AJFAND ONLINE
Volume 9 No. 3 2009
May 2009
ISSN 1684-5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

DEGRADATION OF DIETARY FIBER BY FAECAL BACTERIA AND POTENTIAL PHYSIOLOGICAL EFFECTS

Uzomah A[1*] and CO OFUYA[2]

Uzomah A

Corresponding author email: abimuzomah@yahoo.com

[1]Department of Food Science and Technology,Federal University of Technology, Owerri, Nigeria.

[2]Department of Microbiology,University of Port-Harcourt, Port-Harcourt, Nigeria

AJFAND ONLINE
Volume 9 No. 3 2009
May 2009
ISSN 1684-5374
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ABSTRACT

Dietary fiber was extracted from Nigerian 'gari', Ex *Mannihot esculenta*; plantain, *Musa paradisiaca*; *Gnetum africana* and *Telfaria occidentalis* and these fiber sources were referred to as GAF, PLF, GF and TF, respectively. *Mannihot esculenta* and *Musa paradisiaca* are rich sources of carbohydrate and the fiber extraction was done using termamyl 120L and amyloglucosidase. *Gnetum africana* and *Telfaria occidentalis* are vegetables and the acetone dried powder method was employed for fiber extraction. The fiber extracted from each source was subjected to degradation by the gut microbial flora and the extent of degradation after 72 h was determined. Water holding capacity (WHC) of each fiber was measured before and after the degradation. The short chain fatty acids (SCFA) produced during degradation were measured chromatographically. Exposure of the fibers to the gut microflora showed that the non-vegetable fibers (GAF, PLF) were more readily degraded than the vegetable fibers (GF and TF). Consequently, the percentage of the undegraded fiber after 72 h of incubation was highest with the vegetable fibers; GF, 80.0 % and TF, 83.3 %, while that of the non-vegetable fibers (GAF and PLF) was 62.0 % and 72.5 %, respectively. The degradation by the microflora affected the WHC of the fibers (except TF). Water holding capacity (WHC) for GF was 11.1 ± 3.3 g H_2O / g fiber, before degradation and 6.3 ± 2.5 g H_2O / g fiber after degradation, indicating a percent decrease of about 43.2%. Similar decrease was observed for GAF (30.4 %) and PLF (13.9 %). Only acetic and butyric acid were detected in the fermenting slurry. The relative composition of acetic acid from each of the fiber source (GAF, 62.0 %; PLF, 70.4%; GF, 62.5%; TF, 52.9%) was found to be greater than that from the slurry (control) (42%). The low pH created by the actions of the microflora in the caecal lumen will decrease the toxicity of luminal contents to the gut mucosa and protect against cancer of the colon

Key words: Dietary fiber, degradation, gut microflora.

INTRODUCTION

The risk of colon and rectal cancer has been associated with the intakes of vegetables and other plant foods rich in fiber [1, 2]. The benefits of fiber against colon cancer were closely associated to both the fermentative and the non-fermentative processes, where the poorly fermentable fibers were regarded to be more influential [3]. Dietary fibers are viscous, indigestible polysaccharides derived from plant foods which are not degraded by enzymes of the alimentary tract; therefore, they reach the colon undegraded and serve as potential fermentation substrates for the colon micro-organisms, particularly bacteria [3, 4]. The nature of the colonic bacterial flora, the transit time through the colon and the physical and chemical composition of the fiber determine the extent of the fiber breakdown by bacterial enzymes. Fiber digestion occurs almost exclusively in the colon: 29-82% of ingested cellulose, 56-87% of hemicellulose and 90% of pectin are lost during passage through the colon [5]. Lignin, by virtue of its polymeric cross-linked structure, is resistant to bacterial digestion and is virtually completely recovered in the stool. Degradation of fiber results in the production of compounds such as methane, carbon dioxide, hydrogen, water and short chain fatty acids [3, 6]. The production of these compounds is catalyzed by enzymes produced in response to the degradation of fiber [6]. The dietary fiber composition and the degree of degradation by gut microflora of most food consumed in Nigeria have not been reported. In this study, Ex *Manihot esculenta* (also called "gari"), *Musa paradisiaca* (matured green plantain), *Gnetum africana* leaves and *Telfaria occidentalis* leaves were analysed for dietary fiber content and bacterial degradation. Gari when made into a semi-solid meal by stirring with boiling water is usually eaten with vegetable soup. It is the main staple food of people in the Eastern States of Nigeria. Matured green plantain when boiled or cooked into porridge form is known for its ability to hold loose bowel. It is also regarded by some consumers to be a good recipe for diabetic patients.

