

Chemical, Nutritive Value and Organoleptic Attributes of In-Built Products of Fermented *Afzelia africana* Tender Leaves and Shoots

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Abstract- *Afzelia africana* was identified in four selected communities in Idemili North Local Government Area as important wild vegetable tree. The tender leaves and shoots are known and consumed as delicacies in yam dish. This wild vegetable that substitutes cultivated vegetables in dry season is on the verge of extinction. This is because farmers produce and consume more commonly known vegetable. Planting, processing, food use and organoleptic attributes of its in-built product for food diversification is scanty in Nigeria literature. *Afzelia africana* tender leaves and shoots were collected from forest in the four study areas. The leaves and shoots were divided into four portions. One portion was sun-dried as control. The other three portions were exposed for 30 minutes to sun to wither, squeezed in partially burnt fresh banana leaves wrapped and allowed to ferment in locally made basket by inherent microflora for 4, 7 and 10 days. The fermented samples were sun-dried until they become brittle for pulverization into fine powder. These samples were used for both chemical analysis and formation of diets for adult rats for a 12-day N balance study and liver composition. The in-built product (yam dish) has a promising food diversification use. A 4-day fermentation of *Afzelia africana* leaves and shoots produced much more nutrient density and desirable organoleptic attributes. The dietary protein based on a 4-day fermented *Afzelia africana* leaves and shoots fed adult rats increased N digestibility and retention, net protein utilization and liver composition.

I. INTRODUCTION

Most of the recent studies reported that the incidence of malnutrition is higher in rural areas and urban slums particularly protein and micronutrient (Vitamin A, iron and iodine) deficiencies. This was attributed to food gap and seasonality (Hart, Azubuike, Barimalaa & Achinewhu, 2005). People in these areas experience food abundance, especially vegetables during rainy season and severe scarcity during dry season. This seasonality is attributed to lack of irrigation facilities and opportunities, ignorance of the nutritional value of wild vegetables, leaves of forest trees and many lesser-known legumes and tubers.

Most staple foods are consumed with some measure of supplementation, complementation and fortification. This is limited in scope due to ignorance, poor food processing methods and poverty. Some of the available food stuffs, fruits and vegetables are neglected due to some socio-culture hindrances

such as taboos, fads, fallacies and poor nutrition education of the people, particularly the rural dwellers. Some popular foods such as fish, meat, crayfish, milk, egg and some vegetables are expensive. However, there are other lesser-known fruits and vegetables traditionally cultivated or collected from forests in Nigeria. These are used to supplement those collected from farms and home gardens.

Afzelia africana is one of them. It grows widely in farmlands and forests in Nigeria. It survives the harshest weather conditions such as harmatan and effects of some global climate change. Fruits and vegetables are major sources of micronutrients. It is imperative to make serious efforts to identify, process and popularize other locally available and cheap, nutritious vegetables and their dishes to meet the protein and micronutrient needs of the nation – Nigeria. It is of need to lay much more emphasis on nutrition education, the importance of vegetables especially green leafy vegetables to the nutrition quality of diets of both children and adults. Traditionally, vegetables are added to foods to improve the “eye” appeal rather than for the nutrients and phytochemicals they contain. In dry season in Nigeria, yam is consumed with plain palm oil, yam porridge without any vegetables, plain sauces thickened with cocoyam or rice without vegetables. This is exactly the case in some of the town in Idemili North Local Government Area where the research therein was conducted on fermented *Afzelia africana* – “akpalataa” tender leaves and shoots.

Afzelia africana has many tender leaves and young shoots readily available in the forests and farmlands during the dry season. The processing methods adopted by the indigenes and the nutrition implication of the use of fermented *Afzelia africana* as vegetable has not been adequately investigated and reported in Nigeria literature. This work investigated the chemical composition and food uses of fermented *Afzelia africana* tender leaves and shoots. This study also investigated the nitrogen (N) and mineral balance as well as liver composition of adult rats fed diets containing fermented *Afzelia africana* tender leaves and shoots as sole source of N (protein).

