PHYTOCHEMICAL, OLIGOSACCHARIDES, *IN-VITRO* STARCH/PROTEIN DIGESTIBILITY AND ELEMENTAL COMPOSITION OF ACHA-MORINGA FLOUR BLEND

Supported by tetfund TERTIARY EDUCATION TRUST FUND

J. A. Ayo*, E. O. Adedeji and A. A. Okpasu
Department of Food Science & Technology, Federal University Wukari, Nigeria
Corresponding author: jeromeayo@gmail.com

Received: December 21, 2017 **Accepted:** February 10, 2018

Abstract: The research investigated into the effects of added moringa seed powder on the phytochemical, oligosaccharide, in-vitro starch/protein digestibility and the elemental content of acha-moringa flour blend. The moringa seed flour was substituted (5, 10, 15, 20 and 25%) into acha flour to produce flour blends. The flour blends and the control (100% acha flour) were analysed for phytochemicals (tanin, HCN, alkaloid, saponin, phytate, flavonoid, steroid, phenol), oligosaccharides (raffinose and starchyose), starch/protein in vitro digestibility, elemental (calcium, phosphorous, magnesium, iron, zinc) and vitamins (vit. A, C and B_{12}. The phytochemical result showed increase in phenol (2.67 mg/g to 3.15 mg/g), steroids (0.35 to 0.52 mg/g) and flavonoid (0.83 to 1.01 mg/g), but decrease in saponin (2.96-1.98 mg/g) with increase in the added moringa paste (5-25%). The antinutrient result show increase in tannin (0.86 to 1.28 mg/g), cyanogenic glycoside (HCN) (2.72 to 3.03 mg/g), alkaloid (1.23 to 1.49 mg/g), alkaloid (1.23-1.43mg/g), but decrease in phytate (1.63- 1.06 mg/g) with increase in the added moringa paste. The invitro-protein digestibility increased from 75.70±0.14 – 82.90±0.18 while the in-vitro-starch digestibility decreases from 68.60±0.14 – 62.03±0.04% with increase in the added moringa paste (0-25%). The starchyose and raffinnose values of the acha-moringa seed flour blends ranges from 0.51±.01 - 0.66±.04 and 0.36±.03-0.41± 01%, respectively. The calcium, magnesium, phosphorous, zinc and iron content of the acha –moringa flour blend ranged from 18.62±.08-20.29±19, 28.94±.09-30.95±.08, 35.29±.08- 42.34±.2, 0.72±.03-1.08 ±.06 and 0.84±.08b-1.39± .05 mg/100g, respectively with addition of moringa flour at 5, 10, 15, 20 and 25%. The effects of added moringa seed flour were generally significant, $p<0.05$, and positive on the assessed qualities consequently improving the nutrient content of the eacha-moringa flour blend.

Keywords: Quality, phytochemicals, anti-nutritional, digestibility, oligosaccharides, acha-moringa flour blend

Introduction

Acha (*Digitariaexillis*) is a cereal grain in the family of gramineae and commonly referred to as fonio or hungry rice (Alamu, 2001, Ayo and Nkama 2006). The major traditional foods from the grain are: thick (tuwo) and thin (kunu), porridge, steamed product (*brabusco* or couscous) and alcoholic beverages (Jideani and Akingbala, 1993). Acha grains may be boiled like rice; flour from acha may be fortified with other cereals flour especially for the production of porridge or pudding (Ayo and Nkama, 2003; Ayo and Nkama 2006). Acha grain can also be milled into flour to produce biscuit and bread with desirable qualities (Ayo and Nkama, 2003; Alain *et al*., 2007).

Acha can be classified based on the color and sizes of the grain. Acha is also one of the most nutritious of all grains; its seed is rich in methionine and cysteine which are vital to human health and deficient in today's major cereals like wheat, rice, maize, sorghum, barley and rye (Jideani and Akingbala, 1993; Ogbona and Abdulkadir 2008). The consumption of cereal based foods like biscuit has triggered required development of an adequate substitute for wheat (Ayo and Nkama, 2003; Jideani and Jideani 2011). Acha is also known for its nutritional properties although the protein content of acha is similar or slightly lower than that of other grains, it contains amino acid like methionine and cysteine which are essential for human health, which are often deficient in today's major cereals. Acha is known to be easy to digest, it is traditionally recommended for children, old people and for people suffering from diabetes or stomach diseases (Jideani and Jideani, 2011; Jideani *et al*., 1996). Acha does not contain any glutenin or gliadin proteins which are the constituents of gluten, making this cereal suitable for people with gluten intolerance (Harlan, 1993; Ayo *et al*., 2007).

