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Proximate and Physicochemical Composition of Food Prepared from Fermented Starch

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Abstract

Fermentation is a metabolic process that produces chemical changes in organic substances through the action of enzymes. This study was designed to evaluate the proximate and physicochemical composition of food prepared from fermented starch. Cassava starch was allowed to ferment for a period of 12 days, proximate and physicochemical compositions were analysed using standard methods at intervals of 3 days. The result of proximate analysis showed that fermentation significantly increased the % moisture and protein content (B2 to B5) when compared to B1 of the starch food. Also a significant decrease in carbohydrate content (42.99±0.03 - 9.02±0.05) of the fermented samples was noted. The fibre and ash contents were significantly higher after 12 days of fermentation (B5) compared to the other batches. Result of the physicochemical parameters showed that the chloride values, solubility and oil absorption abilities of the samples decreased as the fermentation period increased. The wettability of the samples significantly increased in all the fermented samples compared to unfermented samples. The result obtained in this study showed that fermentation significantly enhanced the % protein and moisture content of the food and decreased the carbohydrate content as the fermentation period increased.

Keywords: Fermentation; Microorganisms; Proximate; Physicochemical; Cassava Starch

Abbreviations: WAC: Water Absorption Capacity; WSI: Water Solubility Index.

Introduction

Starch is a white granular which is a natural component of many plants such as vegetables, cereals and fruits. It is a complex carbohydrate, polysaccharides which exist as amylase or amylopectin. It is the major polysaccharide in plants and the most common carbohydrate in the human diet [1]. Plant creates starch polymer in other to store glucose produced during photosynthesis. Unique structures and compositions such as in the length of glucose chains or amylase /amylopectin ratio are formed by starch molecules produced by each plant species, thus, starch differs depending on the plant source [1].

Cereals and root vegetables are the major sources of starch intake worldwide [2]. Starch is abundant in nature and has several functions [3]. Starch is used as food and a source

of energy for humans. It is metabolized by the body into glucose. It passes through the bloodstream and circulates in the body. Starch is used to produce such different products (food, paper, textiles, adhesives, beverages, confectionery, pharmaceuticals, and building materials).

Cassava is a starch- tuber crop that is eaten as a whole root, root chips or grated to make flour. Cassava starch is produced by wet milling of fresh cassava root. It has many characteristics such as high freeze-thaw stability, high paste viscosity, high paste clarity, high level of purity, and desired textural characteristics. These qualities enhance its industrial utilization [3]. Though it is used primarily in the food industry, advancement in techniques has led to its steady relevance in health and medicine, textile, paper, fine chemicals, petroleum engineering, agriculture, and construction engineering sectors [3]. In the food industry, it is used as a food product or as additive for preservation, and serves as a thickener, and quality enhancer in baked foods [1].

Fermentation is a metabolic reaction process by which microbial enzymes turn raw materials such as glucose into useful products such as alcohol. The chemical changes lead to the production of chemical energy in the form of ATP used to drive many metabolic processes. Also, it could be described as the extraction of energy from carbohydrates when oxygen is limited or not available [4]. In food production, it refers to any process in which the activity of microorganisms brings about a desirable change to a foodstuff or beverage. Fermented foods are produced to extend the shelf-life of raw materials and increase their safety. Fermentation promotes the growth of microorganisms which play an essential role, contributing to the physicochemical, sensory and safety characteristics of food products [5]. Fermented foods have a long tradition of making raw materials very useful, extending the shelf-life of raw materials and increasing their safety as such raw materials are converted into useful products. Microorganisms are actively involved in these processes, contributing to the improvement of the physiochemical, sensory and safety characteristics of the final products [5]. Often fermentation not only imparts a pleasant taste but can also increase the nutritional value of a food; and act as a preservative.

Starch-based food is one of the most consumed foods in the world as it is rich in nutrients and is the main source of daily calorie intake of human beings. Cassava-based foods are the major sources of energy for humans in many developing Countries. Literature reports noted that fermentation is one of the oldest biotechnologies used in the production of cereal foods which can improve the quality, texture, nutritional value and shelf life of the food products. Fermentation could therefore be a useful tool that can be used to improve the nutritional quality and safety profile of cassava food. This work therefore makes use of natural fermentation to improve the nutrients in cassava food. The effect of fermentation duration on the physicochemical and proximate composition of food prepared from fermented cassava starch was investigated.

