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Effect of Fermentation on the Nutrient and Antinutrient Composition of African Yam Bean (*Sphenostylisstenocarpa*) Seeds And Pearl Millet (*Pennisetumglaucum*) Grains

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Abstract :

Aims: The study evaluated the effect of fermentation on the nutrient and antinutrient composition of African Yam bean seeds and pearl millet grains.

Methodology: Fermentation was done for 24, 48 and 72h. The flours produced after each fermentation periods were analysed using the standard assay methods. The protein, ash, crude fibre and fat content of the samples were analysed using the standard methods of AOAC (9). Carbohydrate was determined by difference (The summation of all the proximate values was subtracted from 100%). Thus: % carbohydrate = 100% - (% crude protein + % fat + % ash + % Crude fibre + % moisture) The minerals Calcium, (Ca), Iron (Fe), Magnesium (Mg), Zinc (Zn) and Phosphorus (P) were determined by atomic absorption spectrophotometer. The tannin level of the flours were determined by the modified vanillin-HCL method of Price and Buttler⁽⁴⁰⁾, while phytate was estimated by a photometric method of Latta and Eskin⁽⁴¹⁾.

Results: Fermentation increased the protein and ash levels in both flour samples (24.70 to 28.70%). It increased fat in millet (4.06 - 4.68% vs. 3.98%) relative to the control. Carbohydrate content of the flours was decreased due to fermentation. The Fe, Zn, Mg, Ca, and P levels were significantly increased in the fermented flours of AYB and pear millet with the exception of calcium for millet (P < 0.05) Fermentation reduced the tannin levels in both flours as against the control (0.51 to 0.31 mg) and (0.16 to 0.02mg) respectively. The phytate content was also reduced from 0.49 to 0.25mg and 0.26 to 0.06mg.

Conclusion: The importance of fermentation in the processing of plant foods cannot be over emphasized, in that it is one of the simplest and cheap food processing techniques for improving the nutritional quality of local staples in many homes.

Keywords: African Yam Bean, Pearl Millet, Fermentation, Nutrient and Antinutrient.

1. Introduction

Food insecurity and malnutrition is a serious problem in Nigeria, despite all the effort to combat them. Global nutrition survey revealed that the most dietary deficiencies are of protein of high biological value⁽¹⁾. This is because animal food which are rich sources of protein are very expensive and beyond the reach of majority of the population. However, vegetable proteins complement each other, a combination of legume and cereal protein will have a nutritive value as good as animal protein. According to Baldi⁽²⁾ protein deficiency cannot be overcome by using animal products alone. He maintained that the only alternative is to push up the vegetable protein intake, which already makes up 70% of world's protein production⁽²⁾.

The African yam bean is grown for both its edible seeds and its tubers. It is a vigorous vine, which twines and climbs to heights of about 3 m and requires staking. It flowers profusely in 100 to 150 days, producing brightly-coloured flowers, which may be pink, purple or greenish white. The slightly woody pods contain 20 to 30 seeds, are up to 30 cm long and mature within 170 days. The plant produces underground tubers that are used as food in some parts of Africa and that serve as organs of perennation in the wild^(3, 4, 5).

Despite the large number of existing grain legumes in Nigeria, their consumption as staple food have centered mainly on cowpea and groundnut⁽⁶⁾. African yam bean is among the lesser known legumes in the tropics. In Nigeria, African yam bean is still among the underutilized food crops. It is not widely cultivated although, it has a promising nutritional attributes^(7, 8). It is mostly found in

Nsukka area, some part of Benue, Imo and Abia State. The mature and dry seeds of African yam bean seeds are hard to cook⁽⁹⁾. In the traditional set up, the seeds are usually cooked over night before consumption. And also they have a very strong beany flavour which repels those who are not familiar with it. The African yam bean seeds have high lysine (an essential amino acid) content⁽¹⁰⁾. The crude protein levels vary from 21-29% of which lysine comprise up to 8% of the protein, 50% of carbohydrate and 5-6% of fibre. Simple processing techniques such as fermentation are employed to convert the seed into flour with reduced beany flavour so as to use it for other food preparation⁽¹¹⁾.

