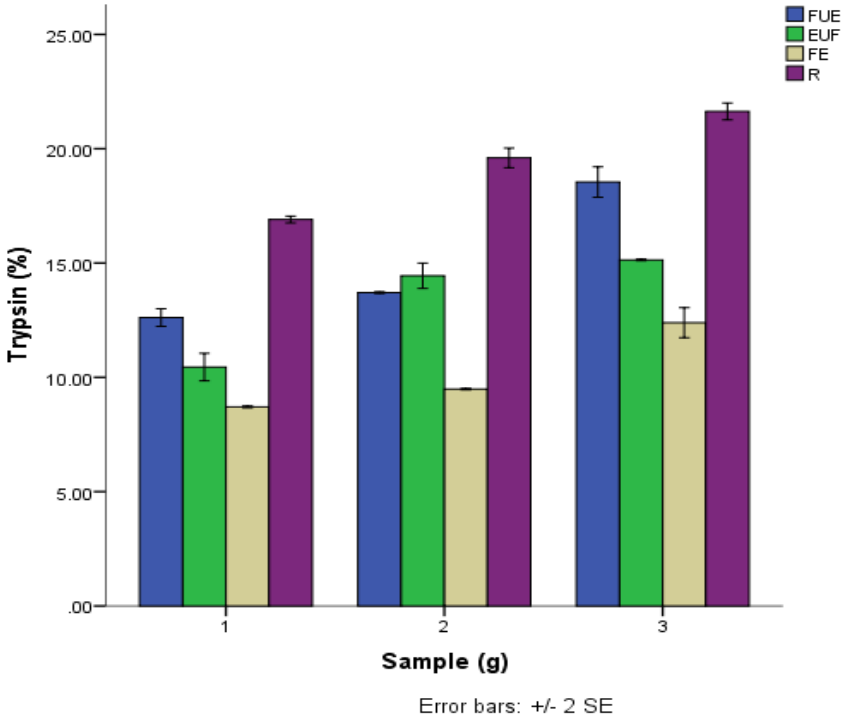

Fig. 7. Trypsin Inhibitor content of the extruded- unfermented, extruded- fermented, fermented- unextruded and raw acha and defatted soybean flour blends

Keys: EUF1=Extruded Unfermented Acha100%, EF1=Extruded Fermented Acha100%, FUE1=Fermented UnExtruded Acha 100%, EUF2= Extruded Unfermentmented Acha70%Soybeans 30%, EF2= Extruded Fermented Acha 70% Soybeans 30%, FUE2=Fermented Unextruded Acha 70% Soybeans 30%. EUF3= Extruded Unfermented Acha 50% Soybeans 50%,
EF3=Extruded Fermented Acha 50% Soybeans 50%, FUE3= Fermented UnExtruded Acha 50% Soybeans 50%. R1= unfermented Unextruded Acha 100% R2= Unfermented Unextruded Acha 70% soy beans 30%,R3= Unfermented Unextruded Acha 50% soy beans 50

The highest carbohydrate content was recorded in Fermented unextrudedted Acha 100% (FUE1) 67.69 ±0.01 while the lowest value was recorded in Extruded fermented Acha 50% Soybean 50% (EF3) 49.94 ±0.01.

3.11 Sensory Evaluation of Acha and Defatted Soybean Flour Blends

The result of organoleptic assessment (Sensory evaluation) obtained indicate that there was no significant difference in the colour of the raw blends. Extruded Fermented blends recorded significant difference in Taste. There was significant difference in texture for Extruded Fermented blends. Extruded Fermented blends were highly singnificant in flavor. Fermented Unextrudede blends recorded significant values in Aroma. Extruded Fermented blends recorded high values for over all acceptability.

3.12 Change in the Mineral Composition of Acha and Defatted Soybean Flour Blends is Shown in Table 6

The mineral analysis result shows that Sodium (Na) had the highest value in sample R2 (unfermented unextruded Acha 70% Soybeans 30%) 13.61±0.01 while the lowest value was in R1 (Unfermented unextruded Acha 100%) 11.21±0.01.

Extruded unfermented Acha 50% and Soybeans 50% (EUF3) recorded the highest level of calcium (Ca) content with the value of 101.0±0.01 the lowest value of calcium was recorded in Unfermented unextruded raw flour blend (R1) 28.27 ±0.01.