Materials and Methodology

Sample Collection

Fresh tubers of cassava were obtained from a farmland situated at Emilaghan community of Abua/Odual Local Government of Rivers State, Nigeria. The plant was identified by M.G. Ajuru of Plant Science and Biotechnology Department, Rivers State University, Oroworukwo-Nkpolu, Port Harcourt and the registration number is RSUPb0102. The coordinates of Emilaghan community are **9.05785°N** and **7.49508°E.**

Sample Preparation

The sample was peeled, washed and ground. The ground powder was mixed with 15 litres of water and poured inside a Muslin bag and the starch water was allowed to drain into a clean bucket. The residue in the Muslin bag was used for other purposes while the starch water was allowed to settle. After 3 hours, the fresh starch settled at the bottom. The supernatant was discarded and the starch sample was collected and prepared for analysis.

Starch Food Preparation

The collected sample was divided into five (Batches 1 to 5) and was prepared for analysis at an interval of 3 days. Batch 1 was food prepared from fresh starch immediately after sample collection, batch 2 was food prepared from raw starch that was allowed to ferment for 3 days, batch 3 was food prepared from raw starch that was allowed to ferment for 6 days, batch 4 was food prepared from raw starch that was allowed to ferment for 9 days, batch 5 was food prepared from raw starch that was allowed to ferment for 12 days.

Procedure

Twenty grams of the raw starch sample was collected and mixed with 1 cup of water. The mixture was poured into a preheated non-stick pot and a tablespoon of palm oil was added. The mixture, while being heated, was stirred continuously until it was cooked.

Proximate Analysis

- **Moisture Content**
  A petri-dish was washed and dried in the oven [6].

Approximately 1-2g of the sample was weighed into a petri dish and the weight of the petri dish and sample was noted before drying. The petri dish and sample were put in the oven and heated at 105^{0}c for 2hr and the result was noted. It was also heated for another 1hr until a steady result was obtained and the weight was noted. The drying procedure was continued until a constant weight was obtained.

$$\%\ moisture\ content = \frac{W1 - W2}{weight\ of\ sample} \times \frac{100}{1}$$

Where, W_1= weight of petridish and sample before drying
W_2 = weight of petridish and sample after drying.

- **Carbohydrate Determination (Differential Method)**

$$100 - (\%Protein + \%\ Moisture + \%Ash + \%Fat + \%Fibre)$$

Ash Content

- **Procedures**

Empty platinum crucible was washed, dried and the weight was noted. Approximately 1- 2g of sample was weighed into the platinum crucible and placed in a muffle furnace at 550^{0}c for 3 hours and the sample was cooled in a desiccator after burning and weighed.

- **Calculation**

$$\%\ Ashcontent = \frac{W3 - W1}{W2 - W1} \times \frac{100}{1}$$

Where,
W_1 = weight of empty platinum crucible
W_2 = weight of platinum crucible and sample before burning
W_3 = weight of platinum and ash.

Crude Fibre

- **Procedure**

2g of material was defatted with petroleum ether (if the fat content if more than 10%). The solution was boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution. The solution was filtered through linen and washed with boiling water until the washings were no longer acid. The residue was transferred to a beaker and boiled for 30 minutes with 200ml of a solution containing 1.25g of carbonate-free NaOH per 100ml. The final residue was filtered through a thin but closed pad of washed and ignited asbestos in a Gooch crucible. It was dried in an electric oven, incinerated, cooled and weighed.

$$\%\ crude\ fibre = \frac{weight\ of\ fibre}{Weight\ of\ sample} \times \frac{100}{1}$$

Crude Fat

- **Soxhlet Fat Extraction Method**

This method is carried out by continuously extracting a food with non- polar organic solvent such as petroleum ether for about 1 hour or more.