II. MATERIAL AND METHODS

The *Afzelia africana* tender leaves and shoots were obtained from gardens and farms in Ogidi, Abatete, Uke and Umuoji in Idemili North Local Government Area of Anambra State, Nigeria. The purpose was to obtain baseline information concerning their knowledge of availability, processing,

preparation and use of fermented *Azelia africana* tender leaves and shoots as vegetable.

About 8kg of *Azelia africana* tender leaves and shoots were obtained and divided into four equal portions. A portion was sun-dried and the other three portions were fermented by its microflora content, and sun-dried. Fermentation was as follows: Fresh leaves and shoots were sun-dried for 30 minutes to whither, wrapped in heat-treated fresh banana leaves and fermented in locally made wooden basket for 4, 7 and 10 days, respectively as shown in figure 1. After fermentation, the *Azelia africana* leaves were spread on trays, sun-dried and pulverized into fine powder. The samples were packaged in name-labeled polythene bags and stored until used for various analyses.

III. CHEMICAL ANALYSIS

The proximate, mineral and anti-nutrient content of the samples were determined using AOAC (2000) procedures. Nitrogen and mineral balance was determined using adult rats to determine the bioavailability of nutrients in the fermented samples. Four diets were formulated to furnish 10% of the dietary protein. Mineral, vitamins, vegetable oil, and sucrose were added to meet the adequate nutrient requirements of the rats. The composition of experimental diets is shown in Table 1. Each diet and rat served as its own control in a 12 day study. A 12 day animal feeding, collection of metabolic wastes (feces and urine), analysis of diets, feces, urine and determination of liver composition of the rats were the methods of Obizoba and Atii (1994). The data generated was analyzed using Steel and Torrie (1960) statistical methods.

Azelia africana tender leaves

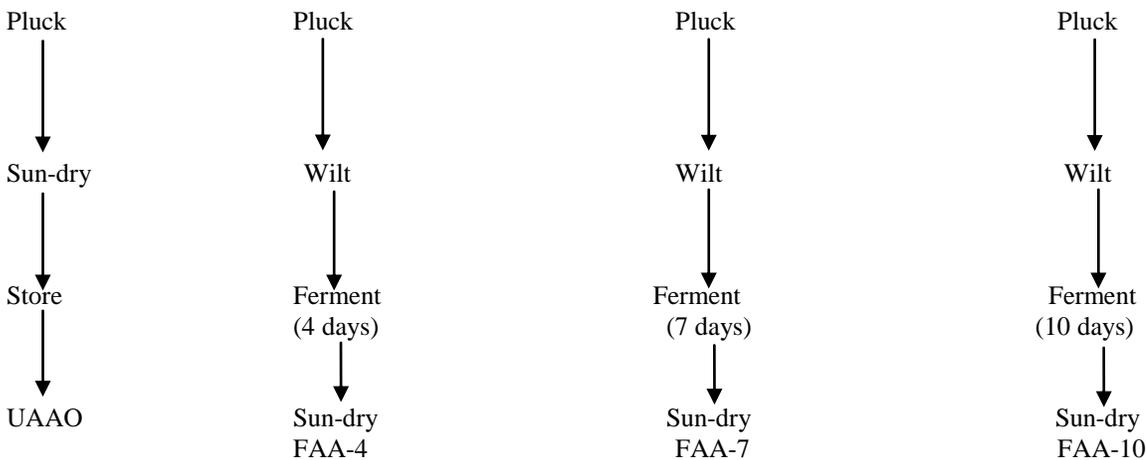


Fig. 1: Flow chart for processing *Azelia africana* leaves

Key:
 UAAO Unfermented *Azelia africana* leaves
 FAA4 Four-day fermented *Azelia africana* leaves
 FAA7 Seven-day fermented *Azelia africana* leaves
 FAA10 Ten-day fermented *Azelia africana* leaves

Table 1: Composition of the four all-vegetable protein diets based on 10% protein from fermented *Azelia africana* leaves (g).