Potassium (K) content was highest in extruded fermented Acha 50% and Soybeans 50% (EF3)

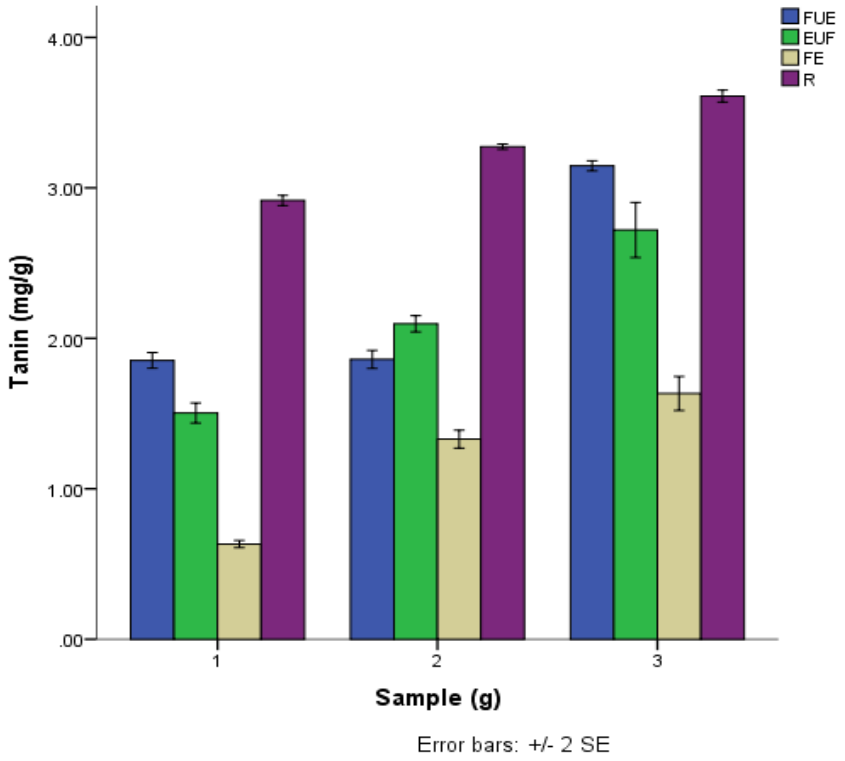

Fig. 8. Tannin content of the extruded- unfermented, extruded- fermented, fermented-unextruded and raw acha and defatted soybean flour blends

Keys: EUF1=Extruded Unfermented Acha100%, EF1=Extruded Fermented Acha100%, FUE1=Fermented UnExtruded Acha 100%, EUF2= Extruded Unfermentmented Acha70%Soybeans 30%, EF2= Extruded Fermented Acha 70% Soybeans 30%, FUE2=Fermented Unextruded Acha 70% Soybeans 30%. EUF3= Extruded Unfermented Acha 50% Soybeans 50%, EF3=Extruded Fermented Acha 50% Soybeans 50%, FUE3= Fermented UnExtruded Acha 50% Soybeans 50%. R1= unfermented Unextruded Acha 100% R2= Unfermented Unextruded Acha 70% soy beans 30%,R3= Unfermented Unextruded Acha 50% so y beans 50

with the value of 268.33±1.67 while t he lowest value was recorded in the Raw flour blend of Acha 100% (R1) 17.88±0.02.

The highest iron (Fe) content was recorded in the Extruded Fermented Acha 50% Soybeans 50% (EF3) 2.63±0.02 while the lowest value was recorded in Unfermented unextruded Acha 100% (R1) 0.42±0.01.

Highest Zinc (Zn) content was recorded in the Extruded Fermented Acha 50% Soybeans 50% (EF3) 0.85±0.02 and was lowest in Unfermented unextruded Acha 100% (R1) 0.36±0.2

Highest Magnesium (Mg) content was recorded in Extruded unfermented Acha 50% Soybeans 50% (EUF3) 39.00±0.58 and was lowest in Unfermented unextruded Acha 100% (R1) 1.81±0.01

3.13 Antinutritional Composition of Acha and Defatted Soybean Flour Blends

3.13.1 Trypsin inhibitor content (%) of the extruded- unfermented, extruded-fermented, fermented-unextruded and raw acha and defatted soybean flour blends is shown in Fig. 7

The highest trypsin inhibitor content was recorded in FUE3 (Fermented unextruded Acha 50% Soybeans 50%) with a value of 18.54 while the least trypsin inhibitor content was recorded in FE1 (Fermented Extruded Acha 100%) with a value of 8.71.

The result of the trypsin inhibitors in the raw flour blends recorded the highest content in R3 (Unfermented unextruded Acha 50% Soybeans 50%) with a value of 21.63, while the least was recorded in R1 (Unfermented unextruded Acha 100%) with a value of 16.90.