The in-vitro starch digestibility and glycemic property of acha, iburu and maize porridge has been investigated (Jideani and Podgorski 2009; Jideani and Ibrahim 2005). The study showed that the total starch (TS) for maize, acha and iburu flours were 45.3, 43.6 and 41.5%, respectively. The resistant starch (RS) was 2.9, 2.1 and 1.2, respectively for maize, acha and iburu flours and the digestible starches (DS) 43.7, 41.4 and 40.0%. The authors conclude that acha and iburu may have potential in a low GI food as porridge from both grains had low estimated value of 40 (Jideani and Podgorski, 2009; Jideani *et al*., 2005).

Moringa oleifera tree is widely cultivated due to its high adaptability to environmental conditions (Teixira *et al*., 2014). It's considered as one of the most useful trees in the world because almost all parts of this plant can be used as in food, in medicines and for industrial purposes (Anwar *et al*., 2007). In many countries, there are huge efforts to spread the use and cultivation of *M. oleifera*, since it is a significant source of fats, proteins, beta-carotene, vitamin C, iron, potassium, and other nutrients with low toxicity of seeds and leaves (Saini *et al*., 2014).

Moringa plant have drawn much attention and have been studied for its various biological activities, including antiatherosclerotic(Chumark *et al*., 2008), immune-boosting (Miyoshi *et al*., 2004), anticardiovascular diseases (Faizi *et al*., 1994), antiviral (Khalafalla *et al*., 2010), antioxidant and antimicrobial Kumar *et al*., 2012, anti-inflammatory (Kumar *et al*., 2013; Lister and Wilson 2001; Kumar *et al*., 2013) properties and tumor suppressive effects in skin papillomagenesis, hepatocarcinoma cancer, colon cancer, and myeloma (Khalafalla *et al*., 2010; Budda *et al*., 2011; Bharali *et al*., 2003; Brunelli *et al*., 2015).

Pinto *et al*. (Pinta *et al*., 2015) demonstrated that vegetable proteins are less susceptible to in vivo digestion than animal proteins. The low content of sulfur containing amino acids, compact structure, presence of non-protein components (dietary fiber, tannins, phytic acid) and antiphysiological proteins (protease inhibitors, lectins) can affect digestion. Teixeira *et al*. (2004) found that whole leaf flour contained 28.7% crude protein, 7.1% fat, 10.9% ashes, 44.4% carbohydrate and 3.0 mg 100 g^{-1} calcium and 103.1 mg 100 g^{-}

[1] iron. The protein profile revealed levels of 3.1% albumin, 0.3% globulins, 2.2% prolamin, 3.5% glutelin and 70.1% insoluble proteins. Otherwise, the most recent investigations reported that a flocculating protein (6.5 kDa, IEP pH 10) from the seeds of *M. oleifera* was isolated and purified. Amino acid analysis and sequencing showed high contents of glutamine, arginine and proline, and a total of 60 residues (Freire *et al*., 2015; Kwaambwa *et al*., 2015; Gasschmidt *et al*., 1995 and Pavankumar *et al*., 2014).

In general, there are low concentrations of antinutritional factors in the plant, although the seeds possess glucosinolates (65.5 µmol/g dry matter), phytates (41 g/kg) and hemagglutination activity while the leaves have appreciable amounts of saponins (80 g/kg), besides low quantity of phytates (21 g/kg) and tannins (12 g/kg), hypotensive activity, strong antioxidant activity and chelating property against arsenic toxicity (Arabshahi *et al*., 2007; Ghasi *et al*., 2000; Mehta *et al*., 2003; Santos *et al*., 2009). This research work was aimed at evaluating the phytochemical, anti nutrient, starch/protein digestibility, elemental and oligosaccharide composition of Acha-moringa flour blend.

Materials and Methods

Material and material preparation

Moringa olieferal was obtained from Wuakri Main market, Nigeria. The hulls of moringa seeds were removed manually milled (attrition mill) and passed through a sieve of 0.35 nm aperture to produce the moringa flour. The acha grains were washed, destoned (sedimentation method using local calabash as described by Ayo and Nkama (2003), oven dried (45^{o}C), milled (attrition mill), and pass through a sieve aperture of 0.35 nm to produce acha flour. The moringa seed flour was substituted into acha flour at 5, 10, 15, 20 and 25%, while 100% acha flour served as control or standard. A Kenwood mixer was used for mixing samples at speed 6 for 3 min to achieve uniform blending.