Researchers who did a nutritional evaluation of 44 genotypes of AYB reported that the crop is well balanced in essential amino acids and has higher amino acid content than pigeon pea, cowpea, and bambara groundnut. Apart from the use of soybean as an alternative to animal protein, protein from other plant sources is not often exploited⁽¹¹⁾. The protein content in AYB grains ranged between 21 and 29% and in the tubers it is about 2 to 3 times the amount in potatoes^(11, 12). AYB produces an appreciable yield under diverse environmental conditions^(13, 14). Another positive contribution of the crop to food security is the identification of the presence of lectin in the seeds, which could be a potent biological control for most leguminous pests⁽¹⁵⁾.

African yam bean is being increasingly consumed by diabetics, hypertensive and cardiovascular patients in some Nigerian communities. However, the processes involved in making it ready to eat are cumbersome and time consuming, thereby denying vulnerable groups' instant consumption access. Therefore, it is of great industrial and commercial importance to develop the technology for its processing into ready-to-eat breakfast cereals, in combination with other local food materials, which will eliminate the associated drudgery and add value to the product⁽⁹⁾.

Pearl millet (*Pennisetum glaucum*) is a major food staple in semi-arid and arid lands of Africa and Asia. Pearl millet is well adapted to drought and sandy acid soil of low fertility⁽¹⁷⁾. The crop ranks as world's fourth most tropical food cereal. The nutrient composition of pearl millet indicates that it is a good source of energy and protein⁽¹⁸⁻²⁰⁾. Essential amino acid profile revealed that pearl millet is 40% richer in lysine and methionine and 30% richer in threonine than in protein of corn⁽²¹⁾. Nutritional studies indicated that metabolized energy of pearl millet for non-ruminant animals is approximately equal to that of maize⁽²²⁾. As with other cereals certain nutritional inhibitors, such as enzyme inhibitors, phytic acid and tannins are associated with pearl millet. These factors affect the nutritional value of the grain by inhibiting protein and starch digestibility and mineral bioavailability. Several methods have been employed to improve the nutritional quality of cereals. Fermentation is one of the oldest methods and widely used process of cereals such as sorghum and millets. Many investigators have reported that fermentation can be effectively used for improving nutritional quality of cereal grain by increasing protein content and digestibility^(23 - 25) and available lysine content and relative nutritive value⁽²⁶⁾. Fermentation was also found to decrease trypsin inhibitory activity (TIA), amylase inhibitor, activity, phytic acid, and tannins^(27 - 30).

Millet is one of the underutilized cereals in Nigeria. They are often regarded as coarse grain or poor people's crop and are not usually traded in the international market or even in local market in many countries as other cereals such as rice, wheat, maize and so on⁽¹⁶⁾. Millet consumption and utilization is common in the Northern part of Nigeria than in Southern or Eastern Parts. The Hausa's use Millet in the production of their local beverages. Pearl Millet is high in fat, carbohydrate and energy value⁽¹⁶⁾.

It is obvious that plant foods contain a lot of antinutritional factors that affect the bioavailability of nutrients. However, Simple food processing technique such as fermentation can significantly reduce the level of these antinutrients⁽³¹⁾.

Millets have the following anti-nutrient components: polyphenols and tannin, phytic acid and phytate, goitrogens, and oxalic acid. Polyphenols and tannin compounds are concentrated in the bran. There is a strong relationship between the tannin levels and in-vitro protein digestibility. Decortication significantly decreases the amount of tannins with a corresponding increase in protein digestibility⁽³²⁾.

Fermentation has great advantage in the processing of legumes and cereals, since it improves the bio-availability of both macro and micro nutrients. Fermentation is required to improve the quality of this food processes. This involves metabolic process whereby electrons released from nutrients are ultimately used to release energy and generate simpler products or nutrients. In food processing, typically, fermentation can be described as the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria or a combination thereof under anaerobic conditions. A more restricted definition of fermentation is the chemical conversion of sugars into ethanol⁽³³⁾. There are three main kinds of fermentation which are alcoholic, acetic and putrefactive. Each is caused by the presence of bacteria, yeasts or molds which produce the particular enzymes responsible for chemical change⁽³⁴⁾.

