ANALYSIS OF FONIO AND THREE SELECTED BOTANICALS USED FOR DIABETES CARE

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CHAPTER I

INTRODUCTION

The treatment of diabetes in Africa. Until about the middle of the twentieth century diabetes mellitus was believed to be rare on the African continent because of the scarcity of foods and the farm-like living style that involved intensive physical activity and little obesity. Infectious diseases were the leading cause of deaths. But today, improved sanitation and health care systems, vaccine development and increased quality and quantity of food have raised life expectancy. However, with increased life expectancy, a higher living standard, the growing western life style and abundance of food, chronic diseases are as common as in Europe, Asia, or America. Among them, diabetes mellitus has the highest incidence with its associated complications.

In the USA, populations have medical choices between the traditional FDA-approved therapies and the use of botanical products usually called "alternative medicine". But in most of the developing countries, drugs rigorously tested at a cost of hundreds of millions of dollars are simply not available or are cost-prohibitive for the majority of populations. The only alternative offered to populations is to use what is found growing on their own land, a practice propagated generation after generation.

Finding local solutions to treat diabetes is currently of major concern. And this is all the more important as we know that some epidemiological studies have suggested that dietary modification and treatments are fundamental to the successful treatment of both type I and type II diabetes (Harris, 1990; National Research Council, 1989). Compared to non-diabetic, diabetic clients have a higher incidence of deaths from cardiovascular

diseases (60-70% versus 20-25% in matched non-diabetic controls) due to dietary disturbances (Harris, 1990; Schuster, 2002). This disease incidence has led to a new interest in botanicals whose activities may sometimes be beneficial.

Purpose of the study The purpose of this study was to assess key components of some African foods used for the treatment of diabetes. The foods include the cereal fonio (*Digitaria exilis*) as a staple food combined with some botanicals, namely nebedaay (*Moringa olifera)*, beer (*Sclerocarya birrea)* and darkassou (*Anacardium occidentale).* Some of these botanicals are gaining scientific recognition for the treatment of different conditions other than diabetes (Caceres et al, 1992; Galvez et al, 1991; Price, 1985). Yet, there is no study found in the literature related to the use of these plants for diabetes treatment. Therefore an investigation needs to be undertaken in order to understand how the components in fonio and these botanicals may affect diabetes. Many questions need to be addressed: Is the active substance located in stems, roots, or leaves? What is the correct solvent to use for its extraction? Is it dissolved in the watery extract or the alcoholic extract? What is the major function of the isolated compound? Does it retain full activity when isolated? Is it a polyphenol (Bravo, 1998: Rastelli et al, 2000), an enzyme inhibitor (an antagonist) or a stimulating enzyme (an agonist) (Blickle, 2000)? In what site of the body does it react? Does it help with handling fasting glucose, postprandial glucose or with sorbitol resorption for preventing atherosclerosis via aldose reductase inhibition (Yoshika, 1998)? Does it retard the breakdown of long sugar polymers such as starch via α-glucosidase inhibition (Yoshika, 1999)? Does it induce triglyceride removal (Bopana et al, 1998) via sulfur- containing compounds for instance?

Does it potentiate insulin via its chromium content (Anderson et al, 1997, Mertz 1993, Vincent, 1999)? Does it contain other important minerals and vitamins useful for diabetes treatment? Does it help with the recovery of damaged beta cells or simply allow their re-proliferation (Ahmed et al, 1998)? Is it an insulin or arcarbose analog (Blickle, 2000)?

These are some of many unanswered questions paving the way for various research investigations that should be addressed step by step. The present study, considered the first step, will be essentially confined to the analysis of some key components of the individual plants.

Research objective. The objective of this project was to analyze fonio and the set of botanicals used for diabetes treatment in Senegal for their selected trace elements, fatty acids, crude fiber, dietary fiber, β-glucan, sulfur-containing amino acids and polyphenols. Former studies have suggested that these components may be of interest in improving the health status of diabetic clients.

The following analyses were completed:

a) Selected minerals including chromium, zinc, magnesium, copper, iron and calcium by atomic absorption spectrophotometry,

b) Fatty acids: myristic, palmitic, stearic, oleic, linoleic, linolenic, behenic, lignoceric, arachidic by gas chromatography,

c) Crude and dietary fiber content using the Fibertec System E,

d) Sulfur containing amino acids by the amino acid analyzer,

e) Total polyphenol content by UV/visible spectrophotometry

f) Beta–glucan content by enzymatic analysis coupled with the clinical analyzer (Langston U niversity).

Research hypothesis. If concentrations of any of the analyzed components are high relative to needs or requirements, those components may be the basis for beneficial effects of fonio and the selected botanicals in diabetes care. Our purpose in this initial study is to identify such components so that further tests could be conducted.

Background of the study. For many years, fonio cereal (*Dactylaria exilis*) as a staple food combined with nebedaay (*Moringa oleifera*), beer (*Sclerocarya birrea*) and darkassou (*Anacardium occidentale*) have been used as diet and treatment for diabetic clients in Western Africa especially in the rural areas of Senegal and its neighboring countries, namely Mali and Guinea. Presently, use of fonio with botanicals has gained momentum because of the high incidence of diabetes in this part of the world. Up to 7% of the hospitalized population suffers from the disease and among them 73 to 80% are non insulin-dependent diabetic clients (Sidibe, 1996).

Although there is not yet much literature on their functions, there is an increasing scientific interest in the cereal and the cited botanicals. Today an ongoing study on the interaction of the cereal fonio and diabetes is being conducted in Casamance, Senegal. However, the mechanisms by which the cereal combined with the cited botanicals (which are thought to be more efficient for diabetes treatment according to the traditional healers and the indigenous population) may affect diabetes are scientifically unknown. Dr Seck, one of the specialists in diabetes at the "Centre National des Diabetiques Abass Ndaw",

stated that in some cases they had to stop or drastically reduce the use of insulin or other drugs because patients who were using those botanicals experienced a sharp decrease of their blood glucose level (Personal communication, summer 2001). The physicians at the center welcomed and expressed the importance and necessity of this study.

Limitations of the study. Due to budget restriction, samples of fonio and botanicals were purchased in three markets of the capital Dakar where diabetic clients regularly supply themselves. But the investigator did not get samples from the remaining eight regions. It is also important to mention that the Moringa leaves are sold fresh, Anacardium bark and Sclerocarya are sun-dried and sold ground or non-ground. Our samples were not dried.

Samples were not stored into liquid nitrogen during transport and flight to the USA.

We did not weigh the samples after we purchased them in the markets because good scales were not available. But samples were stored in the freezer just after our arrival at Oklahoma.

Also samples may have undergone some slight changes because of overextended storage time (two years).

CHAPTER II

LITERATURE REVIEW

There were few papers related to the use of Digitaria, Moringa, Anacardium, and Sclerocarya for diabetes care; instead we found several studies on the mentioned botanicals related to the treatment of other pathological conditions. Therefore, two different but complementary reviews are presented. The first part (entitled: Plants and Compounds/Elements Used for Diabetes Care) was devoted to botanicals different from those of the present study and confined to the treatment of diabetes. Thus, the first part of the literature review was to reveal the main compounds that researchers have found important for diabetes treatment. Eventually some of the same compounds were investigated in our samples. The second part (entitled: Selected Plant Characteristics and Traditional Uses) of the review focused particularly on the cited botanicals (Moringa, Anacardium, Sclerocarya) and the cereal (Digitaria) in order to determine the important nutrients and phytochemicals they contained and the related conditions they were supposed to treat (especially conditions related to diabetes complications such as joint inflammation, hypertension etc). Thus inference from the combination of these two important reviews dictated our research rationale. But it is important to mention that among all the compounds discussed from this comprehensive review, only a few of them were analyzed for the purpose of this thesis.

I. PLANTS AND COMPOUNDS/ELEMENTS USED FOR DIABETES CARE

Sulfur-containing compounds. The property of lowering blood sugar has been attributed to onions and garlic (Sheela and Augusti, 1992). The active principles are believed to be the sulfur containing compounds, allyl propyl disulphide (APDS) and diallyl disulphide oxide (allicin), although other constituents such as flavonoids may play a role. Experimental and clinical evidence suggests that APDS lowers glucose level by competing with insulin (a sulfur containing hormone) for insulin-inactivating sites in the liver (Sharma et al, 1977). This results in an increase of free insulin. APDS administered in doses of 125 mg/kg to fasting humans caused a marked drop in blood glucose levels and an increase in serum insulin. Allicin at doses of 100 mg/kg produced similar effects (Sharma et al 1977). In addition many hypoglycemic oral drugs are sulfonylureas that are known to lower plasma glucose by stimulating insulin secretion and to a lesser extent, by improving peripheral and hepatic insulin sensitivity. Because of the important role played by sulfur in diabetes treatment, the selected botanicals were analyzed for their sulfur amino acids concentrations.

Fiber. It has been suggested that diabetes mellitus is highly correlated with the fiber-depleted, highly-refined carbohydrate diet of the "civilized man" (Burkitt and Trowell, 1981). Population studies, as well as clinical and experimental research, showed diabetes to be one of the diseases most clearly related to inadequate dietary fiber intake (Burkitt and Trowell, 1981; Vahouny and Kritchevsky, 1981). These results indicate that

while the intake of refined sugars should be curtailed, the intake of complex carbohydrate sources that are rich in fiber should be increased.

The term "dietary fiber" refers to the components of the plant cell wall as well as other indigestible residues from plant foods. The types of fiber that exert the most beneficial effects on blood sugar control are the water-soluble forms. Included in this class are hemicelluloses, mucilage, gums, and pectin substances. As early as 1983, one study (Simpson et al, 1983) showed that these types of fibers were capable of slowing the digestion and absorption of carbohydrates, thereby preventing rapid rises in blood glucose; increasing the sensitivity of tissue cells to insulin, thus preventing the excessive secretion of insulin; and improving uptake of glucose by the liver and other tissues, therefore preventing a sustained elevation in blood sugar.

In the study cited earlier, a high carbohydrate, legume rich, high fiber diet improved all aspects of diabetic control. Other studies showed that when diabetic patients were supplemented with 14 and 26 grams of guar gum per day, they required less insulin and had less glycosuria (Vahouny and Kritchevsky, 1981; Jenkins et al, 1980).

Both crude fiber and total dietary fiber contribute to the lowering of the glycemic index of foods (Jenkins et al, 2002) and are inversely related to body mass index (MCkeown et al, 2002), Hb_{A1c} levels and ketoacidosis risk (Toeller, 2002). The glycemic index concept, an extension of the fiber hypothesis, suggests that fiber consumption reduces the rate of nutrient influx from the gut, and as a result significantly improves glycemic control in diabetic clients. Fiber also reduces urinary C-peptide excretion in healthy people as well as circulating insulin and serum lipids in hyperlipidemic subjects (Jenkins et al, 2002; Giacco et al, 2002). And consumption of high fiber diets has been

associated with higher HDL-cholesterol concentrations in cohort studies, with decreased risk of developing diabetes and cardiovascular diseases (Lui et al, 2000; McKeown et al, 2002). Determination of the fiber (crude and dietary) content in fonio and the botanicals was of interest because consistent values are not available from the literature.

Trace elements. Diabetes treatment may require nutritional supplementations, as patients have an increasing need for some nutrients. Supplying the diabetic clients with additional key nutrients has been shown to improve blood sugar control and to prevent many of the major complications (Barbagallo et al, 2003; Anderson, 1992). The following section reviews the specific trace elements considered to play an important role in improving the diabetes condition.

Chromium: Many studies have shown that this trace element is vital to proper blood sugar control. The studies showed that it works closely with insulin in facilitating the uptake of glucose into cells. Without chromium, insulin action is blocked and glucose levels are elevated (Anderson, 1992; Mooradian et al 1994). In some clinical studies, supplementing the diet with chromium has been shown to decrease fasting glucose levels, improve glucose tolerance and lower insulin levels and decrease total cholesterol and triglyceride levels while increasing HDL-cholesterol levels (Anderson 1992; Mooradian et al 1994). More recently, Vincent and his team made a major breakthrough regarding the structure and role of chromodulin or low- molecular-weight chromium -binding substance. They showed that the oligopeptide composed of residues of glycine, cysteine, glutamate, and aspartate binds four atoms of chromium. The resulting holo-form can potentiate as much as seven times the action of insulin without increasing its

concentration, by binding insulin receptor kinase. And insulin stimulation is directly dependent on the number of bound chromium atoms (Vincent, 1999; Vincent, 2000).

Magnesium: Magnesium by modulating insulin-mediated glucose uptake is involved in several areas of glucose metabolism and there is considerable evidence that diabetes clients need supplementation with the element (Barbagallo et al, 2003). Magnesium deficiency is common in diabetic patients and its supplementation may prevent some of the complications of diabetes like retinopathy and heart disease (White and Campbell, 1993). In addition, according to the "ionic hypothesis", some responses reported in human hypertensive disease can be explained by a steady-state elevation of cytosolic free calcium and decreased magnesium level altering the functions of many tissues. For instance, blood vessels can exhibit vasoconstriction, and there may be cardiac hypertrophy and skeletal muscle insulin resistance (Resnik, 1999)

Zinc: This element is present in almost all aspects of insulin metabolism from synthesis and secretion to utilization. It is well documented that zinc also has a protective effect against beta-cell destruction and displays antiviral effects. Diabetic patients excrete an excessive amount of zinc in the urine and therefore may need supplementation (Mooradian and Morley 1987), which has been shown to improve insulin levels in both type 1 and type 2 diabetes. (Hegazi, 1992). In addition, zinc helps improve the poor wound healing observed in diabetes (Engel et al, 1981).

Calcium: The Ca^{2+}- Mg^{2+} - ATPase pump is an important regulator of intracellular calcium concentration. Migdalis and colleagues demonstrated that there were abnormalities of the Ca^{2+}- Mg^{2+} - ATPase pump activity and therefore Ca^{2+} metabolism.

They noted that the study group displayed lower levels of Ca^{2+} compared to the control group at Alpha= 0.05 and $P < 0.001$ (Migdalis et al, 2000).

Furthermore, Oliveira and colleagues showed impaired calcium metabolism in cardiac cells from streptozotocin-induced diabetic rats due to an enhanced mitochondrial transition resulting in decreased mitochondrial calcium accumulation. This fact may be one explanation for the cardiac dysfunction associated with diabetes (Oliveira et al, 2003).

The antioxidant vitamins. In Senegal, Mali and Guinea, people with diabetes take a pinch (approximately 0.5 g) of the dried and ground raw leaves of Moringa, before each principal meal (breakfast, lunch and dinner); the regimen also includes palm oil-mixed sauces with fonio. Palm oil is known to be rich in carotenoids and Moringa leaf powder is one of the richest plants in carotenoids (Nambiar and Sheshadry, 2001). In the USA, at the Centers for Disease Control and Prevention (CDC), carotenoids alpha and beta-carotene, cryptoxanthin, lutein/zeaxanthin, and lycopene were inversely related to fasting serum insulin. Hence CDC has suggested investigation of carotenoids for their use in diabetic clients (Ford et al 1999).

Vitamin C: It is well established that vitamin C plays an important role in the production of collagen, a key protein for connective tissues. It is vital for wound repair and is critical to immune function. Since the transport of vitamin C into cells is facilitated by insulin, many patients with diabetes do not have enough intracellular vitamin C. Therefore, they develop a relative vitamin C deficiency despite adequate dietary consumption (Cunningham, 1991). Vitamin C at high doses (2000 mg/day) has been

shown to reduce sorbitol accumulation in erythrocytes and inhibit at the same time glycosylation of proteins (Davie et al 1992; Vinson et al 1989).

Vitamin E: The requirement for this vitamin may be very high in patients with diabetes. High doses of vitamin E (900 IU) improved insulin action and exerted a number of beneficial effects that may help in preventing the long-term complications of diabetes (Paolisso et al, 1993). A known action of vitamin E is to improve endothelial function in general and particularly in diabetic patients. Also vitamin E improved metabolic control in diabetic clients (Paolisso et al, 2001).