- **Procedure**

250ml clean boiling flasks was dried in oven at 105 - 110^{0}C for about 30 minutes. It was transferred into a dessicator and allowed to cool. It was weighed correspondingly in labelled, cooled boiling flasks. The boiling flasks were filled with about 300ml of petroleum ether (boiling point 40 - 60°C). The extraction thimble was plugged lightly with cotton wool and the Soxhlet apparatus was assembled and allowed to reflux for about 6 hours. The thimble was removed with care and petroleum ether was collected in the top container of the set – up and drained into a container for re-use. When the flask was almost free of petroleum ether, it was removed and dried at 105°C - 110°C for 1hour.It was finally transferred from the oven into a desiccator and allowed to cool; then weighed.

$$\%\ fat = \frac{wt\ of\ \ flask + oil - wt\ of\ \ flask}{wt\ of\ \ sample} \times \frac{100}{1}$$

Crude Proteins

- **Principle**

The method is the digestion of a sample with hot concentrated sulphuric acid in the presence of a metallic catalyst. Organic nitrogen in the sample is reduced to ammonia. This is retained in the solution as ammonium sulphate. The solution is made alkaline and then distilled to release the ammonia. The ammonia is trapped in dilute acid and then titrated.

- **Procedures**

Exactly 0.5g of sample was weighed into a 30ml Kjeldahl flask (gently to prevent the sample from touching the walls of the side of each and then the flasks were shaken. Then 0.5g of the Kjeldahl catalyst mixture was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling was made up to 100ml with distilled water was added to avoid caking and then, 5ml was transferred to the Kjeldahl distillation apparatus, followed by 5ml of 40% sodium hydroxide. A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of

Bromocresol blue and 1 drop of methylene blue was placed added under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution and distillation commenced immediately until 50 drops get into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid.

- **Calculations**

$$\% \ Nitrogen = Titrevalue \times 0.01 \times 14 \times 4$$

$$\% \ Protein = \% \ Nitrogen \times 6.25$$

Estimation of Total Carotenoids

Total carotenoids and lycopene were estimated by the method described by Evans WC [7].

- **Procedure**

The experiment was carried out in the dark to avoid photolysis of carotenoids once the saponification was complete. The sample (0.5g) was homogenized and saponified with 2.5ml of 12% alcoholic potassium hydroxide in a water bath at 60^0C for 30 minutes. The saponified sample was transferred to a separating funnel containing 10-15ml of petroleum ether and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was collected. The sample was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether sample to remove excess moisture. The final volume of the petroleum ether sample was noted. The absorbance of the yellow colour was read in a spectrophotometer (Genesys 10-S, USA) at 450nm and 503nm using petroleum ether as blank. The amount of total carotenoids and lycopene was calculated using the formulae,

$$Amount\ of\ total\ carotenoids = \frac{A450 \times Volume\ of\ the\ sample \times 1 \times 4}{Weight\ of\ the\ sample}$$

$$Amount\ of\ lycopene = \frac{3.12 \times A503 \times Volume\ of\ the\ sample \times 1}{Weight\ of\ the\ sample}$$

The total carotenoids and lycopene were expressed as mg/g of the sample

WAC and Water Solubility Index

The water absorption capacity (WAC) and the water solubility index (WSI) of the flours are determined according to the methods of Phillips RD and Andersen RA [8,9]. Two (2) g of flours (M_0) are dissolved in 50 ml of distilled water contained in a centrifuge tube. This mixture is stirred for 30 minutes with a stirrer and then kept in a water bath at 37°C. for 30 minutes. It is then centrifuged at 5000 rpm for 15 minutes. The pellet obtained (M_2) is weighed and then dried at 105°C to a constant mass (M_1). WAC and WSI are calculated from the following relationships:

$$WAC\ \% = \frac{M2 - M1}{M1} \times \frac{100}{1}$$

$$WSI\ \% = \frac{M0 - M1}{M0} \times \frac{100}{1}$$

Wettability

The wettability of the flours is determined according to the technique of Onwuka GI [10]. One (1) gram of flour is placed in a graduated 25 ml test tube having a diameter of 1 cm. A finger is placed on the opening of the specimen (to avoid pouring the sample by reversing it). The finger closing the specimen is placed at a height of 10 cm from the surface of a 600 ml beaker containing 500 ml of distilled water. The finger is removed and the sample is poured into the beaker. Wettability is the time needed for the sample to become completely wet

