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Research Article

Effect of Traditional Fermentation Process on the Nutrient and Anti-nutrient Content of Maize and African Locust Beans

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Abstract

The nutrient content of cereals and legumes are not readily bioavailable due to the presence of anti-nutrients in them. This study examined the effect of traditional fermentation process on the proximate nutritional composition (moisture, ash, protein, fat and fibre content) and anti-nutrient (tannic acid) content of maize and African locust beans (ALB). The samples were processed by soaking, milling (maize), boiling and de-hulling (ALB) and were fermented for 0 h, 24 h, 48 h and 72 h. Samples were taken on each period for the various analysis. Traditional fermentation increased protein and fat content of maize by 35.76% and 25.91% and that of ALB by 14.18% and 8.84% respectively. Ash, fibre and tannic acid content decreased by 24.12%, 10.2% and 18.29% for maize and 15.86%, 37.14% and 70.458% for ALB respectively. It was observed in this study that 72 h of fermentation had significant effects on almost all proximate nutritional compositions and anti-nutrient analyzed with the exception of moisture content of both samples and ash content of ALB while 48 h of fermentation had significant effects only on protein content of maize and tannic acid content of both samples ($P<0.05$). There was no significant effect observed for 24 h of fermentation ($P<0.05$).

Keywords: Traditional fermentation; Proximate nutritional composition; African locust beans; Anti-nutrients

1. Introduction

Cereals and legumes are widely and frequently consumed in Africa and beyond due to their nutritional complementary value and potential to reduce malnutrition [1]. They serve as important sources of proteins, vitamins, calories and other nutrients. Cereals such as maize, rice, millet and wheat among others, are staple foods for many localities globally. Legumes such as groundnut and beans are used frequently in many African diets to complement

cereal diets. Cereals and legumes are by far the main household diets in Ghana and Africa as a whole [2]. Maize (*Zea mays*) is a major staple food crop grown in diverse agro-ecological zones and farming systems, and consumed by people with varying food preferences and socio-economic backgrounds in Sub-Saharan Africa [3]. Maize accounts for almost half of the calories and proteins consumed in Eastern and Southern Africa, and one-fifth of the calories and proteins consumed in West Africa [4]. The traditionally fermented flour of maize is widely used by Ghanaians in the preparation of several meals such as *Kenkey, Banku, Aboloo and Koko* [4].

The African locust beans plant (*Parkia biglobosa*) is a perennial deciduous tree of the Fabaceae family. Mostly found in Africa and also in the savannah and transitional zones of Ghana [5]. *Dawadawa* is the name given to traditionally fermented African locust beans. The fermented beans are used as flavor enhancers (spices) in foods in Ghana and other West African countries [6]. The unprocessed forms of these foods contain anti-nutrients such as phytic acid, tannins, trypsin inhibitors, gluten, oxalates, etc. These anti-nutrients reduce the bioavailability of nutrients in foods by forming complexes with the nutrients or inhibiting the activities of certain enzymes resulting in the indigestibility of foods, decreasing the nutritional value of the foods [7]. These anti-nutrients could be eliminated or reduced by some food processing techniques such as soaking, de-hulling, germination and fermentation [8]. Fermentation is one of the oldest and most economical methods of producing and preserving food. Fermentation is the conversion of substrates to desired products with the help of microorganisms under desirable conditions. Food fermentation amongst other things enriches diets by developing different kinds of flavors, aromas and textures in food substrates. Fermentation has also been known to enrich food substrates with proteins, essential amino acids and vitamins, eliminate anti-nutrients and also reduces cooking time [8]. The production of organic acids like lactate and acetate during fermentation also helps to preserve and extend their shelf lives. Natural fermentation or traditional fermentation, a very old food processing method, is used extensively by Ghanaians in the preparation of several kinds of diets. Traditional fermentation means leaving the foods in a closed container at room temperature for one to seven days. No microorganisms are deliberately added to the foods and no heating process is used [9].

The main aim of this study amongst other things was to investigate the effects of the fermentation process and the duration of fermentation on some nutritional (protein, fibre, crude fat, ash, moisture) and anti-nutritional (tannins) contents in maize and African locust beans. In this study, protein, fibre, crude fat, ash content, moisture content and tannins in maize and African locust beans were measured using various analytical methods before and during the different durations of fermentation.

2. Materials and Methods

2.1 Sample collection

The maize samples were obtained from the Abura Market in the Cape Coast Metropolitan Assembly of the Central Region of Ghana and the African locust beans were obtained from Bimbilla in the Nanumba North District of the Northern Region of Ghana.