The bitter principle. In this section all studies cited will essentially focus on the bitter principle and all plants or other botanicals mentioned in this section display a bitter taste.

Currently some studies suggest that plants displaying the "bitter principle" may be of benefit for diabetics (Bennett 1999). In one study, the bitter melon (*Momordica charantia)* normalized some enzymatic defects in diabetic rats (Ahmed et al, 1998). Other studies also showed that charantia fruit juice caused an increase in the number of beta cells in the pancreas of rats (Ahmed et al,1998, Welihinda et al, 1982). These researchers suggested that the fruit juice either renewed beta cell function or alternately permitted the recovery of partially destroyed beta cells.

In Viet Nam and India the juice of the immature small melon/gourd (very bitter taste) and the bitter melon are used as foods for traditional nutrition therapy for persons with diabetes. Bitter melon is composed of several compounds with confirmed anti-diabetic properties. Also, charantin contains steroids and is very rich in polyphenols;

when extracted by alcohol, it displays a better hypoglycemic effect than the drug tolbutamide (Welihinda et al 1982). Momordica also contains an insulin-like polypeptide, known as polypeptide-P that lowers blood sugar levels when injected subcutaneously into type 1 diabetic rats. Since it appears to have fewer side effects than insulin, it has been suggested as a replacement for some patients (Welihinda et al, 1982). In one study, the bitter principle (not the exudates) of *Aloe barbadensis* showed a highly significant hypoglycemic effect in mice that was at a maximum at 8 hours and extended over a period of 24 hours (Ajabnoor 1990).

In India, some researchers have investigated the effects of defatted fenugreek seeds on blood glucose and serum lipid profiles in type 1 diabetic patients and found significant benefits (Sharma et al 1999). When 2.5 grams were given twice a day for 3 months to Indian individuals with type II diabetes, both fasting and postprandial blood glucose levels were significantly reduced (Bordia 1997).

Other researchers have shown that the plant *Biophytum sensitivum* has significant hypoglycemic effects in rabbits, possibly due to pancreatic beta-cell stimulation (Puri and Baral, 1998). In addition, extracts of the plant *Cleome droserifolia* suppressed the rise in peripheral blood glucose concentrations in rats and lowered low-density lipoproteins (LDL), which consequently raised the HDL/LDL ratio, thus adding an antiatherogenic effect to its antidiabetic effect (Nicola,1996). In China, scientists have shown that baicalin and licorice extracts have dramatic results in rats in the reduction of aldose reductase activity that has been found to contribute to chronic diabetic complications (Zhou and Zhang, 1989). All these botanicals cited share the same characteristic of being bitter. Furthermore the bark of Anacardium used for diabetes treatment, like Moringa

leaves and seeds, also displays a bitter taste. Therefore an investigation of polyphenols (which are mainly bitter) content may be of interest.

The fatty acids. The following section reviewed how dietary fat (quantity and distribution of fatty acids) affects diabetes. In 1999, Lovejoy stated that the effect of dietary fat on insulin varies depending on its fatty acid composition and that saturated fatty acids have been consistently associated with insulin resistance whereas, medium and long-chain fatty acid intakes are associated with insulin sensitivity as are high intakes of omega (n-3) fatty acids (Lovejoy, 1999).

In human experience it has been shown that n-3 fatty acids may improve many of the metabolic impairments associated with insulin resistance by lowering blood pressure and triacylglycerol concentrations. In animals, n-3 fatty acids may cause less weight gain than other fats although they may raise low-density-lipoprotein concentrations, increase hepatic glucose output, and lower insulin in non-insulin-dependent diabetes mellitus (Berry 1997).

Giron and his research team compared the function of three diets supplemented with 5% olive oil, sunflower oil, or fish oil in streptozotocin-induced diabetic rats and responses were measured in terms of liver Δ-9 and Δ-6-desaturase enzymatic activities and levels of triglyceride production. Both enzymatic activities decreased in fish oil-fed rats. The Δ-9 desaturase activity and production increased in olive oil-fed rats and they had the lowest triglyceride levels. These findings supported the hypothesis that olive oil improves hypertriglyceridemia accompanying diabetes (Giron et al 1999).

In 2000, Ryan and colleagues stated that abnormalities in endothelial function might be associated with increased cardiovascular risk in diabetic clients. They compared the effects of an oleic–acid-rich diet versus a linoleic-acid-rich diet, on insulin resistance and endothelial-dependent vasoreactivity in type 2 diabetes patients. Although there was no difference in diabetic control, they found that the change from polyunsaturated (linoleic acid) to monounsaturated diet (oleic acid) significantly reduced insulin resistance and restored endothelium-dependent vasoreactivity in type 2 diabetes (Ryan et al, 2000). The beneficial effect of oleic acid in diabetes may be related to the oleic/linoleic ratio (O/L ratio). In studies where subjects were fed with a fat of high O/L ratio, there was a positive effect on lipoprotein profile, that is, an increase in high-density lipoprotein (HDL) without any increase of LDL (O'Bryne et al. 1997; Renaud et al. 1995). In addition when this ratio decreased, the positive effects gradually disappeared (Ochoa et al, 2002; Ochoa et al, 2001). Oils rich in n-3 fatty acids displayed the same effects but raised serum LDL. The outcomes of studies that have focused only on the oleic acid content of the oils without taking into account the O/L ratio are controversial. Some of them did not find any beneficial effect. It would be interesting to investigate the O/L ratios of oils from the cited studies that did or did not show a beneficial effect.

Another study in the food science area focused towards the modification of physical and chemical properties of vegetable oils through genetic modification of oilseed crops such as canola, soybean, corn and sunflower (Liu et al, 2002) leading to an improved oxidative stability by reducing the content of linolenic and linoleic acids and increasing the content of oleic acid. As a result of raising the O/L ratios, oils can have increased storage and frying stability. Only a few studies have assessed the fatty acid

profiles in Moringa seeds. Thus fatty acids profiling in Moringa seeds was of great interest in order to compare its O/L ratios with olive oil and high oleic peanut oil.

In their study Meyer and his colleagues collected data supporting an inverse relationship between incidence of type II diabetes and vegetable oil (after adjusting for age, smoking, alcohol consumption, Body Mass Index (BMI), waist-to-hip ratio, physical activities, demographic factors, dietary magnesium and cereal fiber). A similar response was obtained when polyunsaturated fatty acids (PUFA) were substituted for saturated fatty acids and cholesterol. Dietary assessment was achieved using a food frequency questionnaire at baseline in an 11-year follow-up cohort study involving 35,988 non-diabetic older women. 1,890 incident cases of diabetes occurred during the period of the study (Meyer et al 2001).

Other studies have shown that both human and experimental diabetes are associated with a substantial disturbance in essential fatty acid (EFA) metabolism (Keen et al 1993). As a result, a variety of microvascular disturbances are produced which ultimately lead to reduced blood flow and neuronal hypoxia. Endoneuronal hypoxia impairs axonal transport, produces demyelination, and reduces neural ATPase activity. One of the key disturbances was due to the impairment in the process of converting linoleic acid to gamma linolenic acid (GLA).

GLA represents one of the two main families of essential fatty acids. Specifically, GLA is an omega-6 fatty acid and the other important family is the omega-3 fatty acids. The body uses essential fatty acids to make various prostaglandins and leukotrienes. These substances influence inflammation and pain; some of them increase symptoms, while others decrease them. Taking GLA may swing the balance over to the more

favorable prostaglandins and leukotrienes, making it helpful for diseases that involve inflammation. GLA is widely used in Europe to treat diabetic neuropathy and eczema.

In their study Reginato and colleagues reported that fat differentiation was a critical aspect of obesity and diabetes and that dietary fatty acids were converted to arachidonic acid, which serves as precursor of prostaglandins (PGs). One of the PGs, namely PGF_2 blocked adipogenesis, yet other PGs' signals regulated gene expression required for adipogenesis. In conclusion, this study showed that the balance between PGF_2 and PGJ_2 signaling was central to the development of obesity and diabetes (Reginato et al, 1998).

II. SELECTED PLANT CHARACTERISICS AND TRADITIONAL USES

Moringa oleifera (or nebedaay, the Senegalese name): There are about 13 species of Moringa trees in the family Moringaceae (Price, 1985). They are native to India, the Red Sea area and some parts of Africa including Madagascar. *Moringa olifera* remains the most widely known species. Price states in *Survival and Subsistence in the Tropics* that: "Among the leafy vegetables, one stands out as particularly good, the horseradish tree (Moringa). The leaves are an outstanding source of vitamin A and when raw, vitamin C. They are a good source of B vitamins and among the best plant sources of minerals".

The Moringa tree is well known within West Africa. Different ethnic groups in this area use similar names derived from the English expression " never die'' characterizing its high resistance to drought and semi arid conditions. The Wolof ethnic groups call it "nebedaay'', whereas the Mandingo have it as "nenedayo", and for the

Fulani, the term "neboday" is used (*Pharmacopee Senegalaise Traditionnelle.* Published in Paris, France 1970),

Habitat. Moringa originated from India and Arabia and is widely cultivated and adapted in many countries of the tropics. It is a slender tree with drooping leaves and grows to about 10 meters tall (Palada, 1996). Moringa grows in ecologies ranging from subtropical dry to moist. The tree is reported to tolerate annual precipitations of 760 to 2250 mm, annual temperatures of 18.7 to 28.5 Celsius and soil pH of 4.5 to 8 (Duke 1978, Palada 1996).

In *Pharmacopee Senegalaise Traditionnelle* (Published in Paris, France 1970), Kerharo explains that the Moringa tree (root, leaves and bark) is very valuable and is most used in the Senegalese folk medicine. Its leaves are also used for sauce preparation. In his book he wrote that in 1968, Sarfraz from Pakistan did a study on the local species showing a hypoglycemic effect in dogs (*Pharmacopee Senegalaise Traditionnelle.* Published in Paris, France 1970).

Although the leaves are processed to remove their bitter taste for preparation of couscous sauce, traditional healers use the leaves raw for diabetes treatment. Seeds of Moringa are preferred by some traditional healers to treat their patients; these are dosed as one seed in the morning and another in the night just before meals. Seeds display a deep bitter aftertaste as well.

Different Uses. According to Kerharo and Adam, the multipurpose Moringa is one of the most famous botanicals used in Senegalese traditional medicine. In addition to its medicinal value it also has agronomic, horticultural, nutritional and industrial purposes (Palada, 1996). The daily use of its leaves in sauce preparation of staple dishes such as

couscous is traditional in Senegalese households (*Pharmacopee Senegalaise Traditionnelle.* Published in Paris, France 1970). Currently the Church World Service in collaboration with "Agir Autrement pour le Developpement en Afrique" (AGADA) is developing a project for women's nutritional rehabilitation with Moringa in the southern Senegal.

Medicinal uses. The most common forms of medicinal use are for the treatment of chronic malnutrition, bronchitis, and flu in stunted infants suffering from cephalic and neuralgic troubles. There are many medicinal preparations from Moringa to treat various conditions including: fresh leaf extracts as eye drops, dried ground leaves dispensed in the nostrils, drinks or skin ointments from leaves, roots or bark extracts. In adult persons, Moringa is used for the treatment of edemas, arthritis, and rheumatism. The gum extracted from an incision of the bark is also used for medicinal purposes.

Antitumor effects. Three known thiocarbamate (TC) and isothiocyanate (ITC)-related compounds have been found and isolated from the leaves of Moringa. All of them displayed anti-tumor effects. One of the TC compounds, niaziminin (sic niazimicin) inhibited Epstein-Barr virus (EBV) activation in Raji cells, when compared to 10 other TCs including 8 synthetic ones (Murakami et al, 1998). Moreover, the naturally occurring 4-[(4′-O-acetyl-alpha-L-rhamnosyloxy) benzyl] ITC, and both commercially available allyl and benzyl ITC compounds depressed activation suggesting that the isothiocyano group plays a key role for antitumor activity.

Another study that focused on Moringa seeds isolated several compounds including a benzyl ITC, the same TC niazimicin mentioned above, and two different sisterols. These four compounds were tested against Epstein-Barr Virus-early antigen

(EBV-EA) activation in Raji cells, induced by the tumor promoter, 12-O-tetradecanoyl-phorbol-13-acetate (TPA). All compounds showed anti-tumor effects, with the benzyl ITC, and the niazimicin displaying very significant activities. Niazimicin was also tested in vivo and found to have potent antitumor promoting activity. Niazimicin was thus proposed to be a potent chemo-preventive agent in chemical carcinogenesis (Guevara et al, 1999).

A third study to test the radioprotective effect of Moringa leaves was conducted (Rao et al, 2001). A first group of Swiss albino mice was pre-treated with single doses of 150 mg/kg of methanolic extracts (ME) one hour before their whole body was irradiated (RT, 4 Gy) and a second irradiated group was treated with five daily doses of 30 mg/kg each. Bone marrow protection was evaluated by scoring aberrations in metaphase chromosomes and micronucleus induction in polychromatic erythrocytes and normochromatic erythrocytes. Pretreatment with a single dose of 150 mg ME /kg body weight significantly reduced the percent aberrant cells by two-thirds on day one and brought the values to normal range by day 7 post-irradiation. Thus pretreatment with methanolic leaf extract of Moringa significantly protected bone marrow chromosomes in mice against lethal whole body irradiation.

Hypocholesterolemic effects. Ghasi and his colleagues reported that Indians use leaves of Moringa in their herbal medicine to lower cholesterol in obese patients (Ghasi et al, 2000). In their study, they showed that when crude leaf extract was given to Wistar rats fed with high-fat diet, serum liver and kidney cholesterol decreased significantly.

Other medicinal effects. Methanolic extract (ME) of Moringa root was shown to potentiate sleeping time and to have analgesic properties. Pretreatment with ME also led

to significant protection against strychnine and leptazol-induced convulsions suggesting the central nervous system (CNS) depressant nature of ME (Gupta et al, 1999). A high dose of methanolic root crude extract was also found to increase WBC count and to significantly decrease blood-clotting time (Mazumder et al, 1999).

Nutritional aspects. Moringa leaves are widely used as food throughout the world, especially in Asia and Africa (Palada 1996; Lockett et al 2000).

Vitamin A content. A study was conducted on male albino rats to test β-carotene bioavailability from fresh and dehydrated drumstick leaves of Moringa. The rats were given a vitamin A deficient diet for four weeks. During this period six rats died. The remaining rats were divided into three groups; the first group was repleted with vitamin A in the form of vitamin A acetate, the second group was repleted with the drumstick leaves and the third was fed with the vitamin A deficient diet. After 4 weeks, not only serum and liver vitamin A returned to normal ranges for groups 1 and 2, but they also recovered normal weights when compared to the control groups. For group 3, weight, serum and liver vitamin A decreased drastically. The gain in body weight was highest for the group fed with the leaves although serum vitamin A was slightly lower when compared to the group fed with the synthetic vitamin A. The authors concluded that in developing countries drumstick leaves are valuable in overcoming the problem of vitamin A deficiency (Nambiar and Seshadry, 2001).

Digitaria exilis (fonio) is a variety of millet mainly grown in West Africa, consisting of tiny kernels with a size approximating 700 μm of diameter. According to

the geographic location, it is given different names such as "Acha" or "Fundi"; its English common name is "hungry rice" (Sartelet et al, 1996).