Cyanogenic Glycoside (Acid Titration Method)

- **Procedure**

The collected sample was ground and sieved, in an 800ml Kjeldahl flask, and 100ml H2O was added [11]. It was macerated at room temperature for 2 hours. 100ml of H_2O was added and steam distilled, collecting distillate in 20ml 0.02N $AgNO_3$ acidified with 1ml HNO_3. Before distillation adjust appropriately, so that the tip of the condenser dips below the surface of the liquid in receiver. When 150ml has passed over, the distillate was filtered through the gooch wash receiver and gooch with little H_2O. Excess $AgNO_3$ was titrated in combined filtrate and washings with 0.02N KCN, using Fe alum indicator. 1ml 0.02N $AgNO_3$ = 0.54mg HCN.

All data were presented as mean ± standard deviation. Data were analyzed with a one-way analysis of variance (ANOVA). Results were compared among groups with Scheffe's post hoc test and considered significant at $p<0.05$.

Result and Discussion

Parameters	B1	B2	B3	B4	B5
Carbohydrate content	42.99±0.03bdfh	18.54±0.66*adfh	9.02±0.05*bcfh	19.76±0.12*bdeh	12.76±0.33*bdfg
Fat Content	6.85±0.20acfh	6.57±0.05adfh	6.92±0.12bcfh	6.04±0.08*bdeg	5.99±0.08*bdfg
Ash content	0.59±0.03adfh	0.69±0.01adfh	0.26±0.02*bcfh	0.15±0.00*bdeh	2.43±0.08*bdfg
Moisture content	45.27±0.56bdfh	67.17±0.26*adfh	75.93±0.11*bcfh	64.83±0.25*bdeh	69.71±0.17*bdfg
% Protein	4.66±0.16bdfh	6.37±0.12*adfh	7.69±0.01*bcfh	8.19±0.29*bdeh	6.90±0.14*bdfg
Fibre Content	0.50±0.02bdfh	0.99±0.06*adeh	0.30±0.01*bcfh	1.14±0.01*adeh	1.92±0.13*bdfg

Key B1: Starch food immediately after grinding, B2- Starch food after 3 days of fermentation, B3- Starch food after 6 days of fermentation, B4- Starch food after 9 days of fermentation, B5- Starch food after 12 days of fermentation.
Note: Values are expressed as mean ± standard deviation (n=3). Values with * showed significant difference at the 0.05 level when comparing B1 with others (B2, B3, B4 & B5). Values with different superscript (a,b) show significant difference when comparing B2 with others (B1, B3, B4 & B5). Values with different superscript (c,d) show significant difference when comparing B3 with others (B1, B2, B4 & B5). Values with different superscript (e,f) show significant difference when comparing B4 with others (B1, B2, B3 & B5). Values with different superscript (g,h) show significant difference when comparing B5 with others (B1, B2, B3 & B4).
Table 1: Proximate Composition of Food Prepared from Fermented Starch.

Starch-based food is among the most consumed foods in the world; it is rich in nutrients and is the main source of daily calorie intake of human beings. Table 1 showed the proximate composition of food prepared from cassava starch. Carbohydrate is an essential nutrient that supplies energy for nutrition and they are readily fermented by microorganisms to release carbon dioxide. The result obtained in this study showed that fermentation decreased the carbohydrate content of the starch food as there was a significant decrease in the carbohydrate content of B2 to B5 samples compared to its value for the B1 sample. High carbohydrate content facilitates the manufacturing of foods such as cassava strips, snacks, gruels, short biscuits, porridges, instant flours and gluten-free pasta [12]. The low level of carbohydrates recorded in starch foods could be attributed to the fact that bacteria eat up sugars and starches of the starch food, thus converting them to lactic acid and carbon dioxide. Low carbohydrate content implies that fermented starch food may not be effectively utilized in formulating composite flour blend in the food manufacturing industry [12].