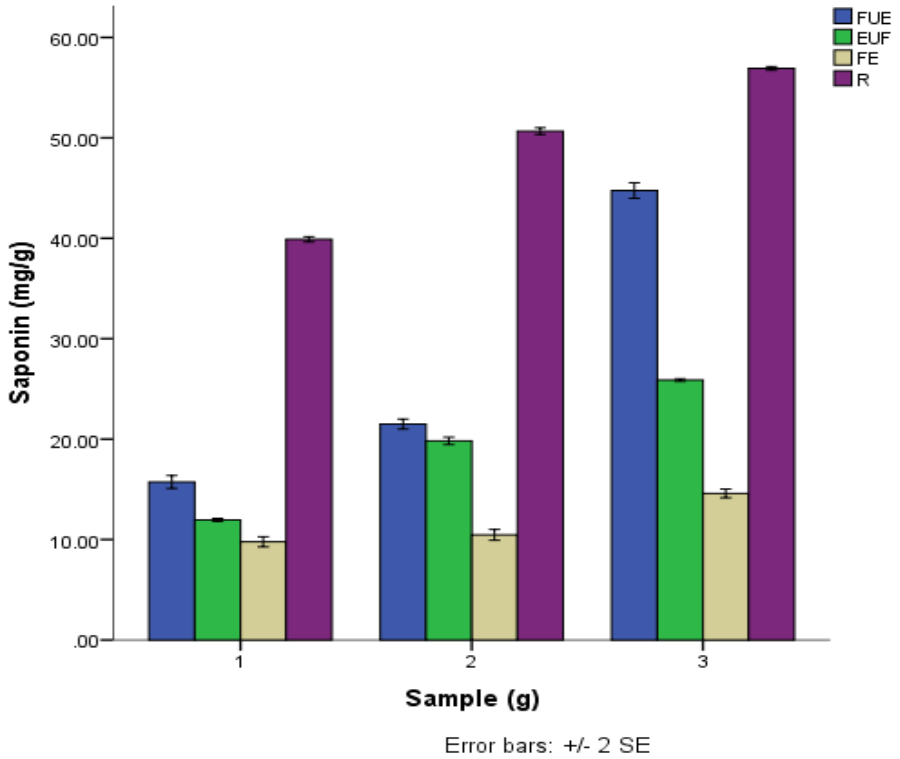

Fig. 9. Saponin content of extruded- unfermented, extruded- fermented, fermented-unextruded and raw acha and defatted soybean flour blends

Keys:EUF1=Extruded Unfermented Acha100%, EF1=Extruded Fermented Acha100%, FUE1=Fermented UnExtruded Acha 100%, EUF2= Extruded Unfermentmented Acha70%Soybeans 30%, EF2= Extruded Fermented Acha 70% Soybeans 30%, FUE2=Fermented Unextruded Acha 70% Soybeans 30%. EUF3= Extruded Unfermented Acha 50% Soybeans 50%, EF3=Extruded Fermented Acha 50% Soybeans 50%, FUE3= Fermented UnExtruded Acha 50% Soybeans 50%.

R1= unfermented Unextruded Acha 100% R2= Unfermented Unextruded Acha 70% soy beans 30%,R3= Unfermented Unextruded Acha 50% so y beans 50

3.13.2 Tannin content (mg/g) of the extruded- unfermented, extruded- fermented, fermented- unextruded and raw acha and defatted soybean flour blends is shown in Fig. 8

Tannin content recorded the highest value in FUE3 (Fermented unextruded Acha 50% Soybeans 50%) with a value of 3.15 while the least tannin content was recorded in FE1 (Fermented Extruded Acha 100%) with a value of 0.633.

The results of the tannin in the raw flour blends recorded the highest content in R3 (Unfermented unextruded Acha 50% Soybeans 50%) with a value of 3.61, while the least was recorded In R1 (Unfermented unextruded Acha 100%) with a value of 2.91.

3.13.3 Saponin content (mg/g) of extruded- unfermented, extruded- fermented, fermented- unextruded and raw acha and defatted soybean flour blends is shown in Fig. 9.

Saponin content recorded the highest value in FUE3 (Fermented unextruded Acha 50% Soybeans 50%) with a value of 44.76 while the least Saponin content was recorded in FE1 (Fermented Extruded Acha 100%) with a value of 9.78.