Habitat. According to the National Academy of Science (1996), Fonio is probably the oldest African cereal cultivated across the arid savannas. This cereal in fact was used as the major staple food. Nowadays the crop still remains important in areas scattered from Cape Verde to Lake Chad. In certain rural regions of Mali, Burkina Faso, Guinea and Nigeria, it is still heavily consumed. Compared to the other cereals, fonio has received little attention due to the fact that scientists and other decision makers have misunderstood the plant. This neglect, characterized by few publications, was brought about by English colonialists who knew little about the cereal and called it "hungry rice", a misleading term. In fact local populations harvested fonio not because of hunger but because they liked its taste (National Academy of Sciences, 1996); and because of its ability to withstand infertile soils and low rainfalls in the Sahelian regions (Sartelet et al, 1996). In some places fonio is particularly reserved for chiefs, royalty and special occasions such as ancestor worship. Harlan (1993) reported that according to the Dogon tribe in Mali, the whole universe emerged from a seed of fonio.

Harlan made the following statement on fonio: "First, let me call attention to some of the other cereals, both wild and tame. Fonio or acha, *Digitaria exilis*, has been given the misnomer "hungry rice" by English colonials. It is not grown to relieve hunger but because of its quality. It is a chief's food, a gourmet item, and couscous made of fonio is better than couscous made from wheat''.

Biochemical properties. Fonio is reported to be different from other cereals for containing important concentrations of methionine, cysteine (sulfur-containing amino

acids) and leucine. The concentrations of the three amino acids in fonio are higher than those defining the FAO reference protein (Sartelet et al, 1996; Thompson, 1993; Becker, 1986).

Also, fonio has lower flavonoid concentrations when compared to the other cereals with 500 mg/kg against 1 to 1.2 g /kg from *Pennisetum millet* and *Pennisetum americanum*. But in the latter cereals, the types of flavonoids are composed of C-glucosyl derivatives (less potent in antithyroid peroxidase activities), so fonio once again differs from those species by containing two aglycones (150 mg/Kg of apigenin and 350 mg/Kg of luteolin) that are approximately seven times more potent than C-glucosyl derivatives (Sartelet et al, 1996). This feature implies that the lower content of flavonoids in fonio is compensated by the presence of the most active ones. But, contrary to other millet species, fonio did not contain thioglucosides or cyanogenic glucosides that are transformed after hydrolysis or bioconversion into thiocyanates known to be goitrogenic compounds (Van Etten and Wolf, 1973; Delange and Ermans, 1976).

In addition, Sartelet and his colleagues showed that luteolin from fonio showed a regulatory effect on thyroid functions of pig thyroid cells. First, they demonstrated that both luteolin and apigenin had inhibitory effects on thyroid hormone production by impairing thyroid peroxidase activity; however, luteolin displayed a highly stimulatory effect on thyrotropes on day 4 of treatment, through deactivation of cAMP phosphodiesterase, thus allowing a highly significant overproduction of the second messenger cAMP nucleotide that is dependent on the thyrotropin-stimulating hormone (TSH) action on thyrotropes. But the net result of these opposing effects was slightly goitrogenic. They concluded that further studies should be conducted to better elucidate

the role, function and mechanism of action of the *fonio* aglycones on thyroid hormones (Sartelet et al, 1996).

Nutritional aspects. The proximate composition of white fonio is comparable to wheat with the husked grain containing 8 percent protein and 1 percent fat. In some black fonio samples, the protein content was 11.8 (National Academy of Sciences, 1996).

Becker and later on Thompson showed that the difference compared with the other cereals was the amount of the essential amino acids, especially the sulfur containing ones: approximately 7.3-8.0 % methionine plus cystine is found in the fonio protein. The amino acid profile compared to that of whole-egg protein showed that except for the low score for lysine of 46%, the other scores were high: 72 for isoleucine, almost 100 for valine, tryptophan, threonine and phenylalanine; 127 for leucine; 175 for total sulfur and 189 percent for methionine (Thompson, 1993; Becker, 1986). These figures show the important potential of fonio, not in terms of survival crop but as a standard diet (Thompson, 1993; Becker, 1986). Moreover, as previously mentioned, sulfur plays an important role in diabetes treatment through compounds such as sulfonylureas drugs.

Anacardium occidentale. A member of the family, *Anacardiaceae* is an important crop of the tropical regions of Africa and South America. Its nuts are commercialized world wide for their taste and nutritional components. Its leaves, bark and roots have long been used in folk medicine (Price, 1985; Kerharo and Adam, 1974).

Habitat. Anacardium originated in the *cerrados* of central Brazil and was seemingly brought to East Africa and India by the Portuguese in the sixteen century (Price, 1985).

Medical properties. Crude extracts of Anacardium usually used for the treatment of infectious and septic diseases in both humans and animals were screened in vitro for antibacterial activity. The plant was found to exert good antibacterial activity against two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* (Kudi et al, 1999).

Infusions of the bark and leaves are used to relieve toothache and sore gums while the young leaves are used for the treatment of dysentery, diarrhea and piles and the stem bark is used for pellagra treatment (Akinpelu, 2001)

Most important, anacardium was shown to play a protective role against experimental diabetes in streptozotocin (STZ)-induced diabetic rats. The rats were fed with 175 mg of the aqueous extract of the bark per kg body mass twice a day, beginning two days before STZ injection. Three days after the STZ injection, a 48%-increase of blood glucose over normal was recorded in rats pretreated with the aqueous extract of Anacardium compared with a 208%-increase in diabetic rats treated with STZ alone. In addition, the pretreated group presented no glycosuria, a normal weight gain and a non significant increase in food and fluid intake at the end of the treatment contrary to the

STZ-alone group which displayed glycosuria, polyphasia, polydypsia and body weight loss (Kamtchouing et al, 1998).

Sclerocarya birrea. Another member of the Anacardiaceae family is a plant widely used in Africa for various purposes. It is a medium sized single stemmed tree of up to 15 m in height. The rough flaky stem-bark is brownish with a mottled appearance. The leaves divided into 10 or more pairs of leaflets, are much paler below, with their tips narrowing to a sharp point. The flowers are small with red sepals and yellow petals. The large, rounded slightly flattened fruits of about 30 mm in diameter are born in profusion in late summer to mid winter. The nut-containing fruits have a delicious taste and a high content of vitamin C. Fruits are nowadays being commercialized (Ojewole, 2003).

Habitat. Sclerocarya birrea, with various folk names including marula, or "cider tree", beer (in Senegal), kokwaru, or umganu (Zulu name) depending on the geographical location, is widely spread throughout the African region.

Medicinal uses. The stem, bark, roots and leaves are widely used in African traditional medicine against many diseases and afflictions such as hypertension, dysentery, stomachache or gastroenteritis. Belemtougri et al suggested that the bark and leaves are also used against high glycemic conditions. In their study on calcium signaling in cultured rat skeletal muscle cells, they showed that the different extracts (crude decoction, aqueous, ethanolic, and chloroformic) of the leaves displayed significant antagonistic effect on caffeine –induced calcium release from sarcoplasmic reticulun (Belemtougri et al, 2001).

Also, Eloff showed that acetonic extracts (from 0.15 to 3 mg/ml) of the bark and leaves were very efficient (with a higher potency for the bark) against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Enterococcus faecalis* (Eloff, 2001). Another study on Wistar rats weighing 250 to 300 g used the aqueous and methanolic extracts of *Sclerocarya birrea* stem-bark (injection of 500 mg / kg) to assess the anti-inflammatory effect on rat paw edema induced by sub-plantar injections of fresh egg albumin (0.5 ml /kg). Both extracts reduced progressively edema with the methanolic one displaying a stronger effect (Ojewole, 2003).

CHAPTER III

MATERIALS AND METHODS

Samples preparation. Due to budget restriction, as previously said, samples of fonio and the selected botanicals were purchased in three markets of the capital Dakar where diabetic clients regularly supply themselves. But the investigator did not get samples from the remaining eight regions.

Samples were not stored into liquid nitrogen during transport and flight to the USA. We did not weigh the samples immediately after we purchased them in the markets because good scales were not available, thus accurate water contents from the materials could not be determined. Samples were sold at the open air. For conservation purposes, samples are sun-dried by merchants for days depending on the intensity of the sunrays before taking them to the markets. In spite of these facts, all samples were weighed in the NSCI laboratory just after arrival at Oklahoma and stored in the freezer. All samples were allowed to dry in the oven at 100 °C for 24 hours before analysis, except for the fonio samples reserved for analysis of fibers and beta-glucan. Therefore apart from beta-glucan and fibers, all results obtained in the following determinations were reported on a dry weigh basis. All samples were ground before analyses using the Cyclotec, a Waring blender or porcelain mortar when minerals were being determined.

Crude Fiber Determination. The procedure is essentially based upon the Weende method: an acidic hydrolysis (150 ml of a 0.128 M sulfuric acid solution) followed by an alkaline hydrolysis (150 ml of a 0.223 M potassium hydroxide or sodium hydroxide

solution) are performed on the ground sample. The obtained extract, rinsed with hot water, ethyl alcohol and acetone, is dried and ashed. The difference between the dried extract and the ash content represent the crude fiber. Crude fiber was determined using the Fibertec system E from Tecator (Tecator AB, Hoganas, Sweden) consisting of a Hot Extraction Unit 1010, a Cold Extraction Unit 1011 and accessories from the NSCI laboratory. The vacuum system for the Cold Extraction unit was not operating. Thus we modified the installation by incorporating a vacuum pump and a trap to solve the problem. A Cyclotec mill from the Horticulture and Landscape Architecture Department was used to grind the barks and Moringa leaves.

This method had already been compared to the classical methods of Weende and Wijkstrom, two almost identical procedures with the sole difference residing in the extraction times (10 minutes less in the Wijkstrom). The correlation coefficients were excellent: Weende/Wijkstrom, Fibertec, $r = 0.9995$ in Tecator laboratories and the comparison laboratories analyses gave $r = 0.9993$ as well as a very small standard deviation on the y value (0.27 and 0.48 respectively). The results showed a slightly better reproducibility in the Fibertec system.

The Fibertec system E with several units integrated was faster and more convenient than the methods (Weende and Wijkstrom) that the investigator had used previously in Senegal. The only constraints (the same encountered with the classical methods) were the preparation of the filtration crucibles that are critical for obtaining good results. After a fiber determination, residues stick in the porosities of the crucibles and simple cleaning as usually handled in the laboratory for glassware is not enough to get rid of the material retained in the porosities. In this case, the tare (T) of the crucibles

weighed before analysis is false due to an increase of a small increment (δT) brought about by the presence of the retained material. Thus the tare that the analyst records in the laboratory book is False Tare (Ft) = T + δT. Running the analysis involves the acidic and alkaline hydrolyses of the relatively low sample amount (1 gram) that leaves only a few milligrams of fibers (Sr) after filtration and washing procedures. Note that the retained material (δT) in the porosities can be the same or even higher than the weight (Sr) of the true sample residue on the bed of the crucible after analysis. When running the analysis, the passage of a hot strong acid, a hot strong base, alcohol and acetone would eventually wash out the residual matter in the porosities of the crucible. After the analysis is completed, the weight of the tare + the sample residue is taken and recorded; but instead of having Ft + Sr, you have T+ Sr for δT was lost. Then when computing your residue percent in your sample after analysis, you actually have the following equation:

% Residue = (T + Sr) – Ft (recorded in lab book before analysis) / sample wt x 100 or Ft = T + δT, thus if δT is equal to Sr, you end up with a 0 % fiber and if δT is higher than Sr you end up with a negative value.

This aspect of fiber determination has led many laboratories to avoid fiber determinations in relatively low fiber materials or when they do, they end up with a fiber content that they approximate to zero.

In order to avoid this problem, it was necessary to run the whole procedure with the empty crucibles. They were put in the muffle furnace at 500°C for 4 hours and inserted in the Fibertec system, under hot base and hot acid for 30 min each. Then they were rinsed with alcohol and acetone. After being dried, the weights were recorded and the same operations were repeated until the weights of the empty crucibles remained

constant and were recorded as the true tare (T). Good results with high reproducibility were obtained. The detailed procedure is in Appendix A1.

Total Dietary Fibers Determination. The method used for total dietary fiber was the Fibertec System E designed by Tecator Company and based on the Association of Analytical Communities (AOAC) method (1984) for total dietary fiber determination. The AOAC committee based their method on Burkitt andTrowell's definition of dietary fiber, "Dietary fiber is the remnants of plant cells resistant to the alimentary enzymes of man" (Burkitt andTrowell, 1976).

The principle is based on the use of termamyl (amylotic), protease (proteolytic) and amyloglucosidase (amylotic) as the active enzymes thus matching the metabolic pathways occurring in the gastrointestinal tract when foods are ingested. The enzymes are incubated with samples and the resulting insoluble extracts plus the matter obtained after precipitation with ethyl alcohol from the soluble portion, constitute the total dietary fibers. Values obtained from the Fibertec system and the AOAC methods are highly comparable. In order to verify the precision and reliability of the Fibertec System E, Tecator used similar samples that were investigated in the AOAC collaborative study of 1982. There was no significant difference between AOAC and Fibertec system E values. The reproducibility of the Fibertec readings was very good (SD<1%). The detailed methodology is in Appendix C.

That method worked very well for moderately protein-rich or moderately starch-rich samples but when samples were high in starch or protein, they ended up with high coefficients of variation (CV = 64.15%). This problem was thought to derive from the

fact that termamyl's activity reaches 70% at 70°C. Since the time allotted by the AOAC committee for digestion was only 15 min, reaction of the sample with the alpha amylase was stopped by the time the temperature reached 70°C, leading to an incomplete digestion of the starch-containing sample. The Fibertec group prolonged incubation time to ensure complete starch digestion. For high-protein samples, they reported the protein contents using the Kjeldahl method that first determined the nitrogen content of samples residues multiplied by the conversion factor 6.25.

We did not run the protein content of the residues for our samples for several reasons. First, our samples were relatively low in protein content (analyses run by Langston University). When we referred to comparative analyses of dietary fiber from fruits, greens and cereals by Miguel and Belloso (1999), the resistant protein of cereals was under the limit of detection. Second, in her determination and validation of fiber analysis, Garbelotti and her colleagues did not include the determination of the resistant proteins (Garbelotti et al, 2003). Third, after Trowell referred to dietary fiber in 1976 as the gross material resistant to human enzymes, the New Zealand Food Regulations confirmed that definition in1984, by defining dietary fiber as the "edible plant material not hydrolysed by the endogenous enzymes of the human digestive tract"; this edible plant material not hydrolyzed was measured by the method of Prosky et al (1985) accepted by AOAC method 985.29 (National Academy of Sciences, 2003). In 1987, the Food and Drug Administration (FDA) of the USA adopted the AOAC 985.29 method for regulatory purposes, stating that dietary fibers were a mixture of non-starch (National Academy of Sciences, 2003) polysaccharides, lignins and some resistant starch. And recently in 2000 the definition was extended and dietary fiber was referred to as that

fraction of the edible parts of plants of their extracts or analogous carbohydrates that are resistant to digestion and absorption in the human intestine, usually with complete or partial fermentation in the large intestine. They include polysaccharides, oligosacharides and lignins (although lignins are non carbohydrate compounds) [National Academy of Sciences, 2003].

In the NSCI laboratory, some modifications of the method were made. The heat-resistant amylase (termamyl) was not available; so another alpha amylase with an activity peaking at 20°C was used. Thus the first step of the Fibertec system E consisting of heating the sample mixture to 100°C with water together with the termamyl for 30 min, was replaced with a step with one from the Nils rapid enzymatic assay (Nils et al, 1983). The sample was first heated with water for gelatinization at 100°C for 30 min and allowed to cool down at room temperature. Then the alpha amylase was put in the sample for digestion for 30 min. The other steps were those from the Fibertec system E. When these changes were made, very good coefficients of variation were obtained for all samples with a very good reproducibility. The detailed operations are in Appendix A2.

Fatty Acids Extraction and Analysis. Moringa seeds oils were extracted and analyzed for fatty acids profile by gas chromatography following the method for quantitative extraction of pecan oil, which has been developed in the Horticulture laboratory at Oklahoma State University (Maness et al, 1995). This method was different from the classical procedure as the combination of BF_3 and methanol was not used to cleave fatty acids from the glycerol backbone before insertion of a methyl group (methanolysis). Instead, methanolic HCl was used for methanolysis and methyl alcohol

served to scavenge water molecules. BF_3 is more hazardous than the reagents in our protocol as it is derived from a weak base [B^{3+}] and a very strong acid [F^-] and almost behaves as HF, which is very corrosive and dangerous to handle (AOACS Official Method, Ce 2-66, 1997).