Gnetum africana and *Telfaria occidentalis* are vegetables usually consumed once or twice a day. *G. africana* is an unusually hard vegetable. It is shredded into tiny strands (about 0.1 mm - 0.2 mm) and may further be pounded in some instances before it is cooked. *T. occidentalis* on the other hand is much softer and serves as a major soup ingredient. Studies on the physiological properties of the dietary fibers from these food sources have not been reported.

Thus the objective of this study was to examine the degradation of the dietary fibers from these four Nigerian foods by the gut microflora and the effect of the degradation on their physiological properties.

MATERIALS AND METHODS

Sample Sources and Preparation

The dietary fiber sources used in this study included 'gari' Ex *Manihot esculenta*, *Musa paradisiaca* (matured green plantain), *Gnetum africana* leaves and *Telfaria occidentalis* leaves. Gari (a gritty cassava product) was purchased from the producers while the other foodstuffs were harvested fresh from a local farm in the University of

Port Harcourt. The processing of cassava root tubers for the production of gari grit has been described [7]. The gari-meal was prepared by the addition of boiling water to the grits to obtain a semi-solid mass. The plantain was diced into cubes and the gari-meal was made into small balls, each sample was frozen (-18 ^{0}C) over night prior to lyophilization. The lyophilized samples were pulverized to obtain fine powder.

Fiber extraction from the gari and the plantain was as described by Theander and Westerlund, [8]. Two grams of the dried residue (previously refluxed with 80 % ethanol to remove free sugars) was suspended in 0.1 M sodium acetate buffer solution (pH 5.2) containing 4 mM calcium chloride. The sample was gelatinized and hydrolyzed with 120 mL of Termamyl. Absolute ethanol was used to precipitate any solubilized dietary fiber component. The precipitate was pelleted by centrifugation (2000 x g; 15 min) and dispersed in DMSO before hydrolyzing with 0.1 ml amyloglucosidase in 0.1 M acetate buffer (pH 4.6). The residue after digestion was recovered by ethanol precipitation, centrifuged and dried at 100 ^{0}C. The residue (non-starch carbohydrate) obtained from the gari and plantain contained soluble and insoluble dietary fiber components [8] and they were referred to as GAF and PLF respectively in this study.

The acetone dried powder method was employed for the fiber extraction of the two vegetables to obtain the *G. africana* fiber (GF) and *T. occidentalis* fiber (TF) [9]. Ten grams of the leafy vegetables were homogenized in 150 ml of water and frozen at -18 ^{0}C for 24 h. To disrupt the cell wall they were thawed and frozen repeatedly. The insoluble residue was washed free of soluble materials using warm water (40 ^{0}C) and dried with acetone. The fibers so obtained were separately pulverized to pass through sieve of mesh size 0.25 mm - 1.00 mm. Grinding of *G. africana* fiber produced a fluffy mass similar to cotton wool fiber and this was used as the GF.

Degradation Studies

The faeces of six healthy adults were used. Each faecal sample was collected in a double layered polyethylene bags and the samples were chilled immediately in an insulated ice-box containing two packs of dry ice. The samples were transported to the laboratory within 2 h of collection and were kneaded to ensure thorough mixing.

The faeces were then treated with an anaerobic dilution solution (ADS) [10, 11]. The ADS was prepared by adding 33 ml of mineral solution 1, 75 ml of mineral solution 2 and 1 ml of resazurin (0.1%, w/v) and the volume was made up to mark with distilled water in a 1 litre flask.. The mixture was boiled under carbon dioxide and autoclaved (121 ^{0}C, 15 min). Sodium thioglycollate, a reducing agent, (2% v/v) was added after autoclaving [12]. Mineral solutions 1 and 2 were prepared as described by Bryant and Burkey [10]. Mineral solution 1 contained 8 g K_2HPO_4 in a litre of distilled water. Mineral solution 2 was prepared by dissolving in 200 ml of distilled water; 4.8 g K_2HPO_4, 12 g NaCl, 12 g $(NH_4)_2SO_4$, 2.4 g $CaCl_2.6H_2O$ and 2.5 g $MgSO_4$. The solution was made to mark with distilled water in a 1 litre flask.

One gram of the faecal material was transferred into a tube containing 9 ml of ADS. The slurry so obtained was used as the stock solution. Nine millilitres of Schaedler's

broth (Oxoid) was inoculated with 1 ml of the faecal slurry (stock solution) in a glass bottle (bijoux bottle) and incubated for 2 days at 37 ^{0}C. The bacterial isolates so obtained were used to seed the supplemented medium.