Methods of Fermentation could be natural or Control. Natural fermentation is one of the oldest means of preservation. It means leaving the foods in a container at room temperature for one to seven days. No additional bacteria are deliberately added to the foods and no heating process is used. It can be achieved by a combined metabolic engineering and transcriptome analysis approach. Lactic acid bacteria subject the foods to a fermentation process which becomes preserved, it develops a pleasantly sour taste and it is rich in vitamins and minerals⁽³⁵⁾.

Control fermentation is a term of food preservation since it generally results in a reduction of acidity of the food, thus preventing the growth of spoilage microorganism. The two most common acids produced are lactic acid and acetic acid. In controlled fermentation, the reactions are usually very complex and involve a series of microorganisms either working together or in a succession to achieve the final product. Controlled fermentation is used to produce range of fermented foods including krant, pickles olives, vinegar, diary and other products⁽³⁶⁾.

Fermentation can also be grouped into two, that is, Solid substrate fermentation (SSF) and submerged liquid fermentation (SLF). Solid substrate fermentation (SSF) is the growth of fermentation fungi on the surface of cereal grains. The filamentous fungi are the best adapted microorganisms for SSF growing to their physiological, enzymological and biochemical properties. The hyphal mode of fungi growth gives the filamentous fungi the power to penetrate in the solid substrate. This also gives them a major advantage over unicellular microorganisms for the colonization of the substrate and the utilization of the available nutrients. In SSF system fungal growth within a solid mash is an important index for the efficiency of the scarification and production of

metabolites.⁽³⁷⁾ Submerged liquid fermentation (SLF) involves growth of microbes in an aqueous medium and it is a process been employed for the production of probiotics. It is traditionally used in the United States for the production of microbially derived enzymes. It involves submersion of the microorganisms in the aqueous solution containing all the nutrients needed for growth⁽³⁵⁾. This study evaluated the effect of fermentation on the nutritional and antinutritional composition of African Yam bean and pearl millet flour.

2. Methodology

2.1. Material

The African yam bean seeds and millet grains used for the study were purchased from Nsukka in Enugu State and Jos in Plateau State all in Nigeria, respectively.

2.2. Processing of Fermented African Yam Bean and Millet Flours

2.2.1. African Yam Bean Seeds

Two kilograms of African yam bean seeds were cleaned and divided into four equal portions. One portion, which served as the control, was washed with tap water, dried, dehulled and milled into fine flour (70mm mesh screen). The remaining three portions were soaked in deionized cold water in a ratio of 1:3 (W/N) and allowed to ferment by the micro flora inherent in the seeds for 24, 48 and 72h at room temperature ($28 \pm 2^{\circ}\text{C}$). The fermented seeds were separately, dehulled and dried in an air oven (Model No. 320, Gallenkamp, England) at 60°C to 96% dry matter. The seeds were milled in a laboratory hammermill (Thomas Wiley mill, model ED-5) into fine flours (70mm mesh screen). The samples were stored in separate air tight polythene bags until analysed.

2.2.2. Millet Grains

Two kilograms of pearl millet were thoroughly cleaned and divided into four portions. One portion was not subjected to any treatment. It served as the control. The other three portions were separately soaked in cold water in a ratio of 1:3 (W/V) and allowed to ferment for 24, 48 and 72h. Both the ferment and unfermented (control) millet grains were dried separately at 55°C to 96% dry matter and hammer milled into fine flour (70 mesh screen). The flours were packed in polythene bags until analyzed.

2.3. Chemical Analysis

The protein, ash, crude fibre and fat content of the samples were analysed using the standard methods of AOAC⁽³⁸⁾. Carbohydrate was determined by difference (The summation of all the proximate values was subtracted from 100%). Thus: % carbohydrate = $100\% - (\% \text{ crude protein} + \% \text{ fat} + \% \text{ ash} + \% \text{ Crude fibre} + \% \text{ moisture})$

The minerals Calcium, (Ca), Iron (Fe), Magnesium (Mg), Zinc (Zn) and Phosphorus (P) were determined by atomic absorption spectrophotometer as described by Ranjham and Gopal⁽³⁹⁾. The tannin level of the flours was determined by the modified vanillin-HCL method of Price and Buttle⁽⁴⁰⁾, while phytate was estimated by a photometric method of Latta and Eskin⁽⁴¹⁾. All the analysis was done in triplicates.