Another change was that tertiary butanol was added to co-evaporate HCl. Other methods use a chromatographic method to elute the obtained fatty acid methyl esters (FAME) on silica gel in order to get rid of the excess HCl or BF_3. Those procedures are time consuming, expensive and need particular attention from the researcher. Our method displayed a good recovery rate of 70% and we obtained sharp peaks on the same baseline with clear separation of the different fatty acids. None of the six samples or the standards showed a broad peak which occurs when different fatty acids with the same number of carbons do not totally separate from each other (almost eluting at the same time). In addition, the retention times recorded for the samples matched exactly those of the standards without any deviation.

Sample preparation. Thirty Moringa seeds (more seeds would have given a better estimate, unfortunately we were short of samples) were dehulled and weighed to determine the average weight of a seed. The average weight was 210.4 mg with a standard deviation of 36.7. Then seeds were sliced with blades and ground for one min with a Waring blender (Waring, New Hartford, CT). The obtained seed flour was sealed in glass jars, blanketed with nitrogen and stored in a dessicator at -20°C awaiting extraction. A one-minute grinding duration of nuts or seeds was sufficient to reduce more than 90% of the sample to particles sizes of 0.3 mm or smaller, with the remainder present as larger (mostly > 1mm) pieces. The remaining larger pieces were re-ground for

10 seconds after detaching the powdered samples from the walls of the blender. This operation was repeated until all samples were ground to desired size. Then oil extraction and oil recovery were performed (organic solvent extraction) by preparing 6 samples (ground) of 100 mg each in 5 mls vials and 6 samples of 100 mg spiked with 5 mg canola oil. Ether (2 ml) was added to each vial and the vials were stirred with small magnetic rods (5mm long). A 20 min extraction was performed 4 times and supernatants were extracted. The collected supernatants were allowed to dry overnight *in vacuo* with a Speed Vac Concentrator equipped with an ultralow sample condenser and an organic vapor trap (Savant Inc., Farmingdale, NY).

Oil % was calculated using the following equation:

[(Oil extract + vial) – vial tare] x 100]/ sample weight

Recovery percent was obtained using the following equation:

% recovery = {(sample weight + spike - plain sample weight)/spike weight} x 100

Fatty acids methyl ester (FAME) preparation and analysis. From previous analyses run in the Horticulture laboratory, the elution times and spectra of the following seven fatty acids myristic, palmitic, heptadecanoic, stearic, oleic, linoleic, linolenic were already known. For the lipid profile of the Moringa seed oil, three more fatty acids (behenic, arachidic, and lignoceric) were added to the previous list. In order to avoid any confusion, each of the new fatty acids was run individually together with the seven already known, displaying a new peak with specific shape and elution time. After the characteristics of behenic, arachidic, and lignoceric acids were detected individually, the ten standards (from Sigma Chemical Co., St Louis, MO) were mixed together and run

and the spectrum obtained was used as reference peaks. The following section describes the procedure that was used to prepare standards and samples for analysis.

A 5 mg spike of heptadecanoic acid (HDA) as internal standard was added to the vials containing the fatty acid standards and samples. The amount of HDA was adjusted to the weight of the eluates (600 nanomoles HDA/mg eluate). Two hundred μL of methanolic HCl (3% HCl in methanol, prepared by adding 0.5 ml of acetic anhydride to 10 ml methanol) and 50μL methyl acetate were added and vials were sealed with teflon lined caps and allowed to incubate for 2 hours at 90°C in a heating block. Vial contents were stirred with a Vortex mixer during the first 15 min of the incubation period to assure equilibrium into a single phase for methanolysis. After incubation vials were allowed to cool at room temperature and 10 drops of tertiary butanol were added to co-evaporate the HCl. Samples were dried under nitrogen gas. Fatty acid methyl esters were solubilized with 700 μL hexane and a 1 μL aliquot was utilized for gas chromatography.

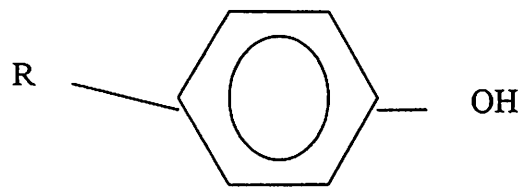
Gas chromatography. A Tracor model 540 gas chromatograph (Tracor Instruments, Austin, TX), equipped with a split injection port (split ratio of 50:1) and flame ionization detector was used. Separations were performed using a DB 23 fused silica capillary column (30 m x 0.25 μm film thickness, J. and W. Scientific Inc., Rancho Cordova. CA) with helium as carrier gas at a linear flow rate of 20 cm/second. The injector temperature was set at 275 °C and the detector temperature was at 300 °C. The initial column temperature was 50 °C for 2 min. Fatty methyl esters separation was performed using a linear temperature program from 50 °C to 180 °C at 10°C/ min, a hold at 180 °C for 5 min, and a second linear temperature program from 180 °C to 240 °C at 5°C/min and a hold at 240 °C for a final 5 min period. Individual FAME peaks were

identified according to co-elution with an authentic standard (FAME preparation 2; Sigma Chemicals Co., St Louis, MO). Peak areas were obtained using a spectra 4270 integrator (Spectra- Physics Inc., San Jose, CA) and quantified against the internal standard.

Determination of Total Polyphenols. Total polyphenols were determined by UV-Vis spectrophotometry, following the procedure described by Capannesi et al (2000) with a few modifications. We added a centrifugation step before filtration of the samples. The nature and texture of our samples made it difficult to filter them directly because they clogged the filtering paper before all the solvent percolated through the filtering paper. The centrifugation step made the filtration easier and faster and practically allowed a total recovery of the filtrates. A second change was brought in concerning the number of extractions. The Capennesi method suggests three extractions; instead our samples were extracted four times, because in chemistry if a solute is indefinitely soluble in solvent, in each extraction 90% of the compounds are recovered (Personal communication with Dr Maness, 2002). Thus we considered that three extractions would extract only 99.9 % of the compound, whereas four extractions, we would theoretically yield 99.99%.

Aqueous extracts of samples and gallic acid standard solutions were allowed to react with the Folin-Ciocalteau reagent (Sigma, St Louis, MO) until full development of a complex giving a specific coloration and a wavelength characteristic of polyphenols. The Folin-Ciocalteau reagent reacts specifically with the phenolic group generating a blue to violet complex at the wavelength of 765 nm. To date, there are approximately 8000 different types of polyphenols (Bravo, 1998), but all of them have in common the phenolic group ϕ-OH. As its structure displays it, the group is polar and thus can be

extracted by polar solvents such as water or alcohols. In addition all chromophores capable of combining with the ϕ-OH group to yield a colored (or uncolored) complex at a wavelength specific to the chemical group can be used as a means of determining total polyphenols. If all polyphenols contain one or more ϕ-OH groups, they are different because of their side chains, which can be composed of other chemical groups (for instance the phenolic compound gallic acid has in addition to the ϕ-OH group, a carboxylic group). In order to separate polyphenols in their different families, the use of high performance liquid chromatography (HPLC) with a diode array detector is needed. For the purpose of this thesis, we focused only on total polyphenols.



General structure of a phenol with the specific group ϕ-OH and its side chain R.

Preparation of calibration curve. A 1 mg/ml stock solution of gallic acid as a reference for polyphenols was prepared and standard solutions ranging from 0.04 to 1 mg/ml were obtained by dilution of the stock solution, subsequently1 ml of each standard solution was dispensed in a 50 ml volumetric tube and 6 ml of methanol was added, followed by 2.5 ml of the Folin-Ciocalteau reagent and 5 ml of 7.5 % Na_2CO_3; and the volume was completed to 50 ml with Millipore water. Standards solutions were then

stored overnight to allow full color development and the spectrophotometric analysis was performed at $\lambda = 765$ nm.

Determination of polyphenols in samples. Approximately 0.5 g of dried and finely ground sample was extracted four times by 10 min centrifugation (4000 rpm) with 2.5 ml of methanolic solution CH_3OH: H_2O 80:20 v/v. Before each extraction, the obtained suspension was vigorously vortexed for 1 min. Next, 2.5 ml of Folin-Ciocalteau reagent and 5 ml of 7.5 % Na_2CO_3 solution were added to the extract in a 50 ml volumetric flask and final volume was reached with purified water. Samples were stored overnight and the spectrophotometric analysis was performed at the wavelength $\lambda = 765$ nm.

Determination of β-glucans in Soluble Dietary Fibers. β-glucans are distributed in both soluble and insoluble dietary fibers, and it is assumed that the more soluble, the more physiologically effective (Manzi & Pizzoferrato, 1999). Thus, we decided to assess solely the β-glucans contained in the soluble dietary fibers of the selected botanicals. For that purpose, three analytical procedures were combined: the total dietary fibers protocol by the Fibertec System was followed up to step 6 (amyloglucosidase reaction), and filtration was performed on the obtained total dietary fibers, allowing for collection of the soluble fraction in the filtrate. Then the soluble dietary fibers in the filtrate were precipitated with ethyl alcohol and their β-glucans content was assessed (on the soluble dietary fractions) using the MC Cleary method (AOAC Method 995.16) with the modifications and advice from Manzi and colleagues (1999). The changes by Manzi and colleagues included different enzyme concentrations, reaction times and the addition of lichenase enzyme. Since lichenase was available from Megazyme in Ireland, we used

cellulase that we purchased in the USA. Both enzymes are specialized for the cleavage of β (1-4) (1-6) mixed linkages abundant in certain vegetables other than cereals that are rich in β (1-3) (1-4) mixed links cleaved by β-glucosidase. Failure to use cellulase or lichenase would affect negatively the β-glucans content in non-cereal vegetables. And since three of our samples were non-cereal plants, it was necessary to add this step as advised by Mc Cleary and Holmes (1998) and Manzi and Pizzoferrato (1999). Finally, glucose obtained from the action of the different enzymes was determined using the clinical analyzer in the NSCI laboratory.

The beta-glucan value was derived from glucose in the following equation:

Beta glucan % = glucose (162/180) x (100/ sample weight)

180 represents the molecular weight of glucose and 162, the corresponding beta-glucan from the condensation of a molecule of glucose to bind another one, resulting in the loss of one molecule of water (18gm): 180 – 18 = 162. The detailed procedure is described in Appendix A3.

Minerals Determination. Minerals were determined by atomic absorption spectrophotometry (by flame with deuterium background correction, or by the graphite furnace AAS with Zeeman background correction) using the Perkin Elmer model 5100 PC (purchased in the US) in the Department of the Nutritional Sciences at Oklahoma State University.

Sample preparation and mineralization were carefully handled following the Hill et al method (Hill et al, 1986) with some modifications. Since chromium was among the trace elements to be determined, all operations were carried out under the hood to reduce

possible contamination from the laboratory environment. Also stainless steel grinders and mixers were avoided due to their composition (presence of chromium in their matrix). Instead, porcelain mortars and pestles were used for the grinding process and mineral free gloves were worn during sample preparation, mineralization and analysis. All the glassware used in the process was acid washed and rinsed with deionized water and then Millipore water. In order to avoid hand contact, small holes were made in plastic bags containing pipette tips just allowing entry of the pipetor when acquiring a tip. During mineralization, doubled-distilled nitric acid (GFS Chemicals, Powell, OH) and Millipore water were used.

Mineralization. Triplicate (0.1 to 0.2 grams) samples and reference material (bovine liver, RF 409 B from the US Dept of Commerce, National Bureau of Standards, Washington DC) were weighed into 10-ml borosilicate glass tubes. Tubes were placed in an acid washed 250-ml beaker and allowed to stay overnight in the oven at 100 °C to obtain dry weights. Then, wet-ashing was performed by treating the dried samples with a combination of 100 µl of Millipore water and 200 µl of concentrated HNO_3, and the mixture was allowed to incubate at 100 °C on the drying block for one hour. After the hour, 50 µl of H_2O_2 was added to the tubes (still resting in the block) at 30-minute intervals during the subsequent 6 hours, and finally drying was allowed to continue overnight on the heating blocks. The whole procedure of wet-ashing was repeated until all samples became thoroughly white. In order to get complete mineralization of Moringa seeds, the two barks and to a limited extent the Moringa leaves, it was necessary to add HNO_3 three times and to repeat the ashing process (three times) at 100°C. This relatively low temperature was necessary, as it is known that higher ashing temperatures may lead

to a loss in zinc. Only fonio became completely white (endpoint of wet-ashing) after the first treatment of HNO_3 and H_2O_2.

Then the beaker containing the tubes was put into the muffle furnace for 24 hours at 375°C. The preset temperature (375°C) was reached by tuning the furnace from room temperature to 100°C, followed by increments of 10°C/min up to the desired temperature.

Dissolution and dilution. Samples were dissolved by adding 2ml of 0.5 % HNO_3 (by aliquots of 0.5 ml) and 100 µl of concentrated HNO_3 in each tube. Then tubes were capped and allowed to stand overnight on the heating block at 45°C. After the first treatment, samples did not completely dissolve and two aliquots of 100 µl of concentrated HNO_3 were further added to achieve complete dissolution. The sample solutions obtained were brought to a volume of 3 mls with Millipore water and analyzed for their minerals and trace elements concentrations with the atomic absorption spectrophotometer. The detailed parameters of the instrument during analysis of the samples are in Appendix A4.

CHAPTER IV

RESULTS AND DISCUSSION

Crude fiber. The determinations of crude fiber for *Moringa oleifera, Anacardium occidentale* and *Sclerocarya birrea* barks and leaves, were not found in the literature. However, results obtained with the Fibertec System E were highly reproducible and the standard deviations were low (**Table 1**). The crude fiber in the barks (44.98% and 24.22% for Sclerocarya and Anacardium respectively), presented the highest values as expected, followed by the Moringa leaves (9.67%), the fonio cereal (3.80%) and the Moringa seeds (2.85%).

Table 1. Crude fiber in grams per 100 grams of sample

Compound	Sample 1	Sample 2	Sample 3	Sample 4	Mean	SD
Fonio	3.85	3.77	3.90	3.69	3.80	0.08
Moringa leaves	9.66	9.46	10.02	9.55	9.67	0.21
Moringa seeds	2.86	2.90	2.79	2.85	2.85	0.04
Sclerocarya bark	44.94	45.13	45.08	44.75	44.98	0.15
Anacardium bark	24.23	23.78	24.53	24.36	24.22	0.28

The dehulled fonio, with a mean of 3.80 % in crude fiber, agreed with the results (3.40 %) found in the scientific review from the National Academy of Sciences (National Academy of Sciences, 1996). However, our results totally disagreed with those from Kansas State University that rounded the values for fiber content in fonio to zero % (Toure, 2000). The slight differences noted with the results reported by the National Academy of Sciences may be explained by the random variations within samples (dehulling rate, types of soil where the samples were grown and the varieties used) and/or random variations due to the techniques based upon to make the determinations. But the

difference with Kansas State University may have been due to the critical steps mentioned in the Materials and Methods section (crucible preparation and treatment before analysis).

The crude fiber content in fonio (3.8%) was higher than those of the other cereals found in the literature. For example, values of 2 % (sorghum), 2.3 % (maize) and 2 % (pearl millet) were reported by the National Academy of Sciences, 1996).

Dietary fibers. No values for dietary fiber for the plants analyzed were found in the literature. Following the same pattern as for crude fiber, barks had the highest values followed by leaves and then by fonio cereal. Dietary fiber was much higher than crude fiber for all samples.