Preparation of Supplemented Medium

Basal supplemented medium 10 (BSM10) was prepared as SM10 but without the addition of the carbohydrates and volatile acids. The following were dissolved in 200 ml distilled water in a 1 litre flask and made up to mark with distilled water, 0.5 g yeast extract; 2.0 g trypticase; 37.5 ml mineral solution 1; 37.5 ml mineral solution 2; 10 ml haemin, (0.1 ml/ml); 1 ml resazurin (0.1%, w/v); liver extract 5% (v/v); chicken faecal extract, 10% (v/v) [13, 14]. The dietary fiber sources (0.1%, w/v) were separately added to the BSM10 medium. The control sample did not contain any carbohydrate. The mixture was autoclaved (121 ^{0}C, 15 min).

In each of the stages described above strict anaerobic techniques were maintained and all manipulations were carried out under a stream of carbon dioxide [12].

Fermentation Studies

The bacterial isolates grown in Schaedler's broth (SB, Oxoid) (1 ml) were used to seed the supplemented medium. Test cultures were incubated at 37 ^{0}C for 72 h, then the cultures were each centrifuged and the non-utilized carbohydrate was estimated using the phenol sulphuric acid method [15]. The coloured complex formed by the addition of phenol sulphuric acid mixture to the culture was cooled and measured at 540 nm [15]. A series of glucose solution (0.01 to 0.06 mg/ml) was used as standard. The amount of sugar in the test sample was determined from the standard curve. This was expressed as a percentage of the total carbohydrate at 0 h in determining the extent of fiber degraded by the micro-organisms.

Determination of the Water Holding Capacity (WHC)

The water holding capacity (WHC) of samples was estimated using the modified method of Eastwood *et al.* [16]. About 0.2 g of the fiber was weighed into a plastic centrifuge tube (25 ml capacity) and 10 ml distilled water containing 0.1 g/litre of sodium azide, a bacteriostat, was added. The fiber was soaked at room temperature (28 ^{0}C) for 24 h before centrifuging at (1000 x g, 30 min). The supernatant was discarded and the tubes were allowed to drain for 30 min after which the pellet was weighed. The difference in weight between the wet and dry fiber weight was taken as the amount of water held by the fiber. By definition, the water-holding capacity is the weight of water that is held by 1 g of the dry fiber material (g H_2O/g fiber) under the described conditions of soaking and centrifugation [16].

Determination of Volatile Acidic Fermentation Products

The volatile fatty acids produced after 72 h of the fiber degradation were analyzed using gas chromatography [17, 18]. The gas liquid chromatography used was Varian 3700 model, equipped with a flame ionization detector with a set temperature of 250 ^{0}C. The Varian recorder was fitted with an automatic integrator, sensitivity set at full scale and a chart speed of 0.5 cm/min, attenuation x 32. A fused silica column (60 m x 0.75 mm) was packed with a polymer of dimethyl silicone (OV-101). The initial

oven temperature was 60 ^{0}C. The injection block was maintained at 250 ^{0}C to provide rapid vaporization of the injected fluid. Nitrogen was used as a carrier gas with a flow rate of 25 ml per min.

Two millilitres of supernatant fluid were acidified to pH 2 by adding 0.2 ml of 50 % H_2SO_4. One millilitre of diethyl ether was added to 4 ml of the acidified supernatant in a screw cap test tube. The tube was inverted gently 20 times to extract the acids and then centrifuged at (700 x g, 1 min) to obtain rapid phase separation. The fatty acid containing upper layer was separated from the lower aqueous layer by placing the test tube in a deep freezer at -18 ^{0}C until the aqueous layer was frozen. The still-ether layer was rapidly poured off into a clean vial and few granules of anhydrous $CaCl_2$ were added to remove residual water. The ether extract (0.1 µl) was injected into the gas chromatography equipment for the analysis of the volatile fatty acid. The standard solution containing (10 µmol/ml) acetic acid, propionic acid and n-butyric acid were extracted in the same manner as above [17,18].

Measurement of the Head Space Gas

The gas solid chromatography (gsc) was the same model as described above, but equipped with a thermal conductivity detector with a set temperature of 200 ^{0}C. The column material was stainless steel (18.29 m x 3.175 mm outside diameter) packed with molecular sieve 13x and the mesh size was 60/80. The carrier gas was helium with a flow rate of 30 ml/min. Initial oven temperature was 50 ^{0}C while that of the injection block was 100 ^{0}C. The recorder model and other set conditions were as given above. Fifty microlitre of a sample of the head space gas was injected after proper conditioning of the column.