2.2 Sample preparation

2.2.1 Maize: The maize samples were soaked in water for two days and milled, after which they were mixed with water, tendered and then fermented. Samples were taken on 0 h, 24 h, 48 h and 72 h of fermentation and kept frozen to stop the fermentation.

2.2.2 African locust beans (Dawadawa): The African locust beans were first boiled for 24-40 h, de-hulled and boiled again for 1-2 h. The seeds were then fermented for 0 h, 24 h, 48 h and 72 h under ambient temperature (25-37°C). Samples were taken on the specified hours of fermentation and kept frozen [6]. The beans were later ground into powder for the various analysis.

2.3 Proximate nutritional composition

The proximate nutritional composition of maize and African locust beans were determined as per the standard protocols [10].

2.3.1 Moisture content: Moisture content was determined according to the Association of Official Analytical Chemist (AOAC) [10] approved method 7.007 with slight modifications. The moisture dishes were cleaned and dried in an oven for 24 h at 107°C and cooled in a desiccator, after which they were weighed. A mass of 2-3 g of each sample was weighed into separate dishes. Each dish containing sample was heated in an oven for four days at a temperature of 107°C and then cooled in a desiccator for 45 min. The cooled samples were weighed and the % moisture was determined using the equation [A÷B] × 100. Where A=Moisture loss and B=Original weight of sample.

2.3.2 Ash content: Ash content was determined according to AOAC, [10] approved method 7.009 by weighing 1-2 g of each sample into clean crucibles. The samples in the crucibles were placed in a muffle furnace at a final temperature of 575°C and incinerated overnight until a light gray ash color was obtained. The ash was cooled in a desiccator and then weighed. The % ash content was calculated as: (mass of ash/ mass of sample) × 100

2.3.3 Crude fat: The crude fat content was determined using the Soxhlet extraction method [10] by weighing 10g of each sample into labelled thimbles and covered with cotton wool. Each sample was placed into a Soxhlet unit containing petroleum ether and extraction was carried out for 4 h at 40°C. The residual ether was dried by evaporation. The extraction flask was placed in an oven till drying was complete then cooled in a desiccator and weighed. The fat content in each sample was calculated using the following equation:

FC (%) = [W2 – W1 × 100] ÷ W5. Where: FC (%)=Fat content, W1=Weight of extraction flask, W2=Weight of extraction flask with oil, W5=Weight of sample.

2.3.4 Protein content: The protein content was estimated using the Kjeldahl nitrogen method 2.057 [10]. A total of 0.2 g of each of the sample was mixed with 4 mL of concentrated H_2SO_4 in a Kjeldahl digestion flask. A tablet of selenium catalyst was added to each sample and then digested inside a fume chamber until a clear solution was

obtained in a separate flask in each case. Also, a blank was made by digesting the above reagents without any sample in it. Each digest was carefully transferred into a 100 mL volumetric flask and made up to the 100 mL mark by adding distilled water. A 20 mL portion of each digest was mixed with 10 mL of 45% NaOH solution in a Kjeldahl distilling unit. The resulting mixtures were each distilled and the distillates collected in each case into 10 mL of 4% boric acid solution containing three drops of mixed indicators (bromocresol green and methyl red). A total of 50 mL of each distillate was obtained and titrated with 0.02 M H_2SO_4 solution. Titration was done from the initial green colour to a deep red end-point. Protein content was determined as:

% N = [(V1 – V2) N1.f]/ E mg × 1400

% Protein = % N × CF

Where:

V1=Consumption of acid from titration,

V2=Consumption of acid, blank determination

N1=Normality of the acid,

f=Factor of the acid,

E=Quantity of the sample in mg,

CF=Conversion factor (A factor of 6.25 was used to convert % N to % protein)

2.3.5 Crude fibre content: Crude fibre was determined according to AOAC [10] with slight modifications as follows: an amount of 1 g of each sample was weighed into a 500 mL beaker. A volume of 100mL of 1.25% H_2SO_4 was added and the mixture was allowed to boil under reflux for 30 min. The solution was filtered with Whatman filter paper (grade 591). Each residue was transferred into a 500 mL beaker and 100mL of 1.25% NaOH was added and boiled for 30 min in a digestion apparatus after which it was filtered and rinsed with distilled water until the filtrate was neutral. Each residue was transferred into a crucible and placed in an oven at 130°C for 3 h to dry. Each residue was then removed and placed in a desiccator to cool before weighing. After weighing, each sample was incinerated and cooled in desiccators and reweighed. The crude fibre content in each sample was calculated as;

CF (%) = [W1 – W2 × 100] ÷W5

Where: CF (%)=Crude fibre,

W1=Weight of crucible with sample,

W2=Weight of crucible with the ash,

W5=Weight of sample.