2.4. Data Analysis

Means and standard deviation were calculated for all the samples using the method of Obi⁽⁴²⁾.

3. Results and Discussion

Sample	Protein%	Fat %	Ash%	Fibre %	CHO%
UAYB	24.70±0.02	2.70±0.02	3.60±0.04	4.46±0.03	64.54±0.02
AYBF ₂₄	27.61±0.02	1.72±0.02	3.55±0.06	2.50±0.04	64.62±0.03
AYBF ₄₈	28.70±0.06	1.50±0.02	4.20±0.06	2.30±0.01	63.30±0.02
AYBF ₇₂	25.81±0.02	1.37±0.06	2.90±0.02	2.00±0.02	67.92±0.02
UM	12.30±0.04	3.98±0.01	2.55±0.03	2.00±0.03	79.17±0.02
MF ₂₄	11.40±0.01	4.06±0.02	2.55±0.03	1.30±0.03	80.69±0.02
MF ₄₈	13.13±0.02	4.50±0.03	2.20±0.02	1.66±0.01	78.51±1.10
MF ₇₂	9.63±0.03	4.68±0.02	1.35±0.02	1.60±0.04	82.67±0.60

Table 1: Proximate Composition of Fermented and Unfermented African Yam Bean and Millet Flours (Dry Weight Basis)

Mean ± SD of Three replications

UAYB	=	Unfermented African Yam Bean Flour
AYBF	=	African Yam Bean Fermented for 24 hours
AYBF ₄₈	=	African Yam Bean Fermented for 72 hours
AYBF ₇₂	=	African Yam Bean Fermented for 72 hours
UM	=	Unfermented Millet Flour
MF ₂₄	=	Millet Fermented for 24 hours
MF ₄₈	=	Millet Fermented for 48 hours
MF ₇₂	=	Millet Fermented for 72 hours

Table 1 presents the proximate composition of the flour samples. Crude protein levels ranged from 24.70 to 28.70% for African yam bean flours and 9.63 to 13.13% for pearl millet flours. Fermentation increased the protein content of the flours except for the MF24 and MF72. The higher protein for the fermented flours, especially the 48h period relative to the control (28.70 and 13.13% vs. 24.70 and 12.30%) might be due to the increased activity of the proteolytic enzymes that released more free amino acids into the fermenting medium. This observation accords the report of many workers on legume^(43, 44). Similarly, Ali et al⁽²⁴⁾ reported a marginal change in protein content of fermented pearl millet⁽²⁴⁾. Contradictory reports on protein content in fermented pearl millet are also available. Inyang and Zakari⁽²³⁾ reported that fermentation significantly increased protein content of pearl millet⁽²³⁾, while El Hag et al.⁽²⁵⁾ observed a decrease in protein in fermented pearl millet⁽²⁵⁾. However, it could be attributed that Fermentation will alter the biochemical structure of protein in the substrate material hence, breaking in down to simpler components. Any increase in “protein” per se might be due to microbial activity increase (single cell proteins) which in turn will affect the protein of the final product analysed. There have been reports on the liberation of soluble amino acids during fermentation of vegetable seeds into condiments⁽⁴⁵⁾ Ogunsheet *et al.*,⁽⁴⁶⁾ reported increased total free amino acids in the controlled fermentation of afiyo. Increases in the level of free amino acids with fermentation have been reported in other leguminous vegetable seeds⁽⁴⁷⁻⁴⁸⁾. This rapid increase in the total free amino acids may be a reflection of the increased protease activity observed in the fermenting seeds. Odunfa,⁽⁴⁹⁾ and Campbell-Platt⁽⁵⁰⁾ also noted a high level of proteolytic activity during *dawadawa* fermentation, which culminated in the formation of peptides and amino acids.