RESULTS

DEGRADATION STUDIES

The Extent of Fiber Degradation

Percent undergraded portion after 72h of gut microflora action was 62.0% for GAF ; 72.5% for PLF,; 80.0% for GF, and 83.3% for TF (Table 1).

Water Holding Capacity of Fibers

The values obtained for the WHC for the various fibers varied from 4.3 g H_2O/g PLF to 11.1 g H_2O/g GF (Table 2). GF held 1,110 g H_2O/100 g of the fiber while TF held 760 g H_2O/100 g of the fiber. The values for the storage organs were smaller (690 g H_2O /100 g of GAF and 430 g H_2O/100g of PLF). After exposure to microbial degradation the WHC decreased appreciably. The percentage decrease in WHC for the GF was 43.2 %; GAF was 30.4 % and PLF was 13.9 %.

Cellular Fatty Acid

The two acids identified from the culture tubes after 72 h of incubation were acetic and butyric acids (Table 3). It was observed that the production of acetic acid in all the culture tubes containing the different fibers was higher than that of butyric acid. In the pooled slurry (without the fiber which served as the control) the relative

composition of acetic acid was 42% and 58% for butyric acid. Plantain fiber (PLF) gave the highest value for acetic acid (70.5%) and TF gave the least (52.9%). GAF and GF had similar values (62.4% and 62.5%, respectively). Propionic acid was not detected in any of the samples.

The Head Space Gas

Nitrogen appeared to be produced in greater proportion than the other two gases, during the fiber degradation (Table 4). The volume of carbon dioxide produced was very low and in trace amount in TF. Other metabolic products such as hydrogen and methane produced *in vivo* were not detected**.**

DISCUSSION

Degradation Studies

The susceptibility of the fibers to microbial degradation varied considerably (Table 1). *Telfaria occidentalis* fiber (TF) appeared to be the least fermentable (83.3%, undegraded fiber) followed by GF (80.0% undegraded fiber), both fibers were derived from vegetable sources. The other fiber sources (GAF, PLF) also showed high percentage of undegraded fiber (62% and 72.5% respectively). The results suggest that each dietary fiber interacted with the microflora to produce different possible effects. The effect registered by each depended largely on its fermentability, which was in the following order, GAF>PLF>GF>TF. The least fermentable (TF) will most likely have the highest increase in stool output when consumed while the most fermentable are usually lost before reaching the colon [19]. It is, however, important to note that this is an *in vitro* study. Within the gut environment there may be some other effects exerted due to the presence of other food components present in the colon. Some studies have shown that the unavailable portion for fermentation is determined by the extent of lignifications of the cell wall polymers and the cutin content that is associated with the cellulose and hemicellulose [20]. Associated silica, cuticular substances and tannins have also been reported to inhibit polysaccharide breakdown in the rumen and can be expected to do so in the colon [20]. The resistance of the fiber to microbial action depends largely on the physical form of the fiber (the three-dimensional arrangement of the different component within the fiber) and the physico-chemical properties. Plaami [21] in his review of dietary fiber in foods reported that the hard red spring wheat neutral detergent fiber showed greater fermentative degradation than corn bran neutral detergent fiber even though their diets contain similar dietary fiber levels and composition [21]**.** Lignification was found to be the major factor preventing the fermentation of the corn bran and this was associated with strong linkage of the polysaccharide molecule of the corn fiber. The fiber fermentability of the foodstuffs investigated in this study may, therefore, be governed by similar factors. Chemical composition and the extent of lignifications were probably the controlling factor for the two vegetable fibers (GF and TF) but the other two fibers (GAF and PLF) in addition to this were also affected by the processing which may have led to the production of resistant starches [22, 23]. The presence of high amylase activity in the gut microbial flora has been reported [24], indicating that fiber component reaching the gut contains some substantial quantity of resistant starch.

The relationship between WHC and stool output is complex. Easily hydrated fiber is more highly digestible, and in turn has less effect on faecal composition and bowel habit [20]. *Telfaria occidentalis* fiber (TF) and GF that gave the high values of WHC (7.6 ±2.0 and 11.1 ± 3.3g H_2O/ g fiber respectively) before exposure to the micro flora were expected to be most readily degraded but the results showed that they were poorly degraded. Similar exceptions have been reported by Cummings [19] who showed that ispaghula with high WHC resisted fermentation and wheat bran, a reliable stool bulker, has very low WHC. Faecal bulking in relation to WHC is thus dependent on the amount of the undegraded fiber and its ability to still hold water after degradation [19].