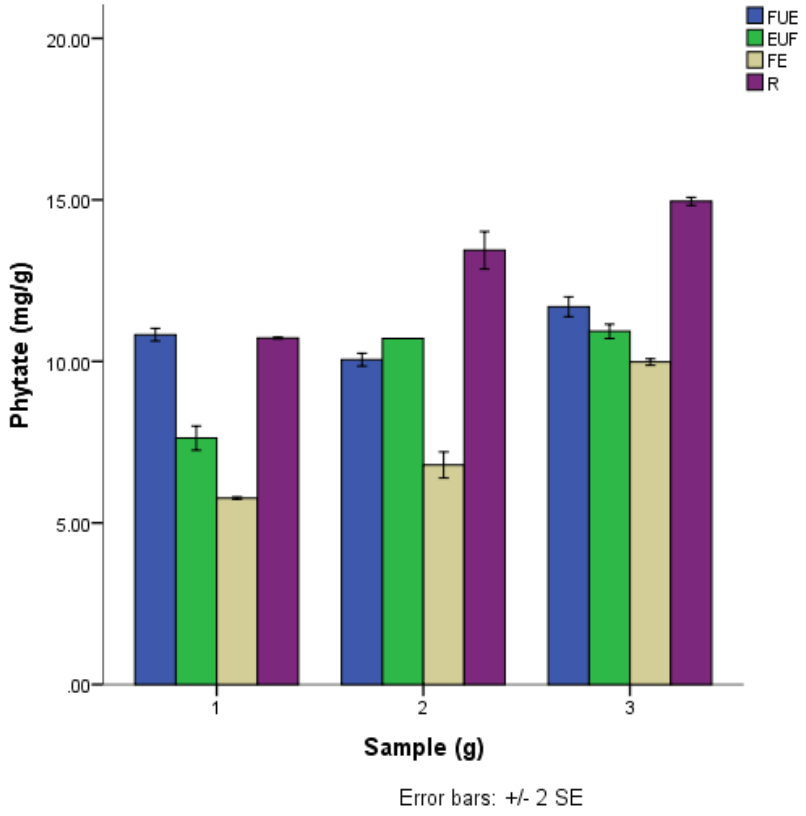

Fig. 10. Phytate content of extruded- unfermented, extruded- fermented, Fermented- unextruded and raw Acha and defatted Soybean flour blends

Keys: EUF1=Extruded Unfermented Acha100%, EF1=Extruded Fermented Acha100%, FUE1=Fermented UnExtruded Acha 100%, EUF2= Extruded Unfermentmented Acha70%Soybeans 30%, EF2= Extruded Fermented Acha 70% Soybeans 30%, FUE2=Fermented Unextruded Acha 70% Soybeans 30%. EUF3= Extruded Unfermented Acha 50% Soybeans 50%, EF3=Extruded Fermented Acha 50% Soybeans 50%, FUE3= Fermented UnExtruded Acha 50% Soybeans 50%.

R1= unfermented Unextruded Acha 100% R2= Unfermented Unextruded Acha 70% soy beans 30%,R3= Unfermented Unextruded Acha 50% soy beans 50

The results of the Saponin in the raw flour blends recorded the highest content in R3 (Unfermented unextruded Acha 50% Soybea ns 50%) with a value of 56.91, while the least was recorded In R1 (Unfermented unextruded Acha 100%) with a value of 39.90.

3.13.4 Phytate content (mg/g) of the extruded- unfermented, extruded-fermented, fermented- unextruded and raw acha and defatted Soybean flour blends is shown in Fig. 10

The highest Phytate content was recorded in FUE3 (Fermented unextruded Acha 50% Soybeans 50%) with a value of 10.70 while the least Phytate content was recorded in FE1 (Fermented Extruded Acha 100%) with a value of 5.77.

The results of the phytate in the raw flour blends recorded the highest content in R3 (Unfermented unextruded Acha 50% Soybea ns 50%) with a value of 14.95, while the least was recorded In R1 (Unfermented unextruded Acha 100%) with a value of 10.72.

4. DISCUSSION

Diverse group of microorganisms were isolated during the process of fermenting Acha and defatted Soybeans. Fifteen organisms were isolated, eight bacteria, five moulds and two yeasts. This is similar to a result reported by [33]. They reported that legumes fortified have higher bacterial population than yeast. The increase in microbial loads could be attributed to the availability of water and source of microorganisms. As the duration of fermentation progresses, a decrease in the growth of bacteria was observed between 48 and 72 hours. The decrease could be du e to reduction in the pH value and utilization of available nutrients such as water.

Temperature of each batch was observed to fluctuate. The fluctuation in temperature may be due to the presence of different microorganisms during fermentation process. The pH of each blend decreased with increase in fermentation time. This is similar to a result reported by [17]. They reported decrease in pH during fermentation process.

Total Titratable Acidity (TTA) of the fermented acha and soybeans increased, this is similar to a report by [15].