Fats are the most energy-dense nutrients providing on average about 9cal/kg of energy. They help in regulating blood pressure and play useful role in the synthesis and repair of vital cell parts [13]. The result obtained in this study showed that the fat content of the starch food of B4 and B5 samples were significantly lower when compared with B1. High-fat content in starch food is an undesirable attribute since too much fat will lead to rancidity and increased cloudiness in the starch food [13]. Low-fat content leads to an increase in storage capacity, thereby reducing the development of rancidity. The decrease in the fat content of starch food observed in this study is in tandem with the findings of Zemenu KT, et al. [14].

Ash refers to the inorganic residue remaining after the complete oxidation of organic matter in a foodstuff and it represents the total mineral content of a food item. The availability of minerals in food helps in the metabolism of macronutrients for the release of energy [15] .The result obtained in this study shows that the highest ash content was observed in sample B5 while sample B4 had the least ash content. The lower ash content recorded in samples B3 and B4 could be a result of the usage of minerals (such as iron) by inherent microorganisms for metabolic activities and this is similar with the findings of Adejuyitan JA, et al. [15]. The moisture content of a food material is of significance to shelf life, packaging and general acceptability. Moisture is one of many environmental conditions that support microbial growth and spoilage. The result revealed that the moisture content increased in the fermented samples B2 to B5 when compared to the B1 sample. The increase observed in the fermented samples could be due to decreased dry matter content as a result of microbial cell proliferation [16]. The high moisture content observed in the food prepared from the fermented samples could reduce their storage stability by enhancing mould growth and increasing moisture-dependent biochemical reactions [17]. High moisture content makes a product susceptible to microbial and enzymatic spoilage [16].

Protein is an important macronutrient and a functional ingredient in food formulations required for the survival of humans and animals for the supply of substantial amounts of

amino acids. Protein is needed to form blood cells, protects, forms, rebuilds, maintains and grows tissue, skin, hair, tissue and vital organs [18]. The result obtained in this study showed that the food prepared from fermented samples (B2 to B5) had a significant increase in protein content. This finding is in consonance with the result of Adejuyitan JA, et al. [15], who reported a decrease in the protein content of tigernut flour.

Crude Fibre is well known for maintaining bulk, motility and increasing peristalsis by surface extension of the food in the intestinal tract [19]. The result obtained in this study shows that fermentation could increase the fibre content of the starch food as shown in B2, B4 and B5. High fibre content of food helps to remove potential mutagens, steroids and xenobiotics by attaching to dietary fibre components. The increase in fibre content observed in most fermented food samples has several health benefits as it will aid in digestion in the colon and reduce constipation [20].

Parameters	B1	B2	B3	B4	B5
Ph	5.5±0.55	5.70±0.07	5.16±0.05	5.22±0.06	5.15±0.08
Temperature	30.50±2.12	29.94±0.08	32.50±0.71	31.93±0.09	30.49±0.71
Chloride	98.50±6.36adfh	91.94±0.08acfh	89.89±0.15*aceh	83.89±0.15*bceg	80.43±0.81*bdeg
Copper	0.10±0.02bdfh	0.06±0.00*aceg	0.07±0.00*aceg	0.06±0.01*aceg	0.06±0.00*aceg
Lead	0.06±0.01	0.04±0.00	1.52±2.09	0.03±0.00	0.02±0.00
Cadmium	0.05±0.00	0.06±0.01	0.05±0.01	0.04±0.01	0.04±0.01
Manganese	0.06±0.01aceh	0.06±0.00aceh	0.05±0.01aceh	0.05±0.00aceh	0.03±0.01*bdfg
Cyanogenic glycoside	1.41±0.59	1.71±0.06	1.55±0.06	1.14±0.05	1.36±0.09
Carotenoids	11.92±0.12adfh	12.18±0.07adfh	13.65±0.14*bcfh	9.95±0.21*bdeh	9.50±0.18*bdfg
Swelling ability	11.81±0.24bdfh	13.55±0.64*acfh	13.37±0.20*acfh	8.87±0.20*bdeg	9.62±0.23*bdeg
Water retent. Capacity	5.19±0.02bdfh	1.87±0.09*adfh	4.71±0.21*bcfg	6.78±0.15*bdeh	4.40±0.03bcfg
Solubility	44.29±0.17bdfh	40.23±0.49*adfh	37.99±1.37*bcfh	6.36±0.09*bdeg	6.29±0.13*bdfg
Oil absorption	29.77±0.46bdfh	24.17±0.39*adfh	20.60±0.15*bcfh	8.53±0.11*bdeh	6.65±0.09*bdfg
Wettability	34.49±0.71bdfh	48.49±0.71*adeh	55.94±0.08*bcfh	49.55±0.63*adeh	37.94±0.08*bdfg