Dehulled fonio flour with a mean dietary fiber of 15.12 % **(Table 2)** presented a higher concentration when compared to oat flour, a cereal praised for its fiber contents: rolled oat flour combined with some bran had dietary fiber of 13.9 %, however whole oat bran was 23.8 % (Miguel et al, 1999). Unfortunately, whole fonio bran was not determined to allow comparison with oat bran.

Table 2. Dietary fiber concentrations in grams per 100 grams of sample

	Samples						
Compound	**1**	**2**	**3**	**4**	**Mean**	**±**	**SD**
Fonio	15.11	15.14	14.89	15.32	15.12	±	0.15
Moringa leaves	62.40	63.55	63.02	61.89	62.72	±	0.62
Moringa seeds	37.68	38.05	37.80	38.18	37.93	±	0.19
Sclerocarya bark	74.86	75.00	73.20	74.40	74.37	±	0.70
Anacardium bark	59.86	60.28	61.50	60.33	60.49	±	0.61

There is a wealth of evidence showing that total dietary fiber is composed of soluble and non-soluble portions. With the Fibertec system E, the method directly obtains the total

dietary fiber by gravimetry. When the assay was being run an important observation was made during the precipitation step with 78% ethanol. We noted a flocculation indicative (qualitatively) of a soluble portion. The soluble portion may have an important biological activity in glucose metabolism and insulin action especially if it is due to the β-glucan content and butyric acid as a by-product from its fermentation (Kumar et al, 2002, Jenkins et al 2002). In her short communication, Slavin reported that the Dietary Reference Intake (DRI) for dietary fiber for adults between 19 and 50 years was 38 gm for males and 25 gm for females and over 50 years of age, it was 30 and 21 gm respectively (Slavin, 2003). A daily consumption of 200 gm (dry weight) of fonio would provide 30.2 gm corresponding to more than 80 % of males from 19-50 years of age and meeting the DRI for others.

Fatty acids.

Extraction: When lipid extraction and recovery were performed gravimetrically on Moringa oil seeds, the data shown in **Table 3** were obtained:

The mean oil content of Moringa seeds was 38.4 % and 99.5 % of the added oil (spike) was recovered attesting to the validity of the procedure. Precision was good (standard deviation = 0.4%) and our results were very similar to those from the literature (Anwar and Bhanger, 2003).

Table 3. Moringa seeds oil concentrations

	Extraction[1]							
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**Mean**	**± SD**
Moringa seed oil	38.9	38.5	38.0	38.4	38.9	37.9	38.4	± 0.4
Percent recovery of spike	102	97	99	101	100	98		

[1]All results represent the mean for four extractions, and oil content is expressed in g/100g

Gas chromatography. The concentration of oleic acid which is a monounsaturated fatty acid was very high (73.7 mol %) with a relative mole percent of 88. 8 % compared to linoleic acid (0.8 mol %), a polyunsaturated fatty acid (**table 4**).

Table 4. Fatty Acid concentration in Moringa seed oil

	Mol%			Rel Mol%		
Fatty Acid	**Mean**	**±**	**SD**	**Mean**	**±**	**SD**
Myristic (C:18:0)	0.3	±	0.04	0.3	±	0.03
Palmitic (C16:0)	8.1	±	0.03	9.7	±	0.5
Oleic (C18:1)	73.7	±	0.1	88.8	±	5.43
Linoleic (C18:2)	0.8	±	0.05	1.0	±	
Linolenic (C18:3)	0.3	±	0.03	0.3	±	0.02
Arachidic (C20:0)	9.7	±	0.04	11.7	±	0.6
Behenic (C22:0)	6.1	±	0.03	7.4	±	0.46
Lignoceric (C24:0)	1.0	±	0.02	1.2	±	0.06

NB. Stearic acid was under detection limit. Recovery rate of the fatty acids was 70%

Fatty acid profile determination in Moringa seeds was important because of their high total lipid content (38.4%) and to assess their oleic/linoleic ratio (O/L ratio) since a total of two seeds (420.8 mg) per day are given as dietary supplements to diabetic patients in Senegal and surrounding countries.

The effects of the O/L ratio on serum lipoprotein profile is an area of active research for it is thought that the higher this ratio, the less the degree of lipid peroxydation in low-density lipoprotein (LDL). Oxidation of LDL in diabetic patients is

thought to be the major cause for joints inflammation. Also, a high O/L ratio has a positive influence on the fatty acids composition of LDL (Ochoa et al 2001; Ochoa et al 2002).

In the comparative **Table 5**, Moringa oil with a value of 92 presented the highest O/L ratio followed by the high oleic peanut (25.9) and olive oil (17.8). Seppanen reviewed data that were consistent about the beneficial effects of decreasing polyunsaturated fats for the benefit of the monounsaturated oleic acid. But the optimal ratio is still unclear. Seppanen suggested that a ratio of 1:2:6 for α-linolenic/linoleic/oleic could be a starting point, since it allows improved metabolism of both n-3 and n-6 PUFA. But he mentioned that the proportion of linolenic acid was critical, and should be lower than 25% since that amount would have a strong suppressing effect on the formation of long-chain n-3 PUFA (Seppanen et al, 2002). Moringa displayed a α-linolenic/linoleic/oleic ratio of approximately 1:3:245 (0.3, 0.6 and 73.7 moles percent for linolenic, linoleic, and oleic acids respectively).

The O/L ratio for Moringa oil could be a reason for its success for diabetes treatment in Senegal. The high oleic content may repress oxidation of the LDL residues in diabetic patients by raising their O/L ratio gradually and progressively (just one seed in the morning and one in the afternoon) to an acceptable range. If a seed weighs an average of 210.4 mg with a fat content of 38.4 % and a 70% recovery from the analysis by the GC, an estimate of total fatty acid content in a seed is 210.4 mg x 38.4/100 x 70/100 = 56. 6 mg fatty acid available per seed. Therefore the consumption of two seeds is equivalent to a daily intake of 56.6 mg x 2 = 113.2 mg of which 73.7% consists of oleic acid or 83.4 mg and 0.8% of linoleic or 0.9 mg.

Table 5. Normal and high oleic peanuts, olive and Moringa seed oils.

Fatty Acid	Normal peanut oil	High oleic peanut oil	Olive oil	Moringa oil
Palmitic C16:0 (Mol%)	12.1	8	9.2	8.1
Stearic C18:0 (Mol%)	1.7	0.0	3.4	0.0
Oleic C18: 1 (Mol%	47.4	88.8	80.4	73.7
linoleic C18:2 (Mol%)	38.8	3.4	4.5	0.8
O/L ratio	1.2	25.9	17.8	92.1

1 Olive oil values are from Seppanen et al, 2002; peanuts and Moringa results are from investigator.

These characteristics perhaps may explain why currently there is a shift from dried powdered Moringa leaves to the consumption of seeds as a treatment for diabetes in Senegal. Furthermore, we noted that the price of the seed had increased 10 fold between summer 2001 and summer 2002.

Polyphenols. Total polyphenol values for our samples were not found in the scientific literature, but the analyzed fonio with a mean value of 57.2 mg/100 gm (**Table 6**) was comparable to wheat (22-40 mg/100gm). Barley values were the highest ranging from 1200- 1500 mg/100gm, followed by millet with a range of 590-1060; oats and rice were as low as 8.7 and 8.6 mg respectively (Bravo, 1998).

Table 6. Polyphenol concentrations in samples

	Sample[1]					
Compound	**1**	**2**	**3**	**Mean**	**±**	**SD**
Fonio	57	56	59	57	±	1.2
Moringa leaves	773	803	798	791	±	13.3
Moringa seeds	54	50	50	51	±	2.1
Sclerocarya bark	650	611.	607	623	±	19.7
Anacardium bark	804	769	728	767	±	30.9

[1]All values are expressed in milligrams per 100 grams of sample.

We selected the Capennesi procedure because it used gallic acid as standard. Based on the literature review, we noted that among the more than 8000 phenolic structures known, tannins were highly present in cereals. Tannins consist of gallic acid and the dimeric condensation product, hexahydroxydiphenic acid, esterified to a polyol that is mainly glucose (Bravo, 1998).

Thus we inferred that gallic acid structure and chemistry would be relevant in relation to glucose metabolism since Bravo further explained that tannins can oxidatively condense to other galloyl or other hexahydroxydiphenic molecules and form high-molecular weight polymers. As a result gallic acid could serve as a chelating agent of the circulating excess glucose in diabetic patients forming a big complex that is less bioavailable for further oxidation in blood thus reducing the possible development of ketone bodies. Polyphenols are traditionally known as antinutrients due to their chelating effects on divalent trace elements (iron, zinc copper etc). This may be the reason why there are no dietary recommendations for polyphenols. However polyphenols are currently gaining recognition in humans as good antioxidants and are used as treatment against cancer and cardiovascular disease (Mehta, et al, 2003; Bharali et al, 2003; Ashok and Pari,2003; Siddhuraju and Becker, 2003).

Beta glucans. The fonio cereal with a mean of 6.9 % (**Table 7**) was the richest sample in beta-glucan among the selected samples and this particular feature may make it very important for use for diabetes care since according to Jenkins and coworkers, dietary fiber and beta-glucan have a strong potential regulatory effect on glucose metabolism and insulin action. (Jenkins et al, 2002; Kumar et al, 2002)

Table 7. Beta-glucan concentrations in samples

	Sample					
Compound	**1**	**2**	**3**	**Mean**	**±**	**SD**
Fonio	7.1	7.0	6.7	6.9	±	0.17
Moringa leaves	1.0	1.1	1.1	1.0	±	0.05
Moringa seeds	4.4	4.2	5.3	4.6	±	0.48
Sclerocarya bark	4.2	4.5	4.2	4.3	±	0.14
Anacardium bark	4.0	4.3	4.1	4.1	±	0.13

[1]All values are expressed in gm beta-glucan per 100 gm of sample

There is no RDA for beta-glucan available in the literature, but we found mushrooms as a reference source rich in dietary fibers and beta-glucan. Beta-glucans concentration in fonio (6.9 %) from our analysis was found to be much higher than values obtained in the richest mushroom in the literature review with a maximum of 0.53% (dry basis) for *Pleurotus pulmunarius* (Manzi and Pizzoferrato in 1999). In addition, a meta-analysis by Plotnikoff and Infanger on the effects of oat products compared the results of twenty studies. All studies evidenced that a daily dose of 3 grams of beta glucan (a quantity comparable to a large bowl of oat bran cereal) significantly reduced LDL levels. Also, the effects of beta-glucan on LDL were dose responsive (Plotnikoff and Infanger, 2000).

Minerals. The values found in the literature were not similar to ours as regards iron values for fonio shown in **Table 8.** The literature gave 8 mg iron per 100 gm of fonio and we recorded 2.4 mg for the dehulled grains and almost ten times the value in the whole grain (22.1 mg per 100 gm).

Table 8. Minerals and trace elements concentrations in samples

Mineral	Fonio (Whole)	Fonio (Dehulled)	Mor (Leaves)	Mor (Seeds)	Anac (Bark)	Scler (Bark)
Cr (ng/gm)	873	415.3	1024	40.6	478	924.7
Fe (µg/g)	220.8	24.5	277	34.5	107.5	107
Zn (µg/g)	25.7	31.6	31.9	38.5	6.4	5.3
Mg (µg/g)	1346	297.3	3515.7	1542	3444.3	2813.3
Cu (µg/g)	4.0	1.8	3.8	1.4	ND	1.4
Ca (µg/g)	178.6	17.2	16220	1533.7	5353	22470

This may have resulted from variations due to the dehulling rate, the varieties and/or the nature of the soils in which the fonio were grown. It is important to note that our values were quite similar in terms of zinc concentrations (25.7µg/g and 31.6 µg/g in the whole and the dehulled samples respectively).

Chromium in the whole fonio was very high with a content of 872.9 ng/gm. However, chromium content in dehulled fonio was also important (415.3 ng/gm) particularly when taking into account the number of fonio servings a diabetic client may have per day. In some areas of Senegal and the surrounding regions fonio replaces rice for diabetic patients as staple food (the cereal is combined with various sauces of fish, meat or with milk). Therefore, fonio is ingested two to three times a day.

Another important source of minerals and trace elements was the Moringa leaves with 1.0 µg/g in chromium, 31.9 µg/gm zinc, 27.7 mg iron per 100 gm, 0.35 % magnesium, and 1.6% calcium. This is all the more important as the most popular couscous sauce (mboum) is made of Moringa leaves (unfortunately ingredients to make the sauce are expensive for common populations).

Preparation of fonio in households involves an average of 1 kg of the cereal for 5 persons. Therefore, one serving of fonio for a single person would be around 200 gm representing: (415.3 µg x 200gm)/1000 gm = 83µg of chromium, more than twice the

adequate intake (AI) for adult men (35 μg) and more than three times the AI of an adult woman (25 μg) [Dietary Reference Intakes book, National Academy Press, 2001]. Although fonio is mainly consumed as dehulled, 200grams of whole fonio would provide: (872.9 μg x 200 g)/1000 = 174.6 μg of chromium, almost five times the AI of an adult man or 7 times that of an adult woman. Thus decreasing the milling rate or simply processing the whole fonio grain into bars or breakfast cereals would be of considerable benefit for diabetic clients

For an average of 20 g of Moringa leaves (dried weight basis) served with the sauce, the corresponding amount of chromium would be: (1023.9 μg x 20 gm)/1000 gm = 20.4μg of chromium which is around the recommendation for women.

The 200 g of dehulled fonio provides 2.45 mg of iron x 2 = 4.9 mg or 27 % of the recommended dietary allowance (RDA) for adult females (18 mg), and 61% of the RDA for adult males (8 mg). Decreasing the dehulling rate would probably cover the needed daily intake for both men and women. Note that a combination with the Moringa leaves (5.52 mg) sauce would cover more than the RDA for adult men.

As regards zinc, the 200 gm of dehulled fonio would yield: 3.16 x 2= 6.32 mg or 79 % of the RDA (8 mg) for adult women and 57% for adult men (11 mg for the RDA). The 10 gm of the Moringa leaves would bring 3.19 x 0.2 = 0.6 mg or 8 % and 6 % respectively for needs of women and men. The combination would cover 87% for women and 63 % for men.

For magnesium, the 200 g of dehulled fonio would yield: 0.2973mg x 200= 59.5 mg equivalent to 14.9 % of the adequate intake (AI) for males (400-420mg) and 19.2 % of the (AI) for adult females (310-320 mg) whereas the same proportion of the whole

fonio would cover 134.6 mg x 2 = 269.2 mg or 67.3 % for males and 86.8 % for women. The 20 g of Moringa leaves sauce would bring 351.6 x 0.2 = 70.32 mg or 17.56 % and 22.68 % for men and women AI's respectively. The combination of Moringa and dehulled fonio would contribute up to 32.46 % and 41.88 % for men and women AI's.

The AI's for adult men and women for calcium is common (1000 mg) and although the contribution of the 200 gm of dehulled fonio to meet the AI is low: (0.0172 mg x 200 = 3.4 mg) less than.0.5 %, the 10 gm of Moringa leaves sauce would bring: 16.22 mg x 10 = 162.2 mg representing 16.22 % of the AI.

The contribution from the 200 gm of dehulled fonio to cover the RDA for copper (900 µg for both adult men and women) would be: 1.80µg x 200 = 360 µg equivalent to 40 % and the 50 gm of the Moringa leaves would add: 3.8 µg x 20 = 76 µg or 8.4%. The contribution of the combination of Moringa leaves and fonio would be 48.4%.

As regards the barks, their contribution would be low although they displayed high concentrations. The dosage for diabetes treatment ranges from 1gm to 2 gm of ground bark per day. For instance the most abundant mineral found in the barks was calcium (in Sclerocarya) with 22.5 mg /g. A dosage of 2 g of bark would yield only 45 mg or 4.5 % of the RDA. For the purpose of this study we mostly ran proximate analyses; investigation at the cellular or biomolecular levels would probably be the best way to assess the potency of the barks for diabetes treatment.