There were variations in the fat content of the African yam bean flours. Relative to the control, fermentation decrease the fat level from 2.70% to 1.39 - 1.72%. It was observed that as fermentation period increased, the fat content decreased and vice versa. However, fermentation increases the fat values of the millet flours as against the control (4.06 to 4.68% vs 3.98%). The 72h fermentation period had the highest fat value (4.68%). As fermentation period increase, the fat content also increased. The lower fat (1.37 to 1.72%) as compared to the control (2.70%) for all the fermented African yam bean flours was not a surprise. Reports have shown that legumes store energy in form of carbohydrate and not fat. It might also be attributed to the metabolic activity of micro organisms. This observation was similar to the findings of Anosike and Egwuatu⁽⁵¹⁾ in fermented castor oil. The increase in the lipid level of the fermented millet flours as against the control might be that the free fatty acids reacted with some components in the fermenting medium to form esters. These produce the characteristics aroma in foods⁽⁵²⁾.

There was increase in the ash value at 48h fermentation period in African yam bean flour (4.20%) as against the control (3.60%). There were decreases in ash at 48 and 72h fermentation period in millet (2.20 and 1.35) respectively. The 24h fermented millet had the same ash with the control (2.55%). The highest increase in ash for the 48h fermented flour (A YBF48) and 24h fermented millet (MF24) as shown in table, suggested that they are the optimum fermentation periods to increase ash in African yam bean and millet. This was in agreement with the reports of Obizoba and Anyika⁽⁵³⁾ as well as the findings of Eka⁽⁵⁴⁾. Fermentation drastically reduced fibre in both fermented flours. The values ranged from 2.50 - 2.00 vs 4.46% for African yam bean and 1.32 - 1.66 vs 2.00 for millet. The 72h fermented flour had the lowest fibre in both samples (2.00 and 1.16%). The lower fibre for the fermented flours is contrary to some reports in literature. However, it might be due to increase in the microflora population and the use of fibre in metabolism. The lower carbohydrate for the AYBF48 and MF48 as compared to the control might be attributed to the increased micro flora activity and their dependence on carbohydrate as source of energy. The carbohydrate content increased significantly after 72hrs of fermentation.

Sample	Calcium (Ca)	Iron (Fe)	Magnesium (Mg)	Zinc (Zn)	Phosphorus (P)
UAYB	0.50±0.25	2.81±0.15	0.60±0.02	1.29±0.10	620.33±2.35
AYBF ₂₄	0.43±0.20	3.88±0.11	0.66±0.02	1.77±0.00	656.67±2.17
AYBF ₄₈	0.67±0.25	6.63±0.12	0.33±0.07	1.73±0.00	316.00±6.44
AYBF ₇₂	0.57±0.15	2.41±0.31	0.77±0.04	1.80±0.16	426.00±2.54
UM	0.90±0.20	6.25±0.02	0.40±0.06	1.60±0.16	602.33±1.24
MF ₂₄	0.05±0.03	6.88±0.02	0.57±0.06	0.06±0.03	676.67±2.82
MF ₄₈	0.23±0.15	7.50±0.01	0.43±0.04	1.60±0.10	775.00±3.19
MF ₇₂	0.30±0.20	5.75±0.01	0.53±0.03	1.47±0.10	643.33±4.09

Table 2: The Mineral Composition of Unfermented and Fermented African Yam Bean and Millet Flours (Mg/100g)

Mean ± SD of Three replications

UAYB	=	Unfermented African Yam Bean Flour
AYBF	=	African Yam Bean Fermented for 24 hours
AYBF ₄₈	=	African Yam Bean Fermented for 48 hours
AYBF ₇₂	=	African Yam Bean Fermented for 72 hours
UM	=	Unfermented Millet Flour
MF ₂₄	=	Millet Fermented for 24 hours
MF ₄₈	=	Millet Fermented for 48 hours
MF ₇₂	=	Millet Fermented for 72 hours

The mineral composition of the flour samples are shown in table 2. The increase in calcium content during fermentation was not continuous in AYB. Fermentation of African yam bean for 48 hours witnessed the highest increase, but reduced at 72 hours fermentation. However, the final result after 72hrs of fermentation was higher than the unfermented AYB flour but this was not