Diets	1	2	3	4
UAAO	571	-	-	-
FAA4	-	623.78	-	-
FAA7	-	-	601.60	-
FAA10	-	-	-	579.71
Fat	48.00	48.00	48.00	48.00
Mineral	4.32	4.32	4.32	4.32
Vitamin	2.40	2.40	2.40	2.40
Sucrose	334.28	281.50	303.68	325.57
Total	960.00	960.00	960.00	960.00

Key:
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 FAA4 Four-day fermented *Azizelia africana* leaves
 FAA7 Seven-day fermented *Azizelia africana* leaves
 FAA10 Ten-day fermented *Azizelia africana* leaves

IV. RESULTS

Proximate composition:

The protein content of the samples differed. Fermentation decreased *Azizelia africana* protein when compared with the unfermented leaves (18.00 to 15.4%). Fat values increased, regardless of fermentation period. There were increased in ash content except for the 4-day fermentation with slightly decreased

ash value when compared with the control (UAAO) (9.94 vs 9.76%). The highest increase occurred in the 10-day fermentation period (11.01%). Fermentation increased crude fibre content of the samples when compared with the control (11.48 vs 10.30%). Fermentation precipitated decreases in carbohydrate except for 7-day fermentation that recorded a slight increase (61.03 vs 61.41%).

Table 2: Proximate composition of fermentation *Azizelia africana* (Aa) leaves based on dry weight (%)

Code	Protein	Fat	Ash	Crude fibre	Carbohydrate
UAAO	18.00±0.01	0.40±0.04	9.94±0.01	10.30±0.01	61.03±0.02
FAA4	16.93±0.01	1.47±0.014	9.76±0.02	11.48±0.02	60.08±0.02
FAA7	15.40±0.01	1.45±0.014	10.86±0.02	11.26±0.02	61.41±0.012
FAA10	17.72±0.00	1.18±0.021	11.01±0.3	10.67±0.01	57.39±0.021

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Micronutrient and anti-nutrient content of fermented *Azizelia africana* leaves

Table 3 presents the micronutrient and anti-nutrient content of the samples. The zinc composition of the leaves was low and varied. Iron values had the same trend as zinc. The values ranged from 0.02 to 0.04mg for zinc and 0.11 to 0.13mg for iron. The 4-day and the 10-day fermented samples had 0.01mg iron more than the control (0.13mg and 0.12mg vs 0.11mg). Iodine values were identified by treatments. The 7-day fermentation decreased

iodine (218.50mg). On the other hand, the other fermentation periods increased the value (Table 3). Fermentation had varied effects on tannins content of the vegetables. The 4-day fermentation of Aa slightly increased tannins (0.85 to 0.93mg). The 10-day fermentation decreased it drastically (0.07mg). The 7-day fermentation surprisingly reduced phytate and oxalate. The 7-day fermentation slightly reduced phytate. However, 10-day fermentation nearly doubled that of the control (313.32 vs 167.72mg). Fermentation influenced oxalate levels.

Table3: Micronutrient and anti-nutrient composition of fermented *Azizelia africana* leaves (mg/100g)

	Zinc	Iron	Iodine	Tannins	Phytate	Oxalate
UAAO	0.03±0.02 ^a	0.11±0.22 ^c	223.09±0.8 ^a	0.85±0.01 ^a	167.72±20 ^b	56.67±0.1 ^a
FAA4	0.02±0.02	0.13±0.02 ^c	234.45±0.4 ^a	0.93±0.1	172.50±20 ^b	48.98±0.1 ^b
FAA7	0.04±0.03	0.11±0.02 ^c	218.50±0.4 ^b	0.71±0.1 ^b	156.87±0.13 ^c	54.75±0.2 ^a
FAA10	0.03±0.03 ^a	0.12±0.22 ^c	236.67±0.2 ^a	0.07±0.1 ^c	313.32±20 ^a	46.30±0.2 ^b

Key:
 UAAO Unfermented *Azizelia africana* leaves
 FAA4 Four-day fermented *Azizelia africana* leaves
 FAA7 Seven-day fermented *Azizelia africana* leaves
 FAA10 Ten-day fermented *Azizelia africana* leaves

Bioavailability studies

Table 4 presents food and N intake, faecal and urinary N, digested and retained N, biological value and net protein utilization of adult rats fed four all vegetables protein diets based on *Azizelia africana* diet had the least food intake (40.90g) followed by that of the group fed the 10-day fermented samples

(47.80g). The group of rats fed the 4-day fermented *Azizelia africana* diet had the highest intake (69.43g). On the other hand, the group of rats fed the unfermented *Azizelia africana* diet had slightly higher food intake than the group fed the control diet (casein) (56.49 vs 54.80g)