Sulfur-containing amino acids. The analysis of the amino acids was performed at Langston University using an amino acid analyzer. Because of the low amount of the

barks used as dosage, no further interpretation of the result would be considered clinically relevant for the purpose of this study. Also, Moringa seeds were of interest uniquely for their fatty acids profile. But as regards fonio cereal, interesting amino acids distribution and content were obtained after analysis.

The values (2.0 % for cystine and 6.8 % for methionine) from the analysis of our samples in **Table 9** were similar to those from the literature (2.5 and 4.5% for cystine and methionine respectively).

Table 9. Sulfur amino acids contents

Amino Acid	Dehulled Fonio	Moringa leaves	Moringa seed	Sclerocarya bark	Anacardium. bark
% Methionine	6.8	2.9	3.3	6.3	3
% Cystine	2.0	0.7	3.3	5.3	1.9
% Sulf amino acids	8.8	3.6	6.6	11.6	4.9
Total aa (mg/g)	72.6	224.3	281	16.2	20.7
Wt sulf aa (mg/g)	6.4	8.1	18.55	1.9	1.0

In fact, the National Academy of Sciences did a comparative study of fonio with the major cereals and with egg (National Academy of Sciences, 1996). The amino acid profile of fonio matched or exceeded the egg profile except for the low score of 46% for lysine. The total sulfur amino acid of fonio (8.8% from our analysis) was more than 3 times higher than sorghum (2.6 %) and more than 5 times higher when compared to maize and almost ten times higher compared to pearl millet. Therefore one serving (200 gm) of fonio could be important as a source of sulfur attached to natural ligands for diabetic clients. Although the Moringa leaves with a total content of 3.6% of sulfur amino acids were slightly lower compared to fonio, the potential contribution of the 50 gm sauce would bring a quite appreciable amount of sulfur.

CHAPTER V

CONCLUSIONS

To answer the research questions, the results of the analyses suggest that *Digitaria exilis* (fonio) cereal and *Moringa oleifera* leaves and seeds may help in diabetes care. As regards *Sclerocarya birrea* and *Anacardium occidentale*, we failed to find any evidence on their potential role in diabetes treatment from a nutritional point of view. Maybe an investigation at the molecular level might be more informative.

Fonio and to a lesser extent Moringa, were a good source of the minerals and trace elements considered important for diabetes treatment. An amount of 200 g of dehulled fonio corresponding to an average daily serving would provide more than twice the AI (35 µg) of chromium for adult men and three times the AI (25µg) for women and the 10 gm of Moringa leaves contained an amount (10 µg) approximately equivalent to 1/3rd and a half of the men and women's AI's.

As for zinc content, dehulled fonio covered 79% and 57 % of women and men's RDA's, whereas the Moringa leaves were 20% and 14.5% of the given RDA's. The combination of dehulled fonio (200 gm, as is) and the leaves (10 gm, dry weight basis) would cover 83 % and 60 % of women and men's RDA's.

The 200 grams of dehulled fonio yielded 27 % and 61 % of women and men's RDA's (18 mg and 8 mg) for iron respectively, whereas Moringa leaves (10 gm) with 2..7 mg, would provide 33.8 and 15 % of men's and women's RDA's. The combination (fonio and Moringa leaves) would almost cover men's RDA.

For magnesium, dehulled fonio covered 14.9 % and 19.2 % of women and men's AI's, but Moringa leaves would contribute up to 8.8 % and 11.3 % of the respective AI's. The combination (200 g fonio and 10 g Moringa leaves) would meet 23.7 and 30.5 % of the respective AI's.

For calcium, dehulled fonio covered only less than 0.5 % of the common AI for women and men whereas the Moringa leaves would cover 16.22 %.

Dehulled fonio contributed up to 40% of the common RDA for copper for both men and women and its combination with the Moringa leaves (10 gm) would cover 61% of the RDA; 21% coming from the leaves.

The high contents of fonio in dietary fibers (15.12%) and beta-glucan (6.9 %) may have a strong potential regulatory effect on glucose metabolism and insulin action (Jenkins et al, 2002) as we know that 200 g of fonio would yield 80% or 100% of the RDA's for all populations in dietary fiber. In addition if the reference dose of a daily intake of beta-glucan to lower efficiently LDL concentration was 3 % from oat (Plotnikoff and Infanger, 2000), the daily serving of a 200-g fonio would provide 13.8 g of beta-glucan that may display a better efficiency since the study mentioned that the effect was dose-responsive. In addition, Wursch and Pi-Suyner showed that meals containing 6-8.4g of beta-glucan reduce post-prandrial glycemic responses by as much as 50% (Wursch and Pi-Suyner, 1997) and the ensuing reductions in post-prandrial blood insulin and glucose concentrations have been reported to last for up to 8 hours (Pick et al, 1996). Also, it is important to mention that analysis of beta-glucan content was conducted solely in the soluble part; its content in the non-soluble portion was not assessed. Crude fiber in fonio (3.80%) was high compared to other cereals. Although

Moringa leaves had a high content of dietary fiber (62.72%) and crude fiber (9.67 %), the corresponding content of beta-glucan was low (1%).

Moringa leaves contained large amounts of polyphenols (798.1 mg/100 gm), compared to the dehulled fonio, which was 57.2 mg/100 gm.

The Moringa seeds had high oil content (38.4%) with an O/L ratio of 92.1 higher than all other oils that we found in the scientific literature and could play an important role against joint inflammation in diabetic clients.

Suggestions for Further Studies. Whole fonio contained a much higher concentration of minerals than the dehulled one. Therefore the dehulling rate may play an important role in concentrating or removing minerals. A important step will be to advocate for a reduced dehulling rate, while taking into account the concentration of phytates, commonly rich in cereals bran, and known to be strong chelators of divalent minerals and trace-minerals.

The fonio cereal, particularly the whole one of which an amount of 200 g would contain as much as five times (174.6 μg) the RDA of chromium for adult men, could be processed into bars, crackers or breakfast cereals for diabetic clients using extrusion techniques. A human trial can be undertaken to assess and to find out ways to enhance the bioavailability of the minerals in diabetic subjects after ingestion of the bars or cereals and to check the effects of fonio on parameters such as blood glucose and insulin levels and activity.

Moreover, many diabetic patients in Senegal take one Moringa seed in the morning and one in the night. An animal study on diabetic rats fed with Moringa seeds

would be important to investigate if the high O/L ratio of the seed oil would affect the fatty acid profile of serum lipoprotein and blocks its oxidation.

Fermentation of the soluble fiber for butyric acid production and other short chain fatty acids is extremely important as some studies are pointing out its many regulatory roles in diabetes and other chronic diseases (Kumar et al 2002; Giacco et al 2000). Glycemic index of fonio and Moringa leaves needs to be determined; their high concentrations in both dietary and crude fibers are indicative of a possible low glycemic index.

The structures of fonio starch should be visually investigated for fiber presence; its content is so high compared to the other cereals that we suspect that the internal structure of the grain may possibly display layers of fibers. Thus it may be important to observe the dehulled grain under the electron microscope.

More detailed analyses of polyphenols and antioxidant vitamins should be undertaken on extracts of the barks of Sclerocarya and Anacardium. The extracts may be tested using an animal trial to check whether they can play a regulatory role on glucose metabolism and insulin activity or just to assess in vitro their possible inhibitory effect on aldose reductase and beta-glucosidase.

BIBLIOGRAPHY

Ahmed, I., Adeghate, E., Sharma, A.K., Pallot, D.J.& Singh, J. (1998) Effects of *Momordica charantia* fruit juice on islet morphology in the pancreas of the streptozotocin-diabetic rat. Diab. Res. Clin. Prac. 40:145-151.

Ajabnoor, M.A. (1990) Effects of aloes on blood glucose levels in normal and alloxan diabetic mice. J. Ethnopharmacol. 28:215-220.

Akinpelu, D. A. (2001) Antimicrobial activity of Anacardium occidentale bark. Fitoterap. 72:286-287.

Anderson, R.A. (1992) Chromium, glucose tolerance, and diabetes. Biol. Trace Element Res. 32:19-24.

Anderson, R.A.; Cheng, N. & Bryden, N. A. (1997) Intakes of supplemental chromium intake improve glucose and insulin variables in individuals with type II diabetes. Diabetes. 46:1786-1791.

Anwar, F. & Bhanger, M.I. (2003) Analytical characterization of Moringa oleifera seed oil in temperate regions of Pakistan. J. Agric. Food Chem. 22:6558-63.

AOACS Official Method Ce 2-66. Reapproved (1997) Preparation of methyl esters of fatty acids. Sampling and Analysis of Commercial Fats and Oils.

Ashok K.N.& Pari, L. (2003) Antioxidant action of Moringa oleifera Lam. (drumstick) against antitubercular drugs induced lipid peroxidation in rats. J Med Food. 6:255-259.

Barbagallo, M., Dominguez, L. J., Galioto, A.,Ferlisi, A., Cani, C., Malfa L., Pineo, A.; Busardo A. & Paolisso, G. (2003) Role of magnesium in insulin action, diabetes and cardio-metabolic syndrome X. Mol. Asp. Medic. 24:1-3, 6:39-52.

Becker, R. (1986) Legumes and a cereal with high methionine/cysteine contents. J. Agri. & Food Chem. 34:361-364.

Belemtougri, R.G., Constantin B., Cognard, C., Raymond G.& Sawadogo L. (2001) Effects of *Sclerocarya birrea* (A. rich) hochst (anacardiaceae) leaf extracts on calcium signaling in cultured rat skeletal muscle cells. J. Ethnopharmacol. 76: 247-252.

Bennett, R. (1999) Current usage of botanically based products in diabetes: a global perspective. Euro-Med Pharmaceutic. Charleston, SC. 20:7-10.

Berry, E.M. (1997) Dietary fatty acids in the management of diabetes mellitus. Am J Clin Nutr. 66:991S-997S.

Bharali R., Tamassum J. & Azad M.R. (2003) Chemomodulatory effect of *Moringa oleifera*, Lam, on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. Asian Pac J Cancer Prev. 4:131-139.

Blickle, J.F. (2000) Les traitements pharmacologiques de l'hyperglycemie pospandriale. Diab. & Metab. 26:20-24.

Bopana, K.N., Balaraman, R. & Nadig, R.S. (1998) Role of S-allyl cysteine sulphoxide in antagonizing deleterious metabolic effects of monosodium glutamate in rats on atherogenic diet. Indian J. of Pharmacol. 30:73-81.

Bordia, A. (1997) Effect of ginger (*Aingiberofficinale* Rosc.) and fenugreek (*Trigonella foenumgraecum* L.) on blood lipids, blood sugar and platelet aggregation in patients with coronary artery disease. Prostaglandins Leukot Essent. Fatty Acids. 56:379-384.

Bravo, L. (1998) Poyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56:317-333.

Burkitt, D. & Trowell, H. (1981) Western Diseases: Their emergence and prevention. Harvard University Press, Cambridge, Ma.

Cáceres, A., Saravia, A., Rizzo, S., Zabala, L., De Leon, E. & Nave, F. (1992) Pharmacologic properties of Moringa oleifera. 2: Screening for antispasmodic, antiinflammatory and diuretic activity, J. Ethnopharmacol. 36:233-237.

Capannesi, C., Palchetti, I., Mascini, M. & Parenti, A. (2000) Electrochemical sensor and biosensor for polyphenols detection in olive oils. Food Chem. 71:553-562.

Cunningham, J. (1991) Reduced mononuclear leukocyte ascorbic acid content in adults with insulin-dependent diabetes mellitus consuming adequate dietary vitamin C. Metab. 40:146-9.

Davie, S.J., Gould, B.J. & Yudkin, J.S. (1992) Effect of vitamin C on glycosylation of proteins. Diabetes. 41:167-73.

Delange, F. M. & Ermans A M. (1976) Endemic goiter and cretinism: naturally occurring goitrogens. Pharmacol Ther C.1:57.

De, L.B.O. & Thompson, S. (1993a) Sulfur amino acid-rich proteins in acha (Digitaria exilis), a promising underutilized African cereal. J. Agric. & Food Chem. 4:1045-1047.

Duke, J. A. (1978) The quest for tolerant germplasm. Crop tolerance to suboptimal land conditions. ASA Special. Symposium. 32.Madison,Wisconsin Ameri. Soc. Agron. P 1-61.

Eloff, J. N. (2001) Antibacterial activity of Marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. caffra (Sond.) Kokwaro) (Anacardiaceae) bark and leaves. J. Ethnopharmacol, 76:305-308.

Engel, E. D., Elrich, N. E. & Davis R,H. (1981) Diabetes mellitus: impaired wound healing from zinc deficiency. J. Am. Pod. Assoc. 71:536-544.

Faizi, S., Siddiqui, B. S., Saleem, R., Aftab, K., Shaheen, F.& Gilani, A. H. (1998) Hypotensive constituents from the pods of Moringa oleifera, Planta Medica, 64:225-228.

Faizi, S., Siddiqui, B.S., Saleem, R., Siddiqui, S., Aftab, K. & Gilani, A.H. (1995) Fully acetylated carbamate and hypotensive thiocarbamate glycosides from Moringa oleifera, Phytochem. 38:957-963.

Ford, E.S., Will, J. C., Bowman, B.A. & Narayan (1999). Diabetes mellitus and serum carotenoids: findings from the Third National Health and Nutritions Examination Survey. Am J. Epidemiol. 149:168-176.

Fung, T. T., Hu, F.B., Pereira, M. A., Liu, S., Stampfer, M. J., Colditz G. A. & Willett W. C. (2002) Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. American Society for Clin. Nutr. 76:390-398.

Galvez, J., Zarzuello, A., Crespo, M. E., Utrilla, M. P., Jimenez, J., Spiessens, C.& Witte, P. (1991) Antidiarrhoeic activity of Sclerocarya birrea bark extracts and its actannin constituents in rats. Phytother. Res. 5:276-278.

Garbelotti, M. L., Marsiglia D.A.P.& Torres, E. A.F.S. (2003) Determination and validation of dietary fiber in food by the enzymatic gravimetric method. Food Chem. 83:469-473.

Garcia, M. J., Mc Namara, P. M.& Gordon, T. (1974)Morbidity and mortality in diabetics in the Framingham population. Sixteen year follow up. Diab. 23:105-11.

Ghasi, S., Nwobodo, E. & Ofili, J. O. (2000) Hypocholesterolemic effects of crude extract of leaf of Moringa oleifera Lam in high-fat diet fed wistar rats, J. Ethnopharmacol. 69:21-25.

Giacco, R., Parillo, M., Rivellese, A. A., Lasorella, G., Giacco, A., D'Episcopo, L.& Riccardi, G. (2002) Long-term dietary treatment with increased amounts of fiber-rich low-glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. Diab. Care.23:1461-66.

Giron, M. D., Sanchez, F., Hortelano, P., Periago, J. L. & Suarez, M. D. (1999) Effects of dietary fatty acids on lipid metabolism in streptozotocin-induced diabetic rats. Metab.Clin. & Exp. 4: 455-60.

Guevara, A. P.; Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., Kozuka, M., Ito, Y., Tokuda, H. & Nishino (1999). An antitumor promoter from *Moringa oleifera* Lam, Mut. Res. 440:181-188.

Gupta, M., Mazumder, U. K. & Chakrabarti, S. (1999) CNS activities of methanolic extract of *Moringa oleifera* root in mice, Fitoterapia. 70:244-250.

Harlan, J R. (1993). Genetic resources in Africa. In: J. Janick and J.E. Simon (eds.), New crops. Wiley, New York. (1996) Lost Crops of Africa. Grains. 1:59-75.

Harris M.I. (1990) Epidemiology of Diabetes Mellitus among the elderly in the United States. Clin. Geriatr. Med. 6:703-719.

Hegazi S. M. (1992) Effect of zinc supplementation on serum glucose, insulin, glucagons, glucose-6-phosphatase, and mineral levels in diabetics. J. Clin. Nutr. 12:209-215.