Moisture content is one of the most important and commonly measured properties of different food products. It is measured for various reasons including legal and label requirements, economic importance, food quality, better processing operations and storagability. The stable moisture content of the raw blends prior fermentation and extrusion indicates the storagability and shelf life of the samples if properly packaged.

Moisture content of all the samples was significantly different from each other. The moisture content of the extruded unfermented flour blends was significantly higher than the Fermented unextruded and the fermented extruded samples. This could be attributed to oven drying of the samples. [22]. Low moisture content of food sample gives product a long shelf life since microbial activity is reduced and increased storage periods of food product reported by [6,2] while high moisture content in food encourages microbial growth.

Ash is an inorganic residue remaining after the removal of water and organic matter which provides a measure of total amount of minerals in food.

Fermentation caused a significant reduction in ash content of the samples. This may be attributed to the leaching of water soluble mineral content of the flour blends during fermentation process. [12] reported a decrease in ash content during fermentation of ‘‘Gowe’’, a traditional food made from sorghum, millet or maize. This also corresponds with the report by [19].

Fat is one of the major components of food that provides essential lipids and energy. Lipid constituents are the major determinants of Over-all physical characteristics of food such as aroma and texture. Fat content was highest in fermented un-extruded blends. This could be as a result of the metabolic activities of the fermenting microorganisms. Reduction in the fat content of unfermented extruded and fermented extruded blends could be due to lipid oxidation. Lipid oxidation can reduce the nutritive quality of food by decreasing the content of essential fatty acids, such as linolenic acid and linoleic acid, which are essential fatty acids [20]. These long- chained fatty acids are highly susceptible to oxidation which results from application of high temperature during extrusion [20].

Table 1. Biochemical characteristics of bacterial isolated during fermentation of acha and defatted soybeans flour blends

Gram reaction	Cellular morphology	Motility	Slope	Butt	H2S	Indole	Urease	Catalase	Methyl Red	Citrate	Coagulase	Starch hydrolysis	Glucose	Sucrose	Lactose	Fructose	Maltose	Gas	Probable microorganism
-	Cocci	-	RY	+	+	-	-	-	+	+	-	-	-	-	-	-	-	+	*Lactococcus* sp
+	Cocci	-	RY	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	*Lactobacillus* sp
-	Rod	+	RY	-	+	-	-	+		+	-	-	-	-	-	-	-	+	*Enterococcus* sp.
+	Cocci	-	RY	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	*Staphylococcus aureus*
+	Cocci	+	YY	-	+	-	-	+	-	+	-	+	+	+	-	+	-	-	*Streptococcus thermophillus*
-	Rod	-	RY	-	-	-	-	-	+	+	-	+	+	-	+	+	+	+	*Proteus* sp.
+	Cocci	-	RY	-	+	-	-	-	+	+	+	-	-	+	+	-	+	+	*Lactobacillus brevis*
+	Cocci	-	RY	-	-	+	-	-	-	-	-	+	-	-	-	+	-		*Pedicoccus* sp.

KEY: += Positive, - = Negative

Protein is needed for normal body growth, repairs and maintenance. A relatively high amount of protein is therefore required for functional foods and nutraceuticals, because they are used basically for supplementation. The consumption of acha alone cannot produce required protein necessary for complementary foods [8]. Protein value increased in the Fermented and extruded blend compared to the raw flour blends [7]. Defatted Soybean flour fortified with acha indicates nutrient enhancement. This could be due to the significant quantity of protein in Soybeans seeds. Soybean 50% and acha 50% blends will be of nutritional importance in most developing countries with increase in the protein content of unfermented extruded blends. This is contrary to the findings of [14] that extrusion cooking caused reduction in protein and carbohydrate. Increase in protein content of unfermented extruded blends corresponds with the findings of [1].

Table 2. Biochemical characteristics of fungi isolated during fermentation of acha and defatted soybeans flour blends

Probable fungi	Cultural characteristics and microscopic appearance
Rhizopus sp	Colonies appeared whitish fluffy and cottony in texture. The colony turned brown as it aged. Microscopic examination revealed erect sporangiospores were smooth walled, aseptate and light brown in colour. The sporangia were globose.
Mucor mucedo	The colonies appeared black cotton-like on PDA.cotton-like at 24 hours turning dirty with development of black spore on mycelium, non-septate hyphae, thin sporangiospore with a sporangium in a club like form.
Aspergillus flavus	Yellow coloured ringed colonies which later developed into brown hyphae. Conidiophore upright, simple, terminating in a globose bearing phialides at the apex
Penicillium frequentans	The colonies appeared dark green on agar. Conidiophores 100-200 µm long, conidia in long, compact columns, globose to sub globose, 3.0-3.5 µm diameter, smooth or finely roughened.
Fusarium merismoides	The colonies appeared white. Colonies slow growing reading 0.9cm diameter in four days at 25℃, mostly without any d istinct aerial mycelium
Saccharomyces cerevisiae	Creamy in colour, slightly smooth in chains
Candida utilis	Creamy in colour, dry and rough surface glossy colonies oval and round yeast cells