2.3.6 Tannic acid content: The tannic acid content was determined according to Katoch, [11] method with some modifications by weighing 2mg of sample into 12.5 mL of 80% methanol and left to stand overnight in a closed container. After 30 min of shaking, the mixture was centrifuged at 1500 rpm for 15 min and the supernatant was kept. Into a test tube, 500 µL of the supernatant was taken and made up to 5 mL with distilled water and then 500 µL of 10% Folin Ciocalteu reagent and 400 µL of 7.5% Na_2CO_3 were added. Each solution was kept in the dark for 15 min and absorbance was taken at 760 nm. A blank was prepared using the same procedure, but using 500 µL of methanol instead of supernatant. A standard curve was prepared using 5 mL of different concentrations of tannic

acid with 500 µL of 10% Folin Ciocalteu reagent and 400 µL of 7.5% Na_2CO_3 and kept in the dark for 15 min and absorbance read at 760 nm. The concentrations of tannic acid in the samples were extrapolated from the standard curve derived.

2.4 Data analysis

Data obtained from the test analysis were analyzed statistically. Statistical Package for the Social Scientist (SPSS) version 20.0 was used for the analysis. The laboratory data were submitted to analysis of variance using one-way ANOVA and was applied to distinguish between means that were statistically different ($P<0.05$).

3. Results

3.1 Proximate nutritional composition of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*)

Moisture content was higher on all the hours of fermentation of African locust bean as compared to the unfermented (0 h); however, the highest moisture content was recorded 48 h into fermentation (Table 1). On the other hand, moisture content in maize decreased on all the other hours of fermentation as compared to the 0h, with the lowest moisture content recorded on the 72nd h of fermentation (Table 1).

Fermentation Period (h)	African locust beans (%)	Maize (%)
0	54.0190 ± 1.803	50.1060 ± 0.418
24	58.1250 ± 1.175	48.6250 ± 4.357
48	58.4856 ± 0.829	48.6134 ± 7.659
72	54.4570 ± 3.592	45.6410 ± 0.176

Values are means ± standard deviations of the means of triplicate determinations.

Table 1: Moisture content of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*).

Ash content was lower during all the hours of fermentation of each sample as compared to the unfermented (Table 2). The lowest ash contents were recorded during 72 h of fermentation of each sample (Table 2). However, ash content decreased significantly during 72 h of fermentation 1.25 ± 0.07 as compared to the unfermented 1.64 ± 0.07 for maize ($P<0.05$) (Table 2).

The fibre content increased during 24 h and 48 h of fermentation and decreased on 72 h of fermentation of both samples as compared to the unfermented (Table 3). However, 72 h of fermentation decreased significantly ($P<0.05$) the fibre content of African locust beans as compared to the unfermented ($P<0.05$). There was also a significant decrease in fibre content from 48 h to 72 h of fermentation of maize ($P<0.05$) (Table 3).

Fermentation Period (h)	African locust beans (%)	Maize (%)
0	2.368 ± 0.032	1.642 ± 0.074[a]
24	2.255 ± 0.081	1.402 ± 0.016
48	2.050 ± 0.089	1.305 ± 0.133
72	1.992 ± 0.145	1.246 ± 0.067[a]

Values are means ± standard deviations of the means of triplicate determinations. Means with the same superscript within the same column are significantly different (P<0.05).

Table 2: Ash content of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*).

Fermentation Period (h)	African locust beans (%)	Maize (%)
0	10.77 ± 0.597[a]	2.512 ± 0.102
24	11.02 ± 0.351[b]	2.570 ± 0.063
48	11.58 ± 0.389[c]	2.688 ± 0.095[a]
72	6.77 ± 0.854[abc]	2.256 ± 0.091[a]

Values are means ± standard deviations of the means of triplicate determinations. Means with the same superscript within the same column are significantly different (P<0.05).

Table 3: Fibre content of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*).

Protein content of each sample increased on all the other hours of fermentation as compared to the 0h, with the highest protein content recorded on the 72nd h of fermentation (Table 4). However, the protein content of African locust beans increased significantly on the 72nd h of fermentation as compared to the unfermented (P<0.05). There was also a significant increase in protein content of maize on 48 h and 72 h of fermentation as compared to the unfermented (P<0.05) (Table 4).

