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Potentials of Naturally Sheathed Young Leaves of Napier Grass (*Pennisetum purpureum*) Varieties as Feeding Materials in Nigeria

Ukpabi Joseph Ukpabi[1, *], Miriam Ahunna Ofoeze[1, 2], Ngozi Uchechukwu[2], Chidi Felix Ezeama[2], Hillary Edoga[2], Kenneth Iheanyichukwu Nwosu[1]

[1]National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria
[2]Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria

Email address

ujukpabi@yahoo.com (U. J. Ukpabi), mimiofoeze@gmail.com (M. A. Ofoeze), ngozivic@yahoo.com (N. Uchechukwu), cfezeama@yahoo.com (C. F. Ezeama), professoredeoga@yahoo.com (H. Edoga), nrcriumudike@yahoo.com (K. I. Nwosu)

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Abstract

There is dearth of information on possible varietal effect on the nutritional characteristics and postharvest storability of edible young leaves of Napier or elephant grass (*Pennisetum purpureum*) in Nigeria. The proximate composition and selected micronutrients of young leaf sheaths with their edible inner whorled leafy bundles collected from two local varieties of Napier grassin Nigeria (Achara Ibekuwith purple sheaths and Achara Ngwa with light green sheaths) were chemically determined. The microbial loads on the diced whorled tube-like pieces (about 0.5cm^3) of the inner edible parts were also determined during a 5 day storage period at ambient room temperature. Results showed that the edible tender sheathed young leaves (known as "Achara" in local Igbo dialects) of these varieties had 87.50-93.50% moisture, 1.66-3.32% protein, 0.90-1.10 % ash, 1.03-1.20% fiber, 1.03-1.30 0% fat and 1.72-5.26% carbohydrate contents on wet matter basis. These fresh "Achara" samples also had appreciable amounts of pro-vitamin Acarotene (34.53-50.25µg/kg), ascorbic acid (22.00-74.04mg/100g), calcium (0.10-0.16g/100g), magnesium (0.02-0.04g/100g) and phosphorous (58-96mg/100g). The concentrations of anthocyanins (an antioxidant) in the outer leaf sheaths (60-120mg/100g) were significantly different (P≤0.05) among the experimental varieties. The obtained bacterial loads on the stored leafy vegetable materials ranged from 2-3x10^{3}cfu/g in 24 hours (1 day of storage) to 4.2-6.4x10^{4}cfu/g after 120 hours, while the respective fungi loads for the samples in the same periods were 2x10^{3}cfu/g and 4x10^{3}cfu/g. From this study, the experimental fresh leaf samples of Achara Ibeku variety are potential sources of dietary protein and antioxidants in Nigeria.

1. Introduction

Pennisetum purpureum (Schumach) commonly known as elephant grass or Napier grass, is a tall grassy perennial plant in Poaceae family (Obi *et al*, 2008; Cowell, 2009; Feedipedia, 2015a). Though a native of sub- tropical Africa, it now exists in most tropical and sub-tropical countries of the world as a weed or planted crop that is largely produced and used for livestock feed (Obi *et al*, 2008). The apical ends of culms or stems

of Napier grass plants usually have whorled tender young leaves before flowering (Cowell, 2009). The common two varieties of the plant found in Nigeria are those with purple stem and light green stem; that are respectively known in local Igbo dialects as Achara Ibeku and Achara Ngwa cultivars.

The naturally sheathed tender young leaves of Napier grass which is cherished as a soup vegetable especially amongst Ngwa and Umuahia-Ibekuclans of Igbo people of Abia State, Nigeria (Okaraonye and Ikewuchi, 2009) and some of their surrounding neighbors is also known as "Achara" in the local Igbo dialects. The ethnic soup that is prepared with this leafy vegetable is called "Ofe Achara" (Okaraonye and Ikewuchi, 2009). The Igbo people consume this soup with complimentary ethnic viscous starchy pastes such as yam "fufu" and "eba" (Ukpabi and Ndimele, 1990; Ukpabi and Oti, 2010).