the case for millet flours. It is true that all the fermented millet flours had inconsistent decrease in calcium as witnessed in AYB but the final result after 72 hours of fermentation was far below the calcium content of the unfermented millet flours. The decrease in calcium for all the fermented flour might be due to increased activity of the enzymes. They hydrolyzed the complex bond of chelating agent as such release free more calcium. However, the inconsistency in the decreased may not be explained, this might require further studies. The lower calcium for fermented millet flour as against the control (0.30 - 0.05 vs 0.90mg) indicated that fermentation had no beneficial effect of calcium availability and or probable leaching of calcium into the fermenting medium. This finding accords the report of Lopez et al. who reported loss of Ca, K and Mg during fermentation⁽⁵⁵⁾. There was increase in the iron level of 24 and 48h fermented African Yam bean flours when compared with the control (3.88 and 6.63mg vs 2.81mg). Also there was a significant increase in the iron level of the millet flours due to fermentation except for the 72h that had iron value slightly lower than the control (5.75 vs 6.25mg). This high iron for all the fermented samples except for the 72h period indicated that 72h is not the optimum fermentation period for the Fe availability in both African Yam bean and millet flours. The high and comparable iron value for the fermented and unfermented millet flour showed that millet is relatively rich in iron.

This observation was in line with the report of FAO that millet has high ash content and rich in iron and phosphorus⁽¹⁵⁾. The increase in the mg level for all the fermented flours against the control, except for the AYBF48 were a function of treatment. It is known that fermentation improves the bioavailability of minerals.

Fermentation increased the zinc content of the flours when compared with the control, except for the 24h fermented millet (MF24) that had lower zinc (1.28 vs 1.77, 1.73 and 1.80mg) and (1.60 vs 0.06, 1.60 and 1.47mg) respectively. Researches have shown that fermentation improves the bioavailability of minerals such as iron, magnesium and zinc as a result of phytic acid hydrolysis⁽⁵⁶⁾.

The higher phosphorus for all the fermented samples except for the 48 and 72h fermented African yam bean suggested that these fermentation periods are optimum for phosphorus availability. Also, the high phosphorus content might be attributed to the type of micro-organism the was responsible for the fermentation, probably favours the release of phosphorus from the samples, however, this need to be subjected to further studies and isolation of the microorganisms will be important.

Sample	Tannins	Phytate
UAYB	0.51±0.02	0.49±0.02
AYBF ₂₄	0.49±0.02	0.31±0.00
AYBF ₄₈	0.40±0.01	0.25±0.01
AYBF ₇₂	0.31±0.01	0.29±0.01
UM	0.16±0.07	0.26±0.02
MF ₂₄	0.15±0.01	0.10±0.01
MF ₄₈	0.02±0.00	0.06±0.01
MF ₇₂	0.10±0.01	0.06±0.01

Table 3: The Anti-Nutrients Composition of Fermented and Unfermented African Yam Bean and Millet Flours (mg/100g)

Mean ± SD of Three replications

UAYB	=	Unfermented African Yam Bean Flour
AYBF	=	African Yam Bean Fermented for 24 hours
AYBF ₄₈	=	African Yam Bean Fermented for 72 hours
AYBF ₇₂	=	African Yam Bean Fermented for 72 hours
UM	=	Unfermented Millet Flour
MF ₂₄	=	Millet Fermented for 24 hours
MF ₄₈	=	Millet Fermented for 48 hours
MF ₇₂	=	Millet Fermented for 72 hours

The tannin and phytate content of the samples were presented in table 3. The tannin values of the flour differed. There was a reduction in the level of tannin in all fermented flour as against their controls (0.51 vs 0.45 - 0.31mg) and 0.16 vs 0.15 - 0.02mg respectively). This decreased in the tannin levels in all the fermented flours was attributed to the hydrolysis of tannin complexes during fermentation⁽⁵⁷⁾. The phytate content of the samples were reduced by fermentation as compared with the control (0.49 vs 0.31 - 0.25mg) and (0.26 vs 0.10 - 0.06) respectively. This significant decrease in phytate levels for all the fermented flour samples as against the control was due to the degradation of phytate. This finding was similar to the observation of Ajayi⁽³⁵⁾.

4. Conclusion

Based on the result of this study, fermented African yam bean and millet flours have high and promising nutrient potentials as such, should be incorporated in many food preparations especially complementary foods and snacks that are widely consumed by children and adolescents. The Production, processing and utilization of this under exploited legume and cereal should be promoted as they could be valuable in the persistent fight against hunger and malnutrition. However, fermentation of this crops has shown that it improve the value of nutrients that is accessible to the body thereby, reducing the antinutritional factor.

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