Nitrogen intake

The N intake of both the control and the test groups of rats fed the four all vegetable protein diets differed. It ranged from 0.66 to 1.11g. The group of rats fed FAA4 had N intake significantly different from that of the rats fed 7-day fermented diet (1.11 vs 0.66g) ($p < 0.05$). On the other hand, the group fed casein diet had N intake that was comparable to those fed UAAO (0.99 vs 0.90g). The group of rats fed FAA10 had lower N intake that those of rats fed FAA4 (0.77 and 1.11g respectively) ($p < 0.05$). The group of rats that had the highest food intake also had the highest N intake (69.43 and 1.11g, respectively).

Faecal N

The values for faecal N for all the groups of rats were 0.07, 0.04, 0.05 and 0.06g for the UAAO, the FAA4, the FAA7 and the FAA10, respectively. There were no differences in faecal N output regardless of the treatment ($p > 0.05$).

Digested N

The digested N values for the group of rats fed casein and four all-vegetable protein diets based on fermented *Afzelia africana* leaves differed. The animals fed casein (control) had 0.94g digested N which was higher than those of the groups fed the FAA7 and the FAA10 diets (0.94 vs 0.61 and 0.71g) ($p < 0.05$). On the other hand, the group of rats fed the FAA4 had the highest digested N (1.07). Nevertheless, the values from CA, UAAO, FAA4 groups were similar ($p > 0.05$). However, the digested N of the group of rats fed the unfermented *Afzelia africana* and casein had comparable values (0.94 and 0.83g respectively). The digested N of the group of rats fed FAA7 and

FAA10 diets slightly differed. However, the difference was insignificant ($p > 0.05$).

Urinary N

The urinary N of the group of rats fed the FAA4 and FAA7 diets were similar (0.002g) and the unfermented sample (UAAO) and 10-day fermented samples were equally similar (0.001g). On the other hand, the group of rats fed casein had the highest urinary N (0.11g) and differed from the rest ($p < 0.05$).

Retained N

The retained N values differed. However, the group of rats fed the FAA4 had higher retained N that was different from those of rats fed both control and test diets ($p < 0.05$). The casein group had 0.83g retained N. The rats fed the FAA7 had the lowest N retention (0.61g) followed by the group fed the FAA10 (0.71g) ($p > 0.05$).

Biological value

The biological value of the group of rats fed the unfermented sample (UAAO) was higher than others fed the samples fermented for varying periods (99.9% vs 99.9, 99.7, 99.96%). However, these differences were not significant ($p > 0.05$).

Net protein utilization (NPU)

The NPU of these groups of rats fed four all-vegetables protein diets based on fermented *Afzelia africana* leaves were 92.1, 92.2, 92.12, 92.08 for the UAAO, the similar ($p > 0.05$) and differed only from that of casein ($p < 0.05$).

Table 4: Food and nitrogen intake, faecal and urinary nitrogen, digested and retained nitrogen, biological value and net protein utilization of adult rats fed four all vegetable protein diet based on fermented *Afzelia africana* leaves.

Diet	CA*	UAAO**	FAA4**	FAA7**	FAA10**
Food intake (g)	54.8±3.3 ^b	56.49±3.39 ^a	69.43±4.91 ^a	40.96±5.4 ^c	47.90±2.7 ^c
Nitrogen intake (g)	0.99±0.1 ^b	0.90±0.09 ^b	1.11±0.08 ^a	0.66±0.09 ^a	0.77±0.00 ^c
Faecal nitrogen (g)	0.05±0.01 ^a	0.07±0.06 ^a	0.04±0.01 ^a	0.05±0.01 ^a	0.66±0.01 ^a
Digested nitrogen (g)	0.94±0.95 ^a	0.83±0.02 ^a	1.07±0.08 ^a	0.61±0.09 ^b	0.71±0.00 ^b
Urinary nitrogen (g)	0.11±0.03 ^a	0.001±0.00 ^b	0.002±0.00 ^b	0.002±0.00 ^b	0.001±0.00 ^b
Retained nitrogen (g)	0.83±0.06 ^b	0.83±0.0 ⁶	1.07±0.08 ^a	0.61±0.09 ^c	0.71±0.1 ^c
Biological value (%)	83.3±0.01	99.9±0.01 ^a	99.9±0.01 ^a	99.7±0.02 ^a	99.96±0.00 ^a
Net protein utilization (%)	83.3±0.06 ^b	92.1±0.57 ^c	96.22±0.06 ^c	92.12±0.48 ^c	92.08±0.06 ^c