Table 3. Bacteria succession during fermentation of acha and defatted soybeans flour blends

Saples	Time (hours)			
	0	**24**	**48**	**72**
A	*Staphylococcus aureus*	*Lactobacillus* spp	*Lactobacillus* spp	*Lactobacillus* spp
B	*Proteus mirabilis*	*Lactococcus* spp. *Lactobacillus* spp *Proteus mirabilis*	*Pediococcus* spp *Lactococcus* spp. *Lactobacillus* spp	*Pediococcus* spp *Lactobacillus* spp
C	*Proteus mirabilis*	*Staphylococcus aureus* *Lactococcus* spp. *Lactobacillus* spp *Proteus mirabilis*	*Lactococcus* spp. *Lactobacillus* spp *Pedicoccus* spp	*Lactobacillus* spp *Pediococcus* spp

Keys: A= Acha 100%, B= Acha 70%, defatted Soybeans 30%, C= Acha50%, defatted Soybeans 50%

Table 4. Fungal succession during fermentation of acha and defatted soybean flour blends

Samples	Time (hours)			
	0	24	48	72
A	*Aspergillus flavus*	*A. flavus* *S. cerevisiae*	*A. flavus* *S. cerevisiae*	
B	*A. flavus* *Rhizopous stolonifer*	*A. flavus* *R. stolonifer* *S. cerevisiae*	*A. flavus* *R. stolonifer* *M. mucedo* *Penicillium freguentons* *F.merismoiedes* *S. cerevisiae* *Candida utilis*	*R. stolonifer* *Mucor mucedor* *P. freguentons* *F. merismoiedes* *C. utilis*
C	*A. flavus* *R. stolonifer*	*A. flavus* *R. stolonifer* *S. cerevisiae*	*A. flavus* *R. stolonifer* *M. mucedo* *P. freguentons* *F. merismoiedes* *S. cerevisiae* *C. utilis*	*R. stolonifer* *C. utilis* *M. mucedo* *P. freguentons* *F.merismoiedes*

Keys: A= Acha 100%, B= Acha 70%, defatted Soybeans 30%, C= Acha50%, defatted Soybeans 50%

Table 5. Proximate composition acha and defatted Soybean flour blend

Sample	Moisture	Ash	Fat	Protein	Crude fiber	Carbohydrate
EUF1	18.19±0.01k	2.06±0.02h	1.52±0.01b	21.52±0.02f	0.03±0.01b	65.22± 0.01j
EUF2	5.72±0.01a	0.10±0.01a	3.17±0.01c	22.81±0.01h	0.16±0.01f	65.02± 0.01i
EUF3	9.62±0.02e	2.93±0.02l	6.82±0.01g	23.92±0.01j	0.10±0.00d	56.59± 0.01e
EF1	8.65±0.01b	0.52±0.01d	4.22±0.01d	16.52±0.01a	0.00±0.00a	67.02± 0.01k
EF2	9.22±0.00c	1.75±0.02g	6.42±0.01f	23.72±0.01i	0.17±0.01ef	59.88± 0.01g
EF3	10.44±0.07e	2.82±0.01k	6.83±0.02g	30.87±0.02l	0.07±0.00c	49.94± 0.01b
FUE1	9.22±0.01c	0.38±0.02c	0.62±0.01a	18.41±0.01c	0.29±0.01h	67.69±0.01i
FUE2	10.53±0.00f	1.01±0.01e	6.88±0.02h	20.22±0.01d	0.08±0.01c	63.52±0.01h
FUE3	12.32±0.01g	2.62±0.01i	11.03±0.02k	21.69±0.01g	1.19±0.01j	52.07±0.01c
R1	15.33±0.01j	0.35±0.02b	5.99±0.02e	18.29±0.01b	0.18±0.01g	59.77± 0.01f
R2	14.53±0.00i	1.32±0.01f	7.81±0.02i	20.33±0.02e	0.51±0.01i	55.45±0.01d
R3	12.75±0.01h	2.70±0.00j	10.37±0.01j	29.55±0.02k	0.09±0.00d	59.87±0.01a

Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly di fferent ($p \leq 0.05$).