Fermentation Period (h)	African locust beans (%)	Maize (%)
0	45.822 ± 1.117[a]	10.313 ± 0.104[a]
24	47.514 ± 0.542	10.875 ± 0.173[b]
48	49.380 ± 1.576	12.875 ± 0.196[ab]
72	52.325 ± 1.628[a]	14.000 ± 0.121[ab]

Values are means ± standard deviations of the means of triplicate determinations. Means with the same superscript within the same column are significantly different (P<0.05).

Table 4: Protein content of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*).

The crude fat content was higher on all the hours of fermentation of both samples as compared to the unfermented (Table 5). However, the highest moisture content of each sample was recorded on 72 h of fermentation (Table 5).

Fermentation Period (h)	African locust beans (%)	Maize (%)
0	35.122	5.05
24	35.981	5.58
48	36.703	5.74
72	38.526	6.82

No statistical analysis for crude fat content.

Table 5: Crude fat content of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*).

3.2 Antinutrient content of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*)

The tannic acid content of both samples decreased on all the other hours of fermentation as compared to the 0h, with the lowest tannic acid content of each sample recorded 72 h into the fermentation (Table 6). However, tannic acid content of each sample decreased significantly on 48 h and 72 h, as compared to unfermented ($P<0.05$) (Table 6). There was also a significant decrease in tannic acid content 72 h into the fermentation as compared to 48h of fermentation of the African locust beans ($P<0.05$) (Table 6).

Fermentation Period (h)	African locust beans (%)	Maize (%)
0	2.450 ± 0.133^{a}	1.667 ± 0.067^{a}
24	1.980 ± 0.082^{b}	1.517 ± 0.126
48	1.073 ± 0.057^{ab}	1.346 ± 0.039^{a}
72	0.720 ± 0.082^{ab}	1.313 ± 0.033^{a}

Values are means ± standard deviations of the means of triplicate determinations. Means with the same superscript within the same column are significantly different ($P<0.05$).

Table 6: Tannic acid content of unfermented and fermented African locust beans (*Parkia biglobosa*) and maize (*Zea mays*).

4. Discussion

Cereals and legumes are usually consumed after processing which tends to increase their palatability, digestibility and safety. During processing, the anti-nutritive value of cereals and legumes are adversely affected and in this study, the effect of natural fermentation on the proximate nutritional composition and the anti-nutritional factors of maize and African locust beans (ALB) were investigated. The moisture content of the unfermented ALB and maize were 54.02% and 50.11% respectively in this study. These values were extremely higher than the moisture content of raw or unprocessed ALB and maize which were 8.6% [12] and 9.39% [13] respectively. The extreme increase in moisture content was due to processing techniques such as soaking in water for both samples and boiling for ALB. The moisture content of unfermented ALB in this study agreed favorably with 56.7% reported earlier by Omafuvbe et al. [12]. The fermentation process in this study did not have any significant effect on the moisture content of ALB as compared to Omafuvbe et al. [12] who observed a decrease in moisture content from 56.7% to 52.0% after 72 h

of fermentation. The difference could be due to different methods of sample preparation and fermentation since ALB is sometimes heated during fermentation. The moisture content of maize decreased as fermentation period increased in this study. This could be due to the soft and porous texture of the sample resulting in moisture loss and microbes might have also utilized some of the water for metabolic activities [14].

The ash content of unfermented ALB and maize in this study was 2.36% and 1.64% respectively. The ash content was similar to the 2.8% reported by Omafuvbe et al. [12] for ALB and these values were lower than ash content of raw or unprocessed ALB reported by Odebunmi et al. [15]. The ash content of unfermented maize was also lower than that of unprocessed maize reported as 2.17% by Gernah et al. [13]. The loss in ash content could be due to the removal of the hull of ALB which may have contained significant amounts of minerals. Soaking of samples could also result in leaching of minerals [12, 14]. Ash content may increase or decrease depending on the type of food sample or fermentation techniques after fermentation [16]. The ash content is generally required to increase after fermentation because minerals chelated by anti-nutrients would be available after the anti-nutrients have been hydrolyzed or reduced after fermentation [16]. However, the ash content reduces when there is a high loss of moisture during the fermentation process [16]. The ash content of maize and ALB decreased by 24.12% and 15.86% respectively as fermentation period increased in this study. These values do not agree with increase in ash content reported by Omafuvbe et al. [12] for ALB and Gernah et al. [13] for maize. However, reports by Odebunmi et al. [15] indicated a decrease in ash content of ALB after 72 h of fermentation. These differences could be as a result of different experimental procedures or differences in cultivars used. The fibre content of unfermented maize in this study was 2.51%, this was slightly higher as compared to 2.17% reported by Gernah et al. [13]. The fibre content of unfermented ALB in this study was 10.77%, which was higher than 4.4% as reported by Omafuvbe et al. [12]. Traditional fermentation increased the fibre content of maize and ALB by 6.269% and 6.99%, respectively, within the first 48 h of fermentation and decreased the fibre content on 72 h of fermentation by 10.2% and 37.14% respectively as compared to the 0h of fermentation in this study. This pattern agrees with observations made by Omafuvbe et al. [12]; and Gernah et al. [13]. Decrease in fibre contents after fermentation is an indication of softening of fibrous tissues and increased digestibility during fermentation due to activities of microorganisms which are known for the bioconversion of carbohydrates and lignocellulose into protein [14].