Presently, this perishable leafy vegetable from Napier grass is being marketed in Nigeria with little or no information in scientific literature about its postharvest shelf life. Furthermore, the nutritional data on this leafy vegetable did not indicate varietal effect on its nutritional composition (Okaraonye and Ikewuchi, 2009).This dearth of information on possible varietal differences on the nutritional characteristics of this local food and feed stuff and their postharvest microbial characteristic before culinary utilization (where necessary) led to this study.

2. Materials and Methods

2.1. Source of Materials

Samples of the experimental young leafy materials (tube-like whorled tender leaves covered with tough leaf sheaths) of two varieties of Napier grass (Achara Ibeku and Achara Ngwa) were all collected from Abia state of Nigeria (approximately within latitude 4º 40′ and 6º 14′ N, and between longitude 7º 10′ and 8º E). The experimental young shoots of Achara Ibekuvariety (with purple colored leaf sheaths) were purchased at Orie Ugba market, Umuahia North Local Government Area while those of Achara Ngwa (with light green leaf sheaths) were purchased at Umuosu Nsulumarket, Isiala Ngwa North Local Government Area (all in Abia State, Nigeria). These botanic shoot samples were authenticated as materials from Napier grass (*Pennisetium purpureum*) by Botany Unit, Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The exterior parts of the sheaths were hand peeled or rolled off to get the soft edible part or matrices also known as "Achara" as shown in Figures 1 and 2.

Figure 1. *Achara Ibeku samples from Pennisetium purpureum (with sheaths, left; edible inner matrices, right)*

Figure 2. *Achara Ngwa samples from Pennisetium purpureum (with sheaths, left; edible inner matrices, right)*

2.2. Preparation of Samples

Approximately1kg of apical sheathed young shoot materials (without leaf blades) of each of the two Napier grass (*Pennisetium purpureum*) varieties (AcharaIbeku and Achara Ngwa) was cleaned and chopped to obtain cylindrical samples of about $1cm^3$as depicted by the left samples of Figures 1 and 2. Another 1kg of each variety was further peeled to expose the young edible matrices before dicing them to about $1cm^3$ cylindrical edible "Achara" samples (types used in the preparation of the ethnic "Ofe Achara" soup) as shown in the right sides of Figures 1 and 2 .The chopped edible *Pennisetum purpureum* samples in the open mouthed polythene bags were stored on wooden shelves at ambient room temperature (25-27ºC) for five days.

2.3. Chemical Analyses

The freshly prepared Napier grass(*P. purpureum*) samples were used for the chemical analyses in triplicates. The standard AOAC methods (AOAC, 2005) were used for the proximate composition (moisture, protein, lipid, fiber, ash, carbohydrate) determinations. Crude protein was expressed as % nitrogen x 6.25, while carbohydrate content was determined by difference. Determination of the mineral content of the digested material was done with atomic absorption spectrophotometer (Unicam 929 AA) for magnesium (Mg) and calcium (Ca); and flame photometer (Jenway, Model PFP 7) for sodium (Na). The phosphorous content of the digested solution was determined by the standard spectrophotometric Vanadomolybdate method as described by AOAC (2005).

The carotene (pro-vitamin A) content of the fresh samples was determined by the Harvest Plus spectrophotometric method (Rodriguez-Amaya and Kimura, 2004). Acetone and petroleum ether were sequentially used as the extraction solvents (with light exclusion), while the readings with the spectrophotometer (Jenway 6406, England) was done at λ450 nm with 1 cm glass cuvette.