Hill, A.D., Petterson, K.Y., Veillon, C. & Morris, E.R. (1986) Digestion of biological materials for mineral analyses using a combination of wet and dry ashing. Anal. Chem. 58:2340-2342.

Irving, D. W. & Jideani, I. A. (1997) Microstructure and composition of *Digitaria exilis* Stapf (acha): A potential crop. Cereal Chemistry {a} USDA, Agric. Res. Serv., Western Regional Res. Cent. 800 Buchanan St., Albany, CA 94710, USA. 74:224-228.

Jamal, G. A. & Carmichael, H. (1990) The effects of gamma-linolenic acid on human diabetic peripheral neuropathy: a double blind placebo-controlled trial. Diab. Med. 7:319-323.

Jenkins, A. L., Jenkins, D. J. A., Zdravkovic, U., Wursch, P. & Vuscan, V. (2002) Depression of the glycemic index by high levels of beta-glucan fiber in two functional foods tested in type 2 diabetes. Europ. J. Clin. Nutr. 56:622-628.

Jenkins, D. J. A., Wolever, T. M. S. & Bacon, S. (1980) Diabetic diets: High carbohydrate combined with high fiber. Am J Clin Nutr 33:1729-1733.

Jolly C. A., Muthukumar, A., Avula, C. P., Troyer, D. & Fernandez G. (2001) Life span is prolonged in food-restricted autoimmune-prone (NZB x NZW) F (1) mice fed a diet enriched with (n-3) fatty acids. J.Nutr 10: 2753-2760.

Kalogo, Y., M'Bassiguié, S. A. & Verstraete, W. (2001) Enhancing the start-up of a UASB reactor treating domestic wastewater by adding a water extract of *Moringa oleifera* seeds, Applied Microbiol. and Biotechnol. 55:644-651.

Kamtchouing, P., Selestin, D., Sokeng, P. F., Moundipa, P.W., Hermine B. J. & Lontsi, D. (1998) Protective role of *Anacardium occidentale* extract against streptozotocin-induced diabetes in rats. J. Ethnopharmacol.. 62:95-99.

Keen H., Payan, J., Jamal, G. A. Weir, A. I. & Henderson, L. M. (1993) Treatment of diabetic neuropathy with gamma-linolenic acid. The gamma-Linolenic Acid Multicenter Trial Group. Diab Care. 16: 8-15.

Kerharo, J. & Adam, J. G. (1974) La pharmacopée sénégalaise traditionnelle: plantes médicinales et toxiques. Paris, Vigot Freres. 1011 pages.

Kostyuk, E. P., Voitenko, N. V., Kruglikov, I. A., Kostyuk, P. G. & Efimov, A. S. (2000) Changes in calcium signalling in spinal dorsal horn neurons in rats with streptozotocin-induced diabetes. Diab. Res. & Clin. Prac. 50:362-363.

Kudi, A. C., Umoh, J. U., Eduvie, L.O. & Gefu, J. (1999) Screening of some Nigerian medicinal plants for antibacterial activity. J. Ethnopharmacol. 67:225 -228.

Kumar, C. M. Rachappaji, K. S., Nandini C. D., Sambaiah, K., Salimah, P.V. (2002) Modulatory effect of butyric acid: a product of dietary fiber fermentation in experimentally induced diabetic rats. J. Biochem. 13:522-527.

Lalas, S., &Tsaknis, J. (2002) Characterization of *Moringa oleifera* seed oil variety "Periyakulam 1", J. Food Comp. and Anal. 15:65-77.

Liu, Q., Singh, S. & Green, A. (2002) High-oleic and high-stearic cotton seed oils: Nutritionally improved cooking oils developed using gene silencing. J. Am. Coll. Nutr.21:205S-211S.

Liu, S., Manson, J. E., Stampfe, M. J., Hu, .F. B., Giovannucci, E., Colditz, G. A., Hennekens, C. H. & Willett, W. C. (2000) A prospective study of whole-grain intake and risk of type 2 diabetes mellitus in US women. Am. J. Pub. Health. 90:1409-1415.

Lockett, C. T.; Calvert, C. C.& Grivetti, L. E. (2000) Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria, International J. of Food Sci. & Nutr. 51:195-208.

Lovejoy, J. C. (1999) Dietary fatty acids and insulin resistance. Current Atheroscler. Rep. 3:215-220.

Lovejoy, J. C., Champagne, C. M., Smith, S. R., DeLany, J. P., Bray, G. A., Lefevre, M., Denkins, Y. M. & Rood, J C. (2001) Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. Metab. & Experimental. 50:86-92.

Makkar, H. P. S. & Becker, K. (1996) Nutrional value and antinutritional components of whole and ethanol extracted Moringa oleifera leaves. Anim. Feed Sci. & Technol. 63:1211-1228.

Maness, N.O., Chrz D., Pierce, T. & Gerarld H. B. (1995) Quantitative extraction of pecan oil from small samples with supercritical carbon dioxide. JAOCS. 72:665-669.

Manzi, P. & Pizzoferrato, L. (1999) Beta-glucans in edible mushrooms. Food Chem. 68:315-318.

Mazumder, U. K., Gupta, M., Chakrabarti S. & Pal, D. (1999) Evaluation of hematological and hepatorenal functions of methanolic extract of *Moringa oleifera Lam.* root treated mice. Indian J Exp Biol. 37:612-614.

McKeown, N. M., James B., Meigs, J. B., Liu, S., Wilson, P. W. F. & Jacques, P. J. (2002) Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. A. J. C. N. 76:390-398.

Mehta, K., Balaraman, R., Amin, A. H., Bafna, P. A. & Gulato, O. D. (2003) Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits. J Ethnopharmacol. 86:191-5

Mertz, W. (1993) Chromium in human nutrition: a review. J. Nutr. 123:626-633.

Meyer, K. A., Kushi, L. H., Jacobs, D. R. & Folsom, A R. (2001) Dietary fat and incidence of type 2 diabetes in older Iowa women. Diab. Care 9: 1528-1535.

Migdalis, I. N., Xenos, K., Chairopoulos, K., Varvarigos, N., Leontiades, E. & Karmaniolas, K. (2000) Ca^{2+}-Mg^{2+}-ATPase activity and ionized calcium in Type 2 diabetic patients with neuropathy. Diab. Res. & Clin. Prac. 49:113-118.

Miguel, N. G. & Belloso, O. M. (1999) Comparison of dietary fiber from by-products of processing fruits and greens and from cereals. Lebensm.- Wiss. U-Technol., 32:503-508. Article No. fstl.1999.0587. www. idealibrary.com

Mooradian A. D., Failla, M., Hoogwerf, B., Maryniuk, M. & Wylie-Rosett, J (1994) Selected vitamin and mineral in diabetes. Diab. Care 17:464-479.

Mooradian, A. D. & Morley, J. E. (1987) Micronutrients status in diabetes mellitus. Am J. Clin. Nut. 45. 877-895.

Murakami, A., Kitazono, Y., Jiwajinda, S., Koshimizu, K. & Ohigashi, H. (1998) Niaziminin, a thiocarbamate from the leaves of Moringa oleifera, holds a strict structural requirement for inhibition of tumor-promoter-induced Epstein-Barr virus activation. Planta Medica. 64:319-323.

Nambiar, V. S. & Seshadry, S. (2001) Bioavailability trials of beta-carotene from fresh and dehydrated drumstick leaves (*Moringa oleifera*) in a rat model.eife. Plant Foods for Hum. Nutr. 56:83-95.

National Academy of Sciences (1996) Lost crops of Africa.1: Grains. 59-75. http://www.nap.edu/openbook/0309049903/html/59.html

National Academy of Sciences (2001) Dietary reference intakes. Definitions of dietary fibers. National Academy Press P. 12-21.

National Research Council (1989) Diet and Health Implications for Reducing Chronic Disease Risk. National Academies Press, Washington, DC. 768 pages.

Nicola W. G. (1996) Role of the hypoglycemic plant extract *Cleome droserifolia* in improving glucose and lipid metabolism and its relation to insulin resistance in fatty liver. Boll Chim Farm.135:507-517, 1996.

O'Bryne, D. J., Knauft, D. A. & Shireman, R. B. (1997) Low fat-monounsaturated rich diets containing high-oleic peanuts improve serum lipoprotein profiles. Lipids. 32:687-695.

Ochoa, J. J., Quiles J., Ramirez-Tortosa, M. C., Mataix, J. & Huertas, J R. (2002) Dietary oils high in oleic acid but with different unsaponifiable fraction contents have different effects in fatty acid composition and peroxidation in rabit LDL. Nutr. 18:60-65.

Ochoa-Herrera, J. J., Huertas, J. R., Quiles J. L. & Mataix, J. (2001) Dietary oils high in oleic acid, but with different non-glyceride contents, have different effects on lipid profiles and peroxidation in rabbit hepatic mitochondria. J. of Nutr. Biochem. 12:357-364.

Ojewole J. A. O. (2003) Evaluation of the anti-inflammatory properties of *Sclerocarya birrea* (A. Rich.) Hochst. (family: Anacardiaceae) stem-bark extracts in rats. 85:217-220.

Okuda, T., Baes, A. U., Nishijima, W.& Okada, M. (2001) Coagulation mechanism of salt solution-extracted active component in Moringa oleifera seeds, Water Res. 35:830-834.

Oliveira, .P J., Seica, R., Coxito, P.M., Rolo, A. P., Palmeira, C. M., Santos M S & Moreno, A. J. M. (2003) Enhanced permeability transition explains the reduced calcium uptake in cardiac mitochondria from sterptozotocin-induced diabetic rats. FEBS Lett. 20:511-514.

Palada, M. C. (1996) Moringa (*Moringa oleifera Lam.*): A versatile tree crop with horticultural potential in the subtropical United States. Hort. Sci. 31: 5.

Paolisso, G, Barbieri, M., Rizzo, M.R. & Manzella, D. (2001) Should we recommend the therapeutical use of vitamin E in diabetic patients? Environ. Toxicol. & Pharmacol. 10:159-165.

Paolisso, G., D'Amore, A & Galzerano. (1993) Daily vitamin E supplements improve metabolic control but not insulin secretion in elderly type 2 diabetic patients. Diab. Care. 16:433-437.

Pick, M. E., Hawrysh, Z.J., Gee, M. I., Toth, E., Garg.M. L. & Hardin, R.T. (1996) Oat bran concentrate bread products improve long-term control of diabetes:a pilot study. J. Am. Diet. Assoc. 96:1254-1261.

Plotnikoff, G. A. & Infanger, D. W. (2000) Preventing CAD in women: complementary/alternative approaches. What is the evidence for "non-medical" interventions? Health Prim.Care 3:91-101.

Price, M L. The Moringa Tree.1985 Revised (2000) by Kristin Davis. Echo Technical Note.

Puri, D., & Baral, N. (1998) Hypoglycemic effect of *Biophytum sensitivum* in the alloxan diabetic rabbits. Indian J. Physiol Pharmacol. 42:401-406.

Rao, A. V., Devi, P. U. & Kamath, R. (2001) In vivo radioprotective effect of Moringa oleifera leaves, Indian J. of Exp. Biol. 39:858-863.

Rastelli, G., Antolini, L., Benvenuti, S. & Constantino L. (2000) Structural bases for the inhibition of aldose reductase by phenolic compounds. Bioorg. & Med. Chem. 8:1151-1158.

Raza, H. & Ahmed I. (1996) Effect of Bitter melon (*Momordica charantia)* Fruit Juice on the hepatic cytochrome P450-dependent monooxygenases and glutathione S-

transferases in streptozotocin-induced diabetic rats. Biochem Pharmacol. 2:1639-1642.

Reginato M. J., Krakow, S. .L, Bailey, S. T. & Lazar, M A. (1998) Prostaglandins promote and block adipogenesis through opposing effects on peroxisome proliferator-activated receptor gamma. J. Biol. Chem. 273:1855-8.

Renaud, S. M., De Lorgeril, J., Delaye, J., Guidollet, F., Jacquard, N., Mamelle, J. L., Martin, I., Monjaud, P. & Toubol, P. (1995) Mediterranean diet for prevention of coronary heart disease. Am. J. Clin. Nutr. 61:1360s–1367s.

Ryan, M., McInerney, D., Owens, D., Collins, P., Johnson, A. & Tomkin, G.H. (2000) Diabetes and the Mediterranean diet: a beneficial effect of oleic acid on insulin sensitivity, adipocyte glucose transport and endothelium-dependent vasoreactivity. Q. J. Med. 93:85-91.

Sartelet, H., Serghat, S., Lobstein, A., Ingenbleek, Y., Anton, R., Petitfrère, E., Aguie-Aguie, G., Martiny, L. & Haye, B. (1996) Flavonoids extracted from fonio millet (*Digitaria exilis*) reveal potent antithyroid properties. Nutr. 12:100-106.

Seppanen-Laasko, T., Laakso, I. & Hiltunen, R. (2002) Analysis of fatty acids by gas chromatography, and its relevance to research on health and nutrition, Analytica Chimica Acta. 465:39-62

Sharma, K K., Gupta R. K, Gupta, S., Samuel, K. C. (1977) Antihyperglycemic Effect of onion: effect on fasting blood glucose and induced hyperglycemia in man. Indian J. Med. Res. 65: 422-429.

Sharma, R. D., Ribes, G., Sauvaire, Y. & Da Costa, C. (1999) Effect of fenugreek seeds on blood glucose and serum lipids in Type 1 Diabetes. J Assoc Acad Minority Phys, 10-14.

Sheela, C. G., & Augusti, K. T. (1992) Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic (*Allium sativum, Linn.).* Indian J. Exp. Biol. 30:523-6.

Siddhuraju, P. Becker, K., (2003) Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam) leaves. J. Agric. Food Chem. 51:2144-2155.

Sidibe, E. H. (1996) Diabetes mellitus in Sub-Saharan Africa. Sante C. 8:342-346.

Simpson, H. C. R., Simpson, R. W. & Lousley, S A. (1983) High carbohydrate leguminous fiber diet improves all aspects of diabetic control. Lancet 1:1-5.

Slavin, J. (2003) Impact of the proposed definition of dietary fiber on nutrient databases, J. of Food Comp. & Anal. 16:287-291.

Toeller, M. (2002) Fibre consumption, metabolic effects and prevention of complications in diabetic patients: epidemiological evidence. J. Agr. & Food Chem. 4: 1045-1047.

Toure, F. (2000) Effect of fish and shrimp on iodine bioavailability in a cassava and millet-based staple food in Guinea. Thesis. 84 pages.

Vahouny, G. & Kritchevsky, D. (1981) Dietary fiber in health and disease prevention. Havard University Press, Cambridge.

Van Etten, C. H. & Wolf, I. A. (1973) Natural sulfur compounds. In: Toxicants occurring naturally in foods. Washington DC: Committee on Food Protection, National Academy of Sciences. 2:210 pages.

Vincent, J. B. (1999) Mechanisms of chromium action: Low-molecular-Weight-Chromium –Binding Substance. J. of the American College of Nutr. 18, 6-12.

Vincent, J B. (2000) The biochemistry of chromium. J. Nutr. 130:715-718.

Vinson, J. A., Staretz, M. A., Bose, P.& Kassm, H. M. (1989) In vitro and in vivo reduction of erythrocyte sorbitol by ascorbic acid. Diab. 38:1036-1041.

Welihinda, J., Arvidson, G. & Gylfe, E. (1982) The insulin-releasing activity of the tropical plant *Momordica charantia.* Acta Biol. Med. Germ 41: 1229-1240.

White, J. R. & Campbell R. K. (1993) Magnesium and diabetes: A review. Ann Pharmacother. 27:775-80.

Wimhurst, J. M. & Manchester, K. L. (1972) Comparison of ability of magnesium and manganese to activate the key enzymes of glycolysis. FEBS Letters 27:321-326.