Key: *Obizoba (1989)

**Mean±SEM of 5 rats = 7-day food intake (means with similar superscript are similar)

- CA Casein
- UAAO Unfermented *Afzelia africana* leaves
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Mineral bioavailability

Table 5 presents mineral intake, faecal and urinary, absorbed and retained in four groups of adult rats fed four all-vegetable protein diets based on fermented *Afzelia africana* leaves.

Iron

The iron intake of the various groups of rats fed different diets differed. It ranged from 0.61 to 1.04mg. The rats fed a 10-day fermented *Afzelia africana* diet had the highest intake (1.04mg). The 7-day fermented *Afzelia africana* diet fed group of adult rats had the least intake (0.63mg). On the other hand, the rats fed the 4-day fermented *Afzelia africana* diet had the second highest iron intake (0.80mg). The faecal iron output of all groups

of rats was comparable ($p>0.05$). The absorbed iron values were influenced by faecal excretions. The group of rats that had higher iron intake also had higher absorbed iron (1.03mg) than the other groups. The absorbed iron followed the same trend as the iron intake and vice versa. The urinary iron output varied. It ranged from 0.18 to 0.27mg. The 7-day fermented samples had the least output (0.18mg). On the other hand, the 10-day group of rats had the highest (0.27mg). The unfermented and 4-day group of rats had slightly comparable values (0.22 and 0.21mg, respectively). The urinary output had considerable values adverse effect on retained iron. Regardless of the higher iron excretion of rats fed the 10-day fermented diet, it still had the highest iron retention (0.76mg) as against the values for the other three groups of rats ($p<0.05$). The group of rats fed unfermented and the 7-day samples had similar values of 0.40 and 0.42mg, respectively ($p>0.05$). The rats fed 4-day had an edge (0.58mg) over the other groups of rats fed unfermented and 7-day fermented diets ($p<0.05$).

Zinc

The zinc intake varied. It ranged from 0.90 to 1.61mg. The group of rats fed the 4-day (1.61mg) consumed more zinc than the other three groups of adult rats. There were difference in zinc intake among other three groups (1.27, 0.90 and 1.01mg). The group of rats fed the 7-day diet had the least (0.90mg) that differed from others. The faecal zinc for the four groups of rats ranged from 0.06 to 0.10mg. The values were comparable ($p>0.05$). The low faecal zinc for the four groups of adult rats influenced absorption values. The rats fed the 4-day fermented diet that had the highest zinc intake and lowest output had the highest absorbed zinc (1.55mg). This value differed from the other three groups of rats ($p<0.05$). The rats fed the 7-day fermented diet that had lower intake and high faecal value had the least absorbed zinc (0.82mg) followed by those rats fed the 10-day fermented diet (0.93mg). On the other hand, the rats fed the unfermented diet had much more absorbed zinc than those

fed the 7-day and 10-day fermented diets (1.17 vs 0.82mg, and 0.93mg each). The urinary zinc of all the four groups varied. It varied from 0.204 to 0.224mg. These values seriously affected retained zinc. The rats fed the 7 and 10-day fermented samples had comparable zinc retention (0.613 and 0.482mg each). On the hand, the 4-day sample had higher zinc retention (1.334mg) irrespective of its high urinary zinc output.