The carotene content was calculated as follows:

$$\text{Carotene content}(\mu g \Big/ g) = \frac{A \times V \times DF \times 10^4}{A_c \times \text{Sample weight}(g)}$$

Where A= absorbance

V= Volume of extract

DF = Dilution factor

10^4 = constant

A_c= Absorption coefficient of β-carotene in petroleum ether (2592)

The ascorbic acid (vitamin C) content of the fresh *P. purpureum* samples was determined using the titration method (James,1995)that use2,6-dichlorophenolindophenol (DCIP) as an indicator to get the titer values (at 15seconds persistent pinkish end point). Freshly prepared standard ascorbic acid solution was used to calculate the equivalent to 1ml of the DCIP dye solution

The modified gravimetric method of Harbone (1973) as explained by Onyeka and Nwambakwe (2007) was used to determine the total anthocyanin content of the periderm of the experimental leafy samples. About 5g of the test sample was hydrolyzed by boiling in 100ml of 0.1NHCl. The hydrolysate was filtered with Whatman No. 42 filter paper and put into a separating funnel with equal volume of ethyl acetate. The mixture was allowed to separate into two layers. The aqueous layer was discarded and the ethyl acetate layer collected. The collected material was dried at 100ºC in a steam bath. The amyl alcohol which was used to extract the anthocyanin was also evaporated out in an oven. Weight of the total anthocyanin was determined and expressed as percentage of the original leafy sample weight.

2.4. Microbiological Analyses

Relevant standard methods (ICMSF, 1988; OXOID, 1990; Fawole and Oso, 1995) were used for the microbiological analyses of the edible samples of Achara Ngwa and Achara Ibeku varieties of *P. purpureum*. The total microbial (bacterial and fungal) analyses of the freshly prepared edible samples were carried out at 0, 24, 48, 96 and 120 hours of storage. Sterile diluent (0.1% peptone + 0.85% NaCl) was used for the 10-fold dilution series while Nutrient Agar (for bacteria) and Potato Dextrose Agar (for fungi) were used to check for microbes in the samples. The bacteria plates were incubated at 37ºC for 24hrs while fungi plates were incubated at 26ºC for 72hrs.

While relevant microbiological and biochemical tests were carried to characterize the coded isolated bacteria (Buchanan and Gibbons, 1995), color and microscopy were used to identify the coded isolated fungi at genus level (Barnett and Hunter, 1987). A Zeiss Standard 25 microscope was essentially used to observe the morphology of the experimental micro-flora and to check for the presence of spores in the stained samples. Freshly purchased hydrogen peroxide was used for the Catalase test.

2.5. Statistical Analysis

Statistics Analysis System (SAS)/PC software (Lincence site 0022206002 of International Institute of Tropical Agriculture, Ibadan) was used for mean separations and standard deviations of data.

3. Results and Discussion

Table 1 shows the proximate composition of the chopped fresh Napier grass (*P. purpureum)* samples. All the samples were observed to have relatively high moisture content of 86-93.5%.Kozloski*et al* (2005) also noticed that young shoots of Napier grass have high moisture content that decreased with age. The fresh samples from Achara Ibeku variety had relatively higher protein content than those of Achara Ngwa variety on wet matter basis. The result obtained by Okaraonye and Ikewuchi (2009) for the proximate

composition of the edible inner matrix of *P. purpureum*is similar to that obtained for Achara Ibekuin this study. On dry matter basis, 26.56% protein content was obtained for the Achara Ibeku sample in this study while Okaraonye and Ikewuchi (2009) got 26.36g/100gprotein content on dry matter basis in their *P. purpureum* experiment. Therefore, dehydrated edible Achara Ibeku sample could be used as a good source of protein for human nutritionas Charney (2006) gave the daily protein requirement of humans as 23-56g based on age, weight and health conditions. The proximate composition of both experimental *P. purpureum* varieties are generally within the range obtained for the plant's varieties by international scientists that use them as livestock forage crop (Feedipedia, 2015b). Obi *et al* (2008) in Nigeria found that the leafy stem of *P. purpureum* with 25.4%dry matter and 6.78% crude protein (that is, with 26.69% protein on dry matter basis) was an excellent livestock feed for grass cutter (*Thryonomys swinderianns*). Napier grass (especially the young grass) has also been identified as a popular forage crop for cattle, sheep, goats and rabbits in some other countries (Feedipedia, 2015a)