According to Tope, [17] and Igbabul et al. [14] fat content increases after fermentation, which could be due to extensive breakdown of large fat molecule to simpler fatty acid units due to the high activity of lipolytic enzymes. The increase in fat content could also be fat from dead microflora or the fact that fermenting microflora do not use fat from these foods (substrate) as a source of energy. The fat content of unfermented ALB in this study was 35.12%, which falls within the range (31-40%) reported earlier by Ouoba et al. [6]. Omafuvbe et al. [12]; Essenwah and Ikenebomeh, [18] and Odebunmi et al. [15] reported an increase in crude fat content as fermentation period increased. This conforms to observations made from this study. Crude fat content of ALB increased on 72 h by 8.836%, which was higher than an increase of 5.47% reported by Omafuvbe et al. [12] and was also lower than 24.45% reported by Essenwah and Ikenebomeh, [18]. According to Essenwah and Ikenebomeh, [18] soaking and boiling of the sample might have led to cleavage of protein-lipid or carbohydrate-lipid linkages thereby facilitating

the easy extraction of oil by the extraction solvent. Fat content of unfermented maize was 5.02%, which is slightly higher than 4.02% as reported by Gernah et al. [13]. The crude fat content of maize increased by 25.91% on 72 h of fermentation in this study and this is not in agreement with a decrease in fat content after 72 h of fermentation reported by Gernah et al. [13].

Tannic acid content of unfermented maize and ALB in this study was 1.667% and 2.45% respectively, and was in accordance with the 2.62% recorded for maize by Gernah et al. [13] and 2.51% for ALB recorded by Essenwah and Ikenebomeh, [18]. In this study, traditional fermentation decreased the tannic acid content significantly in maize and ALB on 72 h as compared to 0h by 18.29% and 70.458% ($P<0.05$). The percentage reduction in ALB was slightly higher than the 59.8% reported by Essenwah and Ikenebomeh, [18] while that of maize was lower than the 83.97% reported by Gernah et al. [13]. The vast difference in percentage reductions of tannic acid in maize may be due to malting of the seeds before fermenting by Gernah et al. [13]. The decrease in tannic acid content may be due to the activities of microflora or enzyme polyphenol oxidase [19]. The decrease can also be attributed to tannase activity by lactobacillus during fermentation that breakdown tannin complex with protein [20].

Protein content of unfermented maize and ALB was 10.31% and 45.82% respectively. The protein content of unfermented ALB reported, falls within the range of 39% - 47% reported by Ouoba et al. [6]. That of maize was also in accordance with 10.64% reported by Gernah et al. [13]. The protein content increased as fermentation period increased. The percentage increase on 72 h of fermentation was 35.76% and 14.18% for maize and ALB respectively. A slightly higher percentage increase (16.1%) for ALB was observed by Essenwah and Ikenebomeh, [18]. The percentage increase for maize was in agreement with range of 29.7% to 43.5% percentage increases for four cultivars of maize as reported by Cui et al. [21]. The increase in protein content could be attributed to the increase in microbial mass during fermentation, causing extensive hydrolysis of protein molecules to amino acids and simple peptides. The increase could also be as a result of the enzymatic hydrolysis of some protein inhibitors [14]. It may also be due to the structural proteins that are an integral part of the microbial cell [22].

It was observed from this study that 72 h of fermentation had significant effects on almost all proximate nutritional compositions and anti-nutrient analyzed with the exception of moisture content and ash content for ALB while 48 h of fermentation had significant effects only on the crude protein content of maize and tannic acid contents ($P<0.05$). There was no significant effect observed at 24 h into fermentation ($P<0.05$).

5. Conclusion

Traditional fermentation increased protein and crude fat content and decreased ash, fibre and tannic acid content of both samples as duration of fermentation increased.

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Conflicts of Interest

No conflicts of interest have been registered on this work to the best of our knowledge.

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