Iodine

The iodine intake of the four groups of rats differed. It ranged from 18.64 to 25.43mcg. The rats fed the 7-day fermented diet had the highest iodine intake (25.43g). However, those rats fed the unfermented and 10-day fermented samples had similar values (18.64 and 18.68mcg, respectively) ($p>0.05$). The rats fed the 4-day fermented diet had the second highest intake (24.42mcg). The faecal iodine for the four groups of rats varied. The variation was from 0.33 to 0.55mcg. The rats fed unfermented and the 7-day fermented diets had comparable values (0.55 and 0.50mcg) as well as those fed the 4 and 10-day fermented diets (0.33 and 0.44mcg each) ($p>0.05$). The absorbed iodine for the four groups of rats ranged from 18.09 to 24.85mcg. The group of rats fed the 7-day and the 4-day fermented diets had 24.85 and 24.09mcg absorbed iodine respectively. On the other hand, the rats fed the unfermented and the 10-day fermented diet had comparable values (18.09 to 18.28mcg, respectively) ($p>0.05$). The urinary iodine values for all the groups of rats were high and comparable. It ranged from 4.75 and 4.95mcg, respectively, respectively. The high urinary iodine again affected its retention. The iodine retention ranged from 12.57 to 19.34mcg. The unfermented group of rats had the least followed by the 10-day fermented group of rats (12.57 and 13.33mcg, respectively). The 4-day fermented diet that had the least urinary output (4.75mcg) in turn had the highest iodine retention (19.34mcg) followed by the rats fed the 7-day fermented diet (14.09mcg).

Table 5: Intake, faecal, urinary, absorbed, and retained minerals in groups of adult rats fed four all-vegetable protein diets based on fermented *Azelia africana* leaves.

Mineral	UAAO	FAA4	FAA7	FAA10
Iron intake (mg)	0.63±4.81 ^c	0.80±5.81 ^b	0.61±10.20 ^c	1.04±7.13 ^a
Faecal iron (mg)	0.01 ^a	0.01	0.01	0.01
Absorbed iron (mg)	0.62±0.03 ^{ca}	0.79±0.2b ^a	0.61±0.4 ^{ca}	1.03±0.03 ^{aa}
Urinary iron (mg)	0.22±0.01 ^b	0.21±0.03 ^b	0.18±0.02 ^b	0.27±0.01 ^a
Retained iron (mg)	0.40±0.03 ^c	0.58±0.04b ^b	0.43±0.02 ^c	0.76±0.05 ^a
Zinc intake (mg)	1.30±0.03 ^b	1.61±0.05 ^a	0.90±0.05 ^c	1.01±0.02 ^b
Faecal zinc (mg)	83.3±0.06 ^b	0.06±0.01	0.08±0.01 ^c	0.08±0.01
Absorbed zinc (mg)	1.17±0.02 ^b	1.55±0.03 ^a	0.82±0.02 ^c	0.93±0.02 ^c
Urinary zinc (mg)	0.20±0.03 ^a	0.22±0.04 ^a	0.21±0.00 ^a	0.22±0.00 ^a
Retained zinc (mg)	0.97±0.02 ^a	1.33±0.03 ^a	0.61±0.02 ^b	0.48±0.01 ^b
Iodine intake (mcg)	18.64±1.13 ^b	24.42±1.76 ^a	25.34±11.5 ^a	18.68±1.2 ^b
Faecal iodine (mcg)	0.55±0.03 ^a	0.33±0.02 ^a	0.50±0.01 ^a	0.40±0.02 ^a
Absorbed iodine (mcg)	18.09±0.01 ^b	24.12±0.02 ^a	24.84±0.02 ^a	19.28±0.03 ^a
Urinary iodine (mcg)	5.52±0.09 ^a	4.75±0.08 ^a	5.25±0.05 ^a	4.95±0.04 ^a
Retained iodine (mcg)	12.57±0.09 ^b	19.37±0.04 ^a	19.59±0.03 ^a	13.33±0.04 ^b

Key:

a,b,c, Values with the same superscript letters are similar (p>0.05)