Keys B1: Starch food immediately after grinding, B2- Starch food after 3 days of fermentation, B3- Starch food after 6 days of fermentation, B4- Starch food after 9 days of fermentation, B5- Starch food after 12 days of fermentation.

Note: Values are expressed as mean ± standard deviation (n=3). Values with * showed significant difference at the 0.05 level when comparing B1 with others (B2, B3, B4 & B5). Values with different superscripts (a,b) show significant differences when comparing B2 with others (B1, B3, B4 & B5). Values with different superscripts (c,d) show significant differences when comparing B3 with others (B1, B2, B4 & B5). Values with different superscripts (e,f) show significant differences when comparing B4 with others (B1, B2, B3 & B5). Values with different superscripts (g,h) show significant differences when comparing B5 with others (B1, B2, B3 & B4).

Table 2: Physicochemical Properties of Food Prepared from Fermented Starch.

Table 2 shows the result of the effect of fermentation on the physicochemical parameters of food produced from starch. The result showed that the pH and temperature values of the samples had no statistical difference. The pH values of the foods prepared from samples (B1 to B5) were within the acidic range (pH range of 5.5 to 5.15). This will contribute to maintaining the acid-base balance in the body [21]. The concentration of copper decreased in all fermented samples, while the wettability of the samples increased in all the fermented samples compared to the unfermented sample. Chloride values, solubility and oil absorption abilities of the samples decreased as the fermentation period increased. Water solubility is related to the presence of soluble molecules and is a measure of starch degradation. A lower solubility index of any food substance suggests a minor degradation of starch and leads to less numbers of soluble molecules in food substances. The decrease in solubility observed in the fermented samples is an indication that molecules of the starch food particles were not degraded by the fermentation process making them less available for digestion [21]. Since water solubility is related to the number of solute particles, the increase in moisture content observed in fermented samples showed that there was a decrease in the number of solute particles during fermentation, thus decrease in the solubility index [21]. Oil absorption capacity is important for retaining the flavour and mouth feel of foods.

The lower oil absorption capacity observed in fermented food samples might be due to low hydrophobic proteins which show superior binding of lipids [15].

Also, there was no statistical difference in the concentration of cadmium, lead and cyanogenic glycoside between fermented and unfermented food samples. Lead and cadmium are very toxic metals that affect human health. The result obtained in this study showed that fermentation decreased the concentrations of these metals in some of the samples, although no significant difference was observed. The decrease observed in some fermented samples proves that fermented samples could be safer than unfermented samples. This result is in agreement with the findings of Alozie D, et al. [22], who reported no significant difference in the lead content of fermented maize flour. The concentration of carotenoids and the swelling ability of the food samples increased in samples B2 and B3 but decreased in samples B4 and B5. For manganese, samples B1 and B2 had the same value, likewise to samples B3 and B4. The water retention value of the fermented samples reduced except in sample B4 where an increase was recorded.

Conclusion

In this study, the result of the proximate analysis showed that fermentation significantly increased the % moisture and protein content (B2 to B5) samples when compared to B1 sample of the starch food. A significant decrease in carbohydrate content (42.99±0.03 - 9.02±0.05) of the fermented samples was noted. Results of the physicochemical parameters showed that the chloride values, solubility and oil absorption abilities of the samples decreased as the fermentation period increased.

Authors Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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