Wursch, P., & Pi-Sunyer, F. X. (1997) The role of viscous soluble fiberin the metabolic control of diabetes. Diab. Care 20:1774-1780.

Yoshika, M. (1998) Antidiabetic principle of natural medicines. Aldose reductase and alpha glucosidase inhibitors from Brazilian natural medecine, the leaves of *Myrcia flora* DC (Myrtaceae): structures of myrciacitrins I and II and myrciaphenones A and B. Chemical Pharm Bull (Tokyo). 46:113-119.

Zhou, Y. P. & Zhang, J. Q. (1989) Oral baicalin and liquid extract of licoricereduce sorbitol levels in red blood cells of diabetic rats. Chinese Medic. J. 102: 203-206.

APPENDIX A

Crude Fiber Determination

Equipment (purchased at Tecator Inc., P.O. Box 405, Herndon, Virginia 220 70, USA):

Hot Extractor 1020 for hot extractions and filtration

Cold Extractor 1021 for deffating and solvent washing

Hot Plate 1022

Cyclotec Mill 1093

Reagents:

0.128 M sulfuric acid

0.223 M potassium hydroxide

95% Ethyl alcohol

Acetone (technical grade)

Procedure:

Weigh 1 g of ground and homogenized sample in pre-tarred crucible.

Insert the crucible in the Hot Extractor and turn it on.

Extract for 30min with 150 ml of hot 0.128 M sulfuric acid solution pre-heated on the Hot Plate.

Extract for 30min with 150 ml of hot 0.223 M potassium hydroxide pre-heated on the Hot Plate.

Rinse with hot distilled water the obtained crude fiber and residual minerals.

Insert crucible with its contents in the Cold extractor and extract 3 times with small quantities of ethyl alcohol.

Extract 3 times with small quantities of acetone.

Remove crucibles from the Cold extractor and put them in the oven to dry overnight at 100°C.

Weigh crucibles that contain crude fibers and minerals.

Put crucibles in muffle furnace and ash extracts at 500°C for 3 hours.

Allow to cool down and weigh ash content.

The percent fiber content in the sample is calculated by subtracting the mineral content from the total weight obtained in step 9, following the equation:

$$\% \text{ Crude fiber} = (W_1 - W_2 / W_0) \times 100$$

Where W_1 represents the extracted sample before ashing, W_2 the weight of the obtained ash and W_0 the weight of the sample as is.

APPENDIX B

Dietary Fiber Determination

Equipment:

Tecator Fibertec System E 1023-001(purchased at Tecator Inc., P.O. Box 405, Herndon, Virginia 220 70, USA) comprising:

a. Filtration Unit 1023-011 with accessories

b. Shaking Water Bath 1024-011 with accessories

c. Incubating Flasks1000-2081

d. Crucibles1000-1172, P2 (40-90 μ)

Cyclotec Mill 1093

Drying Oven 105 °C

Muffle Furnace 525 °C

pH-meter

Reagents:

Celite and following Enzymes: Termamyl (heat resistant α-amylase)

Protease P-5380

Amyloglucosidase A-9268

Chemicals:

Phosphate buffer 0.05 M, pH 6.0: dissolve 0.875 g of $Na_2 HPO_4$ and 6.05 g of $Na H_2 PO_4$, H_2O in approximately 700 ml of distilled water. Dilute to 1000ml.

Phosphoric acid solution 0.205 M: dissolve 23.64 g of $H_3 PO_4$ (85%) in distilled water. Dilute to 1000 ml.

Sodium hydroxide solution 0.171 N: dissolve 6.84 g of Na OH in approximately 700 ml of distilled water. Dilute to 1000ml.

Ethanol 95 % (v/v) technical grade.

Ethanol 78 %: place 207 ml of distilled water in a 1000 ml flask. Dilute to volume with ethanol 95 %.

Acetone: reagent grade.

Procedure:

Experimental blank: run the procedure without sample to measure any contribution from reagents to the residue.

In a cleaned flask, add 1 g of sample, plus 50 ml of pH 6.0 PO4 buffer and 100 μl Termamyl solution and incubate in boiling water bath for 30 minutes. Shake gently at 5 min intervals.

After cooling add 10 ml of 0.171N Na OH and check pH 7.5 +-0.1

Add 5 mg protease and incubate at 60 °C for 30 min. Shake gently in water bath.

After cooling add 10 mls 0.205 M H3PO4. Check pH 4.5 +- 0.2.

Add 0.3 ml Amyloglucosidase and incubate at 60°C for 30 min in shaking water bath.

Add 280 ml of 78% Ethanol (preheated at 60 °C). Precipitate at room temperature for 1 hour.

Insert empty flask in the lower part of the filtration module for collection of filtered solutions.

Tare pre-dried crucibles containing 0.5g celite. Wet the celite with 78%Ethanol to form an even bed on the glass filter.

Mount the crucibles upside down on top of the flasks containing the digested samples. Press the safety button, fold down the upper flasks holders and attach the flasks to the bayonet fittings. Fold up the flasks.

Remove the bottoms of the flasks and turn on water aspiration pump and turn the valves to V position for filtration. In case of clogged residue use "Pressure" to rinse the filters.

Wash the residues with 3 x 20 ml of 78% ethanol and 2 x 10 ml 95% ethanol.

Remove the plastic funnels and fit the crucibles to the dehydration system on top of the unit. Wash with 2x 10 ml of acetone. Filter off the solvent.

Dry the crucibles in a 70°C vacuum oven or at a 105°C air oven overnight.

Cool the crucibles in a dessicator and weigh to nearest 0.1 mg.

Determine resistant Kjeldahl protein in the residue from one sample of the set of duplicates.

Incinerate the second sample of the duplicates for 5 hrs at 525°C. Cool in dessicator and weigh to nearest 0.1.

APPENDIX C

Beta-glucan Determination

Equipment

1093 Cyclotec Mill

pH-meter

Hematologic Analyzer

Reagents

A. Enzymes:

Termamyl (heat resistant α-amylase)

Protease P-5380

Amyloglucosidase A-9268

Cellulase 1. 92 units/mg(from Sigma, St Louis): dissolve 260.42 mg in 50 ml of purified water to obtain 10 units/ml

β-glucosidase (from Sigma, St Louis): dissolve total flask content in 50 ml of purified water and obtain 2 units/ml or 0.8 units in 0.4 ml

B. Chemicals:

Phosphate buffer 0.05 M, pH 6.0: dissolve 0.875 g of $Na_2 HPO_4$ and 6.05 g of $Na H_2 PO_4$, H_2O in approximately 700 ml of distilled water. Dilute to 1000ml.

Phosphoric acid solution 0.205 M: dissolve 23.64 g of $H_3 PO_4$ (85%) in distilled water. Dilute to 1000 ml.

Sodium hydroxide solution 0.171 N: dissolve 6.84 g of Na OH in approximately 700 ml of distilled water. Dilute to 1000ml.

95 % Ethanol (v/v) technical grade

78 % ethanol: place 207 ml of distilled water in a 1000 ml flask. *Dilute to volume with* 95 % ethanol.

C. Experimental:

Experimental blank: run the procedure without sample to measure any contribution from reagents to the residue

Add 1 g of sample in a cleaned flask, , plus 50 ml of pH 6.0 PO4 buffer and 100 μL of Termamyl solution and incubate in boiling water bath for 30 minutes. Shake gently at 5 min intervals.

After cooling add 10 ml of 0.171N Na OH and check pH 7.5 +-0.1

Add 5 mg protease and incubate at 60 °C for 30 min. Shake gently in water bath.

After cooling add 10 ml 0.205 M H3PO4. Check pH 4.5 +- 0.2.

Add 0.3 ml Amyloglucosidase and incubate at 60°C for 30 min in shaking water bath. Filter.

Add 280 ml of 78% Ethanol (preheated at 60 °C). Precipitate at room temperature for 1 hour.

Centrifuge precipitates in polypropylene tubes at 4000 rpm for 10 min and discard the supernatant.

Add sodium phosphate buffer (4.0 ml, 20 Mm, pH 6.5) and stir vigorously on a Vortex mixer.

Immediately place the tube in a boiling water bath and incubate for 2 min. Vigorously stir the mixture on a Vortex mixer and incubate for an additional 2 min and stir again.

Incubate the contents at 50 °C for 5 min.

Add cellulase (1ml, 10 U) and stir the tube contents. Seal the tube with parafilm and incubate for 1 hour at 50 °C on a water bath with constant stirring.Add acetate buffer (5.0 ml, 200 mM, pH 4.0) and vigorously mix the tube contents on a Vortex mixer.

Allow the tube to equilibrate at room temperature (5 min) and centrifuge (1000 rpm, 10 min). Carefully and accurately dispense aliquots (0.1 ml) into the bottom of the analyzer test tubes.

Add β-glucosidase (0.4 ml, 0.8 U) in 50 mM acetate buffer (pH 4.0). Incubate tubes at 50 °C for 30 min

Remove the tubes from the water bath and insert tubes in the analyzer and record obtained glucose value.

Beta-glucan value was derived from glucose in the following reaction:

% Beta glucan = glucose (162/180) x (100/ sample weight).

APPENDIX D

Parameters for Minerals Determination by Atomic Absorption

Chromium

A. Instrument:

Instrument model: Perkin Elmer 5100

Technique: furnace

Wavelength (nm): 357.9

Slit width: 0.7

Signal type: Zeeman AA

Signal measurement: Peak Area

Modified spectrophotometer settings: none

B. Calibration:

Calibration equation: non linear

Calibration units: µg/L

Sample unit: µg/L

Maximum decimal places: 3

Maximum significant figures: 4

Replicates: 2

Sample volume (µL): 20

Diluent volume: (µL): 5

Matrix modifiers volume (µL): 5; modifier #1: 5 µL; modifier #2: 0 µL

Modifier #1 in blank, standards, reagent blank, and samples

Calibration blank: 0µg/L

Reagent blank: 0µg/L

Calibration standard #1: 10 ppb

Calibration standard #2: 20 ppb

C. Furnace conditions

Steps	Temp. (°C)	Ramp time	Hold time	Internal flow	Gas type
1	110	10	40	300	Normal
2	130	1	20	300	Normal
3	1350	3	30	300	Normal
4	20	1	15	300	Normal
5	2400	0	5	0	Normal
6	2500	1	5	300	Normal

Extraction system: none

Injection temperature (°C): 20

Furnace clean-out: run step 6 to 1, after peak area is greater than 2.00 between blanks, samples or standards.

Iron

A. Instrument:

Instrument model: Perkin Elmer 5100

Technique: flame

Wavelength (nm): 248.3

Slit width: 0.2

Signal type: AA

Signal measurement: time average

Modified spectrophotometer settings: none

Read time (sec): 0.2

Read delay (sec): 0.0

Flame type: air/acetylene

Oxidant low (L/min): 10.0

Fuel flow (L/min): 2.0

B. Calibration:

Calibration equation: non linear

Calibration units: mg/L

Sample unit: mg/L

Maximum decimal places: 3

Maximum significant figures: 4

Sample replicates: measure 2 replicates from the same cup

Calibration solutions: measure 2 replicates from all standards and blanks

Sample volume (µL): 20

Diluent volume: (µL): 5

Matrix modifiers volume (µL): 5; modifier #1: 5 µL; modifier #2: 0 µL

Modifier #1 in blank, standards, reagent blank, and samples

Calibration blank: 0µg/L

Reagent blank: 0µg/L

Calibration standard #1: 1 ppb

Calibration standard #2: 2 ppb

Auto-sampler setup: AS91F160

Zinc

A. Instrument:

Instrument model: Perkin Elmer 5100

Technique: flame

Wavelength (nm): 213.9

Slit width: 0.7

Signal type: AA

Signal measurement: time average

Modified spectrophotometer settings: none

Modified flame settings: yes

Flame sensor: on

Read time (sec): 1.0

Read delay (sec): 0.0

Flame type: air/acetylene

Oxidant low (L/min): 10.0

Fuel flow (L/min): 2.0

B. Calibration:

Calibration equation: non linear

Calibration units: mg/L

Sample unit: mg/L

Maximum decimal places: 3

Maximum significant figures: 4

Sample replicates: measure 2 replicates from the same cup

Calibration solutions: measure 2 replicates from all standards and blanks

Calibration standard #1: 0.1 mg/L

Calibration standard #2: 0.2 mg/L

Calibration standard #3: 0.3 mg/L

Calibration standard #4: 0.4 mg/L

Auto-sampler setup: tray type AS91F160

Magnesium

A. Instrument:

Instrument model: Perkin Elmer 5100

Technique: flame

Wavelength (nm): 285.2

Slit width: 0.7

Signal type: AA

Signal measurement: time average

Modified spectrophotometer settings: none

Modified flame settings: none

Read time (sec): 1.0

Read delay (sec): 0.0

Fuel flow (L/min): 2.0

B. Calibration:

Calibration equation: non linear

Calibration units: mg/L

Sample unit: mg/L

Maximum decimal places: 3

Maximum significant figures: 4

Sample replicates: measure 2 replicates from the same cup

Calibration solutions: measure 2 replicates from all standards and blanks

Calibration standard #1: 0.5 mg/L

Calibration standard #2: 1.0 mg/L

Calibration standard #3: 1.5 mg/L

Copper:

A. Instrument:

Instrument model: Perkin Elmer 5100

Technique: flame

Wavelength (nm): 324.8

Slit width: 0.7

Signal type: AA

Signal measurement: time average

Modified spectrophotometer settings: none

Modified flame settings: none

Read time (sec): 1.0

Read delay (sec): 0.0

Flame type: air/acetylene

Oxidant low (L/min): 10.0

Fuel flow (L/min): 2.0

B. Calibration:

Calibration equation: non linear

Calibration units: mg/L

Sample unit: mg/L

Maximum decimal places: 3

Maximum significant figures: 4

Sample replicates: measure 2 replicates from the same cup

Calibration solutions: measure 2 replicates from all standards and blanks

Calibration standard #1: 0.5 mg/L

Calibration standard #2: 1.0 mg/L

Calibration standard #3: 2.0 mg/L

Calibration standard #3: 4.0 mg/L

Calcium:

A. Instrument:

Instrument model: Perkin Elmer 5100

Technique: flame

Wavelength (nm): 422.7

Slit width: 0.7

Signal type: AA

Signal measurement: time average

Modified spectrophotometer settings: none

Modified flame settings: none

Read time (sec): 1.0

Read delay (sec): 0.0

Flame type: air/acetylene

Oxidant low (L/min): 10.0

Fuel flow (L/min): 3.8

B. Calibration:

Calibration equation: non linear

Calibration units: mg/L

Sample unit: mg/L

Maximum decimal places: 3

Maximum significant figures: 4

Sample replicates: measure 2 replicates from the same cup

Calibration solutions: measure 2 replicates from all standards and blanks

Calibration standard #1: 1.0 mg/L

Calibration standard #2: 2.5 mg/L

Calibration standard #3: 5.0 mg/L

Calibration standard #3: 10.0 mg/L.

VITA (1)

Djibril Traore

Candidate for the Degree of

Master of Science

Thesis: ANALYSIS OF FONIO AND THREE SELECTED BOTANICAS USED FOR DIABETIC CARE

Major Field: Nutritional Sciences

Biographical:

Education: Graduated from Cheikh Anta Diop University, Senegal; received a Bachelor of Arts and a Master of Arts. Recipient of the Hubert H. Humphrey Fellowship Program at the University of California at Davis at the Department of Food Science in 1993. Recipient of the Fulbright Fellowship Program at Oklahoma State University in 2000. Completed the requirements for the Master of Science degree with a major in Nutritional Sciences at Oklahoma State University in May 2004.

Experience: Employed by the Institute of Food Technology, Senegal since 1983. Employed by Oklahoma State University as a research assistant at the Department of Soil Science in 2001, the Landscape and Horticulture Department in 2002 and at the Child Development Laboratory from 2003 to present.

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