UAAO Unfermented *Azizelia africana* leaves

FAA4 Four-day fermented *Azizelia africana* leaves

FAA7 Seven-day fermented *Azizelia africana* leaves

FAA10 Ten-day fermented *Azizelia africana* leaves

Liver composition of adult rats fed four all-vegetable protein diets

Table 6 presents the liver composition of adult rats fed four all-vegetable protein diets based on fermented *Azizelia africana* leave. The animals fed the 4-day fermented sample had the least liver weight (1.37g). The animal fed the unfermented sample had 1.64g liver weight. The control had the highest liver N (121.6mg). The rats fed the UAAO and the FAA4 diets had different values. However, the differences were not significant (p>0.05). The other two groups of rats (FAA7 and FAA10) had similar N (0.15 and 0.16mg). The UAAO rats had the highest moisture (75.5%). However, the value was higher than that of casein group and the other test groups of rats. The casein group had the least liver moisture (35.0%). The other three test groups of rats had values that were not different from each other (p>0.05). The liver lipids for all groups of rats ranged from 0.438 to 0.518%. The casein group had the highest (9.3%), which was different from other (p<0.05). The group of rats fed the UAAO, the FAA4 and the FAA10 diets had approximately 0.52, 0.50 and 0.51% respectively. These values were different from that of the FAA7 (0.44%). However, the difference were comparable. The liver ash of the four test groups ranged from 0.32 and 0.41%. The FAA4 and the FAA7 groups had comparable values (0.32 and 0.34%, respectively). The zinc and iron values for all groups of rats were not different from each other (p>0.05). However, the values for iodine were quite high (6.80 and 8.56mg). The UAAO and the FAA10 rats had the highest iodine 7.54 and 8.56mcg, respectively. As one would expect, the FAA4 and FAA7 had varied values that were not different (p>0.05).

Table 6: Liver composition of adult rats fed four all-vegetable protein diets based on fermented *Azizelia africana* leaves

Composition	Casein*	UAAO**	FAA4**	FAA7**	FAA10**
Average Liver wt (g)	5.0±0.01	1.64	1.37	1.59	1.79
Dry Liver N (mg)	121.6±0.37	0.193±0.01	0.178±0.01	0.154±0.01	0.1613±0.02
Liver Moisture%	3.50 ^c ±4.0	75.58 ^a	71.97 ^b	72.72 ^b	70.69 ^b
Liver Lipids %	9.30	0.518±0.05	0.50±0.02 ^b	0.438±0.03	0.508±0.04
Liver ash%	-	0.41±0.04 ^a	0.32±0.02 ^b	0.34±0.02 ^b	0.40±0.03 ^a
Zinc (mg)	-	0.002±0.0002 ^a	0.001±0.0 ^a	0.001±0.0 ^a	0.003±0.002 ^a
Fe (mg)	-	0.0007±0.0003	0.003±0.00002	0.001±0.00006	0.001±0.00002
I ₂ (mcg)	-	7.54±0.74	6.80±0.36	6.98±0.45	8.56±0.61

Key: **Mean±SEM of 5 rats = 7-day food intake (means with similar superscript are similar)

*Obizoba (1989)

CA Casein

UAAO Unfermented *Azizelia africana* leaves

FAA4 Four-day fermented *Azizelia africana* leaves

FAA7 Seven-day fermented *Azizelia africana* leaves

FAA10 Ten-day fermented *Azizelia africana* leaves

V. DISCUSSION

Proximate and mineral composition of fermented *Azelia africana* Leaves

The nutrient content of fermented vegetable which was much more improved was not a surprise. Udofia (2005) reported that many workers had observed similar phenomenon in their studies. The protein content of these fermented vegetables was higher than that obtained in most commonly consumed Nigerian green leafy vegetables and foods other than animal and legume products (Oyenuga, 1968).

Oboh (2005) and Ajibade, Balogun, Afolabi and Rapoati (2006) among other studies reported the proximate value for a very common green leafy vegetable fluted pumpkin. The protein value was 9.0, 11.6 and 20.7%, respectively. This wide disparity was attributed to varietal differences, soil and season. Aletor and Adeogu (1995) reported that the dry vegetables contain 19.3g/100g crude protein and 15.3g/100g crude fibre. Fresh products contain very low nutrients which was attributed to the higher nutrient density of dried products as against the fresh vegetables. Atawodise, Bulus, Ibrahim, Ameh, Nok, Manumom and Galadima (2003) observed that lesser popular vegetables improve nutritional status of rural families, especially during period of scarcity.