Table 1. *Proximate composition of the fresh Pennisetum purpureum chopped samples *.*

Sample	Moisture (%)	Protein (%)	Ash (%)	Fiber (%)	Fat(%)	CHO※ (%)	DM※(%)
Achara Ngwa (edible inner matix)	93.50^{a}	1.66^{b}	0.90^{a}	1.20^{a,b}	1.03^{b}	1.72^{c}	6.50^{c}
Achara Ngwa (with outer sheaths)	86.00^{c}	1.54^{b}	1.00^{a}	1.55^{a}	1.43^{a}	8.49^{a}	14.00^{a}
Achara Ibeku (edible inner matrix)	87.50^{b,c}	3.32^{a}	1.10^{a}	1.03^{b}	1.30^{a}	5.26^{b}	12.50^{a,b}
Achara Ibeku (with outer sheaths)	88.50^{b}	3.52^{a}	0.95^{a}	1.48^{a,b}	0.75^{c}	3.81^{b}	11.50^{b}

* Mean values in a column with the same letter are not significantly different (P=0.05).
※CHO=Total carbohydrate, DM= Dry matter

The selected mineral composition of the Napier grass (*P.purpureum)* is shown in Table 2. The obtained results for these mineral micronutrients are also within the obtained range for them by scientists that had earlier worked on *P. purpureum* (Feedipedia, 2015b). The resultant abundant content of calcium (Table 2) is nutritionally important in mammalian bone formation and some other biochemical phenomena in organisms (Lehninger, 2008).

Table 2. *Selected Mineral composition of the fresh samples of Pennisetum purpureum**

Sample	Calcium (g/100g)	Magnesium (g/100g)	Sodium (g/100g)	Phosphorus (g/100g)
Achara Ngwa (edible inner matix)	0.10 (1.56^{a})	0.02 (0.32^{a})	0.001(0.017^{b})	0.058(0.89^{a})
Achara Ngwa (with outer sheaths)	0.14 (1.00^{b,c})	0.04 (0.31^{a})	0.0014(0.010^{b})	0.099 (0.71^{b})
Achara Ibeku (edible inner matrix)	0.16 (1.24^{b})	0.04 (0.34^{a})	0.002 (0.017^{b})	0.104 (0.83^{a,b})
Achara Ibeku (with outer sheaths)	0.11 (0.96^{c})	0.04 (0.32^{a})	0.008 (0.070^{a})	0.096 (0.83^{ab})

※Values in bracket are on dry matter basis
* Mean values in a column with the same letter are not significantly different (P=0.05).

Experimental results in Table 3 showed that the two Nigerian *P. purpureum* varieties have appreciable quantities of pro-vitamin A (carotene) and vitamin C (ascorbic acid). In addition to their biochemical vitamin activities, carotenes and ascorbic acid are recognized as antioxidants in human nutrition (Okaka *et al*, 2002). These plant antioxidants scavenge systematic damaging oxygen radicals. Amongst the edible experimental pieces of both varieties, Achara Ibeku sample significantly (P=0.05) has higher amount of vitamin C when compared with those of Achara Ngwa. However, there is no significant difference (P=0.05) in their carotene contents.

Table 3. *Carotene and ascorbic acid contents of the fresh samples of Pennisetum purpureum.*

Sample	Carotene (µg/kg)*	Ascorbic acid (mg/100g)*
Achara Ngwa (edible inner matrix)	38.00^{b}	22.00^{d}
Achara Ngwa (with outer sheaths)	50.25^{a}	57.20^{b}
Achara Ibeku (edible inner matrix)	43.00^{a,b}	35.21^{c}
Achara Ibeku (with outer sheaths)	34.53^{b}	74.04^{a}

*Valuesin a column with the same letter are not significantly different (P=0.05).