Keys: EUF1=Extruded Unfermented Acha100%, EF1=Extruded Fermented Acha100%, FUE1=Fermented UnExtruded Acha 100%, EUF2=Extruded Unfermentmented Acha70%Soybeans 30%, EF2= Extruded Fermented Acha 70% Soybeans 30%,

FUE2=Fermented Unextruded Acha 70% Soybeans 30%. EUF3= Extruded Unfermented Acha 50% Soybeans 50%, EF3=Extruded Fermented Acha 50% Soybeans 50%, FUE3= Fermented UnExtruded Acha 50% Soybeans 50%. R1= unfermented Unextruded Acha 100% R2= Unfermented Unextruded Acha 70% soy beans 30% R3= Unfermented Unextruded Acha 50% soy beans 50%

Increase in the protein content of fermented unextruded blends could be as a result of protein synthesis by microorganisms during fermentation [10] which contributes to high value in fermented samples. This increase could also be attributed to the increase in microbial mass during fermentation. Increase in the protein content of fermented extruded blends is similar to a report by [10]. They reported that microorganisms are found to increase the protein content of pearl millet-acha on which they grow.

Crude fibre gives bulk to food and aids in regulating physiological functions in the body, it is an indigestible component of food material that helps in improving roughage and bulk as well as contributes to a healthy condition of the intestine. Fermented unextruded blends had the highest crude fibre content but unfermented extruded and fermented extruded blends had low crude fibre content. This implies that extrusion had negative impact on the crude fibre content of the blends. Increase in fibre content for the fermented samples may be due to the activities of microorganisms similar to the report of [4] who reported increased crude fibre content in fermented maize flour. The reduction in fibre content of fermented flour blends could be attributed to enzymatic breakdown of fibre during fermentation by the lactic acid bacteria [16,18]. Decreased fibre content has been reported for fermented millet flour [21].

Carbohydrates play a critical role in the proper functioning of the immune system and human development; Carbohydrates are one of the main types of nutrients. They are the most important source of energy for your body. Carbohydrate content increased compared to the raw flour blends, Carbohydrate content was high in Fermented Un-extruded sample. This may be because fermentation improved carbohydrate content of the blend, this is similar to report by [5].

Mineral such as sodium (Na) and potassium (K) are essential in food. Fermentation improved the Na content of the blends. Calcium (Ca) is good for strong bone and teeth. Zinc (Zn), Iron (Fe) and Magnessium (Mg) are also essential for growth. Mineral content is a measurement of the amount of specific inorganic component present with in foods, It was deduced from the result that fortification of acha and defatted soybeans improved the mineral component of the blends [5].

Extrusion and Fermentation process reduced anti-nutrient content of Acha and defatted Soybeans blend compared to the Raw blend. [29] Confirmed that extrusion process destroys anti-nutrient content of cowpea. Tannins occurs much in legumes, the seed coat of legumes has been associated with tannin contents. From the results tannin content decreased. Fermented Extruded sample recorded the lowest content of tannin. Phytate content reduced in all flour blends compared to the raw flour blends, the reduction was highly significant in fermented extruded samples.

Saponin content reduced in all flour blends but significantly reduced in fermented extruded (FE) blends. Extrusion and Fermentation r educed Trypsin inhibitor, Trypsin inhibitor are enzymes found in legumes. According to [28] these enzyme inhibitors interfere with digestion.

Table 6. Sensory evaluation of acha and defatted soybean flour blends

Sample	Taste	Texture	Flavour	Aroma	Overall acceptability
EUF1	5.61±0.01^{f}	5.30±0.01^{d}	4.21±0.02^{b}	5.29±0.02^{e}	5.92±0.01^{c}
EUF2	5.50±0.02^{e}	5.41±0.01^{e}	5.41±0.01^{e}	5.60±0.02^{g}	6.11±0.02^{d}
EUF3	5.50±0.01^{d}	5.71±0.01^{f}	5.09±0.01^{d}	5.40±0.02^{f}	6.11±0.01^{d}
EF1	6.99±0.01^{k}	7.61±0.02^{j}	6.28±0.02^{h}	6.51±0.02^{j}	7.11±0.01^{h}
EF2	6.91±0.01^{j}	7.60±0.01^{j}	7.61±0.01^{j}	6.41±0.01^{i}	6.91±0.02^{g}
EF3	7.50±0.02^{i}	7.60±0.01^{j}	7.30±0.02^{i}	6.50±0.02^{j}	7.21±0.01^{i}
FUE1	6.71±0.01^{i}	7.40±0.02^{i}	5.12±0.01^{d}	4.91±0.01^{d}	6.70±0.01^{e}
FUE2	6.10±0.01^{g}	5.91±0.02^{g}	5.92±0.02^{g}	6.02±0.02^{h}	6.80±0.01^{f}
FUE3	6.30±0.02^{h}	6.03±0.02^{h}	5.88±0.02^{f}	6.70±0.02^{k}	6.81±0.02^{f}
R1	3.60±0.02^{a}	3.72±0.02^{b}	4.30±0.02^{c}	3.70±0.02^{a}	4.93±0.02^{a}
R2	4.02±0.02^{c}	4.29±0.01^{c}	4.20±0.02^{b}	4.21±0.01^{c}	5.92±0.02^{c}
R3	3.89±0.01^{b}	3.39±0.01^{a}	4.09±0.02^{a}	4.11±0.01^{b}	5.03±0.02^{b}

Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different ($p \leq 0.05$).