Anti-nutrient content of fermented *Azelia africana* leaves

The anti-nutrient content of fermented *Azelia africana* leaves varied with those of Gupta, Kakshmi, Manjunalaa and Prakash (2005), especially in the oxalate. Fermented *Azelia africana* tannins was lower than in most fresh green leafy vegetables. The values ranged from 61-205mg/100g. The choice to consume tender *Azelia africana* leaves reduces anti-nutrient which are higher in older plants. Heaney, Weaver and Fitzsimons (1991) observed that phytate in foods affects mineral absorption, especially intrinsic calcium due to low normal phytase activity. Seven-day fermentation produces higher phytate which was attributed to high fibre. Bruce, Rossender, Hallberg, Greemp and Sandberg attributed high fibre affects phytate and phosphate composition of bread from cereal fibre flour.

Bioavailability studies

Food intake: The high food intake for the group of rats fed the 4-day fermented *Azelia africana* diet could be attributed to the release of desirable aromatic flavour during fermentation. Flavour is the greatest factor that influence food intake in both rat and man. The higher consumption of the 4-day fermented diet more than the other diets might be associated with flavour. Food intake is affected by many factors. Appearance, odour, taste, texture, temperature and other sensory properties of food determine palatability. The 4-day fermentation appeared to be the optimum time because the 4-day sample had much higher palatability than counterparts, as such, the rats consumed more of it. Fermentation of *Azelia africana* leaves is traditionally for 4 days. The communities ferment *Azelia africana* leaves for 4 days without knowing the nutritional implications. The 4-day fermentation period is most appropriate in rural areas. The high food intake of the rats supports this traditional method of fermentation to obtain the best food potentials of *Azelia africana* leaves. The off-flavour for the 7-day fermented products might be associated with increased production of astringent

compounds. Eka (1990) reported that fermentation of food samples beyond optimum conditions produced unacceptable products in cornstarch porridge and locust bean. Food intake is affected by many metabolic and physiological signals including those which arise from the ingestion of food and its nutrients (Grosvenor and Samoline, 2002).

Digestibility of food materials affects food intake in animal because food that has high satiety value tend to reduce food intake. Higher fibre and fat intake is known to reduce food intake. The major criteria for determination of the optimum period are the development of peculiar aroma in *Azelia africana* leaves and increased tenderness of squeezed leaves. Combination of aroma and texture of the fermenting mass ensure its readiness. A 7-day fermentation has negative impact on food intake. Palatability is the major factor that influence food intake. The higher food intake or rats fed 4-day fermented diet ate more and maintained more body weight than those fed casein. This observation was associated with increased flavour, palatability and protein due to microflora enzyme hydrolysis and release of free and absorbable components during fermentation. Combination of aroma, texture and colour had strongest influence on food intake.

N Intake

The higher N intake of the rats fed the 4-day fermented *Azelia africana* leave diet was due to its high palatability which influenced the rats to eat more. The higher N intake of the group of rats fed 4-day fermented *Azelia africana* diet demonstrated that 4-day is optimum fermentation period for microflora proteolytic enzymes to hydrolyze *Azelia africana* protein to increase its bioavailability. The 4-day fermentation results agreed with the method of processing *Azelia africana* leaves in Idemili North Local Government Area. The higher N digestibility (0.94g) of the same group of rats was solely due to very low faecal N excretion (0.04g). The higher retained N, biological values as well as net protein utilization were attributed to high N intake and low faecal and urinary N excretion. The higher food and N intake, retained and digested N, biological value and net protein utilization that plant protein mixture could be equal to or higher than that of casein, in this work, if properly processed and prepared for consumption.

Faecal and urinary N and biological value

The lower faecal and urinary N excretion of rats fed fermented *Azelia africana* diets indicated its high digestibility and retention. It is known that protein that has lower faecal and urinary N output has high digested and retained N. Equally, the higher biological value and net protein utilization of the group of rats fed *Azelia africana* diets indicates that protein tends to be higher than those of rats fed casein (control). This observation is not a surprise because it is known that when plant protein is properly processed and utilized as source of protein, its protein might be equal to or higher than those of animal sources.

Mineral bioavailability

Iron absorption is often enhance by other components of the diet such as vitamin C, and antinutrients such phytate, calcium, and iron binding phenolic compounds inhibits their absorption (FAO/WHO, 2002). Moreover, many have studied the iron-zinc

interference in human-nutrition. In the present study, the interaction might have contributed to the observed higher retained zinc (1.33mg) when compared with iron (0.59mg).

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