Table 4 shows that the periderm layer of the two samples with leaf sheaths have different amounts of anthocyanin with the purple colored AcharaIbeku variety having double amount than that of the light green colored Achara Ngwa variety. Anthocyanins which are also strong plant antioxidants are known to impart red, purple and blue colorations to plant parts (when present in sufficient quantities) under certain chemical conditions (Gould *et al*,2002).It could therefore be inferred that the purple color of the epidermal layer of AcharaIbeku samples is as a result of the relatively higher level of anthocyanin content in it.

Table 4. *Total anthocyanin content of the periderm of the experimental P. purpureum samples.*

Variety	Total anthocyanin (g/100g)
AcharaIbeku (purple)	0.12 ± 0.00
AcharaNgwa (green)	0.06 ± 0.00

The microbial (bacterial and fungal) loadsof the freshly prepared (0hour) and stored (24-120hours) edible chopped

pieces of *P. purpureum* are shown in Table 5. Due to the daily significant (P=0.05) increase in the microbial loads of the stored samples,the Napier grass food material should as much as possible be consumed fresh by people. This is to avoid food poisoning from the isolated bacteria and fungi shown in Tables 6 and 7.Though the experimental bacterial analysis showed absence of spores(that is. to indicate future cases of botulism), probable bacteria found in the study have pathogenic species (Ezeama,2007). The identified fungi genera are also known to have pathogenic species at high concentration of colony forming units (c.f.u.). We recommend that in future microbial studies with *P.purpureum,* elaborate efforts should be made in advanced laboratories, to properly identify the organisms at specific species levels using relevant molecular biology and taxonomic tools. We also recommend that *P. purpureum* samples that are not to be used immediately after harvest as livestock feed, should as much as possible be dehydrated (to discourage microbial spoilage) or ensiled (Feedipedia, 2015a) as silage fermentative microorganisms are largely non-pathogenic (Woolford, 1984).

Table 5. *Microbial loads (cfu)* of the chopped edible Pennisetum purpureum samples during storage.*

Storage time (hrs.)	Achara Ibeku		Achara Ngwa	
	Bacteria ($x10^3$)	Fungi ($x10^3$)	Bacteria ($x10^3$)	Fungi ($x10^3$)
0	0^a	0^a	0^a	0^a
24	2^b	2^b	3^b	2^b
48	8^c	4^c	9^c	3^c
72	14^d	6^d	15^d	9^d
96	24^e	8^e	26^e	15^e
120	64^f	40^f	42^f	40^f

*Values in a column with the same letter are not significantly different (P=0.05).

Table 6. *Microbial and biochemical tests of isolated bacteria*

Sample code	Cultural characteristic	Gram Reaction	Motility	Catalase	Coagulase	Indole	Methyl red	NO_3	H_2S	Urease	Gelatin	Glucose	Lactose	Sucrose	Manitol	Probable Bacteria
K_P	Yellow colonies, smooth and shiny edges	+	-	+	+	-	N	+	N	N	+	+	+	N	+	*Staphylococcus spp*
K_m	Swarming colonies	-	+	N	N	+	+	+	+	+	N	+	N	N	-	*Proteus spp*
K_L	Smooth, shinning and greenish colonies	-	+	+	N	-	N	+	N	N	N	+	-	+	-	*Pseudomonas spp*

Legend: + = positive; - = negative; N = Not determined

Table 7. *Characteristics of the isolated four fungal species*

Code	Colour	Microscopic description	Identified organism
X_1	Green	Verticillate, cluster of three or more sterigmata.	*Penicillium spp*
X_2	Dark-brown	Fruiting heads bearing chains of conidia with long non-septate mycelia.	*Aspergillus spp*
X_3	White cottony	Sickle shaped, macro- and microconidia and presence of conidiophore	*Fusarium spp*

4. Conclusion

The experimental fresh leafy samples of Achara Ibeku variety of *Pennisetum purpureum* could serve as a good source of dietary protein for human and ruminant nutrition in Nigeria. The purple color of the stem of this variety is probably due to its anthocyanin content. To avoid microbial contamination of this high moisture food material, adequate preservation methods are needed if they are not to be consumed fresh.

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