Fiber hydration occurs by adsorption to the surface of the fibrous particles or by entrapment in macromolecular interstices. The presence of sugar residues increases the capacity to hold water, whereas intermolecular bonding such as the hydrogen bonding between chains of cellulose molecule decreases this ability [25]. Hemicellulose a highly branched polysaccharide and the plant exudates (gums) have greater water holding capacity. Also, the structural features that disrupt close packing and alignment of molecular chains decreasing crystallinity promote water solubility of fibers [26].

Cellular Fatty Acids

The short chain fatty acids (SCFA) were the principal products of fermentation of the dietary fibers. The results showed that there was not much variation in the relative composition of the SCFA from the different fiber sources (Table 3). This was in agreement with the findings of Rubinstein *et al.* [27]. Their studies showed that there was little difference in the molar ratios of the SCFA concentrations from the subjects. However, one major role of the SCFA is in acidifying the caecum [28]**.** Most of the bacterial enzymes responsible for the production of carcinogens/mutagens have pH optimal of 7 or above [29]. The acidification of the caecal lumen would decrease the toxicity of luminal contents to the gut mucosa [30]. The results of this study, therefore, suggest that the fiber sources investigated may protect against colorectal cancer by decreasing luminal carcinogens or promoters.

CONCLUSION

The dietary fiber analysis in this study included the soluble and insoluble fiber components. The high values of the undegraded fibers obtained in this study suggested that the foodstuffs investigated are rich sources of dietary fiber. However, a very important aspect in this study is the physiological properties of the dietary fiber after exposure to colonic micro-organisms. The decrease in the ability of some of the fiber to hold water after exposure to colonic micro-organism and consequent effect on the faecal bulking is worth further investigation. It is possible that the other physiological properties of the fiber such as the mineral adsorption may also be affected and specific fiber components may be implicated. Further research on the physiological roles of the different fiber components after microbial degradation is,

therefore, recommended and this may help towards a better understanding of the complex physiological actions of these fibers in the gut.

AKNOWLEDGEMENT
The authors acknowledge the immense contributions of the late Dr. E. O. Uche of the College of Medicine, University of Port Harcourt Nigeria, who was very helpful in the collection of the faecal samples from the subjects used for this study.

Table 1: The extent of the degradation of the fibers.

Fiber Source	Total carbohydrate (g) Period of Incubation (h) 0	72	Undegraded Fiber (%)
Gari	0.840±0.02	0.520±0.03	62.0
Plantain	0.121±0.11	0.087±0.12	72.5
Gnetum africana	0.109 ±0.11	0.087±0.01	80.0
Telfaria occidentalis	0.102 ±0.10	0.085±0.02	83.3

Table 2: Water holding capacity of the fibers.

Fiber Source	Water Holding Capacity (g H_2O/g fiber)	
	Before degradation	After degradation
GAF	6.9 ± 0.5	4.8 ± 0.3 (*30.4%*)
PLF	4.3 ± 0.5	3.7 ± 0.2 (*13.9%*)
GF	11.1 ± 3.3	6.3 ± 2.5 (*43.2%*)
TF	7.6 ± 2.0	7.6 ± 0.5 (*0.00%*)

Percent decrease in WHC of the dietary fiber is in parenthesis

Table 3: Relative composition of the short chain fatty acids (SCFA) produced after 72 h of fermentation of dietary fiber.

Peak	SCFA	Retention Time (min)	Relative Composition (%)				
			Std	Slurry	GAF	PLF	TF	GF
A	Acetic	5.08 – 5.93	36	42	62.4	70.4	62.5	52.9
B	Propionic	6.36	26	Nd	Nd	Nd	Nd	Nd
C	Butyric	7.5 – 8.63	38	58	37.6	29.5	37.5	47.1

Std- Standard

Nd- Not Determined

Table 4: Relative composition of the head space gas collected during microbial degradation of the dietary fibers

Gas	Retention time (min)	Composition %			
		GAF	PLF	GF	TF
Oxygen	1.09-1.21	12.9	11.9	13.6	14.0
Nitrogen	1.91-1.94	86.6	80.6	81.7	86.0
Carbon dioxide	6.10-6.27	0.5	7.5	4.7	Trace

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