Keys: EUF1=Extruded Unfermented Acha100%, EF1=Extruded Fermented Acha100%, FUE1=Fermented UnExtruded Acha 100%,EUF2= Extruded Unfermentmented Acha70%Soybeans 30%, EF2= Extruded Fermented Acha 70% Soybeans 30%,

FUE2=Fermented Unextruded Acha 70% Soybeans 30%. EUF3= Extruded Unfermented Acha 50% Soybeans 50%, EF3=Extruded Fermented Acha 50% Soybeans 50%, FUE3= Fermented UnExtruded Acha 50% Soybeans 50%. R1= unfermented Unextruded Acha 100% R2= Unfermented Unextruded Acha 70% soy beans 30% R3= Unfermented Unextruded Acha 50% soy beans 50%

Table 7. Mineral composition of acha and defatted soybean flour blend

Samples	Na	Ca	K	Fe	Zn	Mg
EUF1	12.92±0.01i	89.51±0.01i	18.89± 0.02a	0.85±0.01 c	0.63±0.02e	2.96±0.01bc
EUF2	11.98±0.02e	60.21±0.01e	155.67±1.34e	2.37±0.02 j	0.77±0.02f	5.95±0.02d
EUF3	13.59±0.02j	101.02±0.01j	243.±1.67h	1.43±0.02f	0.83±0.02g	39.00±0.58g
EF1	11.42±0.01b	41.21±0.01c	19.28± 0.02a	0.94±0.02d	0.483±0.01b	2.24±0.02ab
EF2	12.03±0.00f	83.01±0.01g	128.66±0.88d	1.76±0.02h	0.61±0.01de	5.62±0.02d
EF3	11.51±0.01c	69.09±0.00f	268.33±1.67h	2.63±0.02k	0.85±0.02g	27.68±0.02e
FUE1	11.53±0.00d	40.42±0.01b	17.68± 0.02a	0.63±0.02 b	0.38±0.01a	3.79±0.01c
FUE2	12.100.00g	69.08±0.02f	107.67±1.33c	1.67±0.02g	0.53±0.02c	5.36±0.01d
FUE3	12.31±0.00h	89.03±0.02h	200.00±1.00f	1.75±0.02 h	0.76±0.02f	39.18±0.02g
R1	11.21±0.01a	28.27±0.01a	17.88±0.02a	0.42±0.02a	0.36±0.02a	1.81±0.01a
R2	13.61±0.01j	50.66±0.02d	93.39± 0.02b	0.99±0.01e	0.57±0.02d	5.52±0.02d
R3	12.92±0.01i	89.48±0.02i	206.00±0.58g	1.940±0.02i	0.78±0.02f	34.67±0.88f

Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly di fferent ($p \leq 0.05$)

Keys: EUF1=Extruded Unfermented Acha100%, EF1=Extruded Fermented Acha100%, FUE1=Fermented UnExtruded Acha 100%,EUF2= Extruded Unfermentmented Acha70%Soybeans 30%, EF2= Extruded Fermented Acha 70% Soybeans 30%,

FUE2=Fermented Unextruded Acha 70% Soybeans 30%. EUF3= Extruded Unfermented Acha 50% Soybeans 50%, EF3=Extruded Fermented Acha 50% Soybeans 50%, FUE3= Fermented UnExtruded Acha 50% Soybeans 50%. R1= unfermented Unextruded Acha 100% R2= Unfermented Unextruded Acha 70% soy beans 30% R3= Unfermented Unextruded Acha 50% soy beans 50%

5. CONCLUSION

This study on the effect of Fermentation and Extrusion on nutrient and anti-nutrient composition of acha and defatted soybeans showed that there was improvement in protein, minerals, nutritional quality of the samples compared with the raw blends. Fermented and extruded acha and defatted soybeans can be used in weaning children in developing countries especially Nigeria.

Acha complemented with defatted soybeans proved to be effective in improvement of protein quality which can help to resolve issue of malnutrition in our Country.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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