Determination of bioactive and ant-nutritional composition of acha–moringa flour blends

Determination of alkaloid

Determination of alkaloid was made by the method described by Oluwole *et al*. (2013). The alkaloid content was determined gravimetrically. Five grams of the sample was weighed and dispensed in 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 h at 28°C. It was later filtered via Whitman No. 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution, and dried in the oven at 80°C. Alkaloid content was calculated and expressed as a percentage of the weight of sample analyzed.

Determination of saponins

The spectrophotometric method was used for saponin analysis as described by Oluwole *et al*. (2013). One gram of the flour sample was weighed into a 250-mL beaker and 100 mL isobutyl alcohol was added. The mixture was shaken on a UDY shaker (UDY Corporation, Fort Collins, CO) for 5 h to ensure uniform mixing. The mixture was filtered through a Whitman No. 1 filter paper into a 100-mL beaker and 20 mL of 40% saturated solution of magnesium carbonate was added. The mixture obtained was further filtered through a Whitman No. 1 filter paper to obtain a clear colorless solution. One milliliter of the colorless solution was homogenized into a 50-mL volumetric flask and 2 mL of 5% $FeCl_3$ solution was added and made up to mark with distilled water and allowed to stand for 30 min for blood red color to develop. Standard saponin solutions (0–10 ppm) were prepared from saponin stock solution and treated with 2 mL of 5% FeCl solution as done for experimental samples. The absorbance of the sample as well as standard saponin solutions were read after color development on a 2lD spectrophotometer (Milton Roy, Houston, TX) at a wavelength of 380 nm. The percentage saponin was also calculated.

Determination of tannin content

Tannin content of the flour samples was determined using the methods described by Swain (1979) and Oluwole *et al*. (2013). The sample (0.2 g) was measured into a 50-mL beaker; 20 mL of 50% methanol was added, covered with homogenizer, placed in a water bath at 77–80°C for 1 h, and the contents stirred with a glass rod to prevent lumping. The mixture was filtered using a double-layered Whitman No. 1 filter paper into a 100-mL volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. One milliliter of the sample extract was homogenized into a 50-mL volumetric flask, and 20 mL distilled water, 2.5 mL Folin-Denis reagent, and 10 mL of 17% Na_2CO_3 were added and mixed. The mixture was made up to mark with distilled water, thoroughly mixed, and allowed to stand for 20 min when bluish-green coloration developed. Standard tannic acid solutions in the range of 0–10 ppm were treated similarly as the 1 mL sample above. The absorbance of the tannic acid standard solutions as well as samples was read after color development on a 21D spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated.

Determination of phyticacid

An indirect colorimetric method of Wheeler and Ferrel (1971) and modified by Oluwole *et al*. (2013) was used for phytate determination. This method depends on an iron to phosphorus ratio of 4:6. A quantity of 5 g of the test sample was extracted with 3% trichloro acetic acid. The phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding sodium hydroxide. The precipitate was dissolved in hot 3.2 N HNO and the color read immediately at 480 nm. The standard solution was prepared from $Fe(NO_3)_3$ and the iron content was extrapolated from a $Fe(NO)_3$ standard curve. The phytate concentration was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio.

Determination of oxalate content

Oxalate was determined by AOAC (2005) method. One gram of the sample was weighed in a 100-mL conical flask. Seventy-five milliliters of 3 mol/L H_2SO_4 was added and the solution was stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whitman No. 1 filter paper. The sample filtrate (extract) (25 mL) was collected and titrated against hot (80–90°C) 0.1 N $KMnO_4$ solution to the point when a faint pink color appeared that persisted for at least 30 sec. The concentration of oxalate in each sample was obtained from the calculation: 1 mL 0.1 permanganate = 0.006303 g oxalate

Determination of steroids

The Steroids was determined by the method described by Okeke and Elekwa (2003).

Total carotenoids determination

According to Yuan *et al*. (2009), 5 g of each defatted moringa Ethanol-Petroleum Concentrate were extracted with a mixture of acetone and petroleum ether (1:1, v/v) repeatedly using the mortar and pestle until a colorless residue was obtained. The upper phase was collected and combined with crude extracts after washed for several times with water. The extracts were made up to a known volume with petroleum ether. Total carotenoids content was determined by recording the absorbance at 451 nm with a spectrophotometer. Total carotenoids were calculated and expressed as mg **g−1** dw.

Flavonoids determination

The total flavonoids content of moringa EPC were determined according to the method of Mohdaly *et al*. (2012). A 0.5 ml aliquot of 2% $AlCl_3$ ethanolic solution was added to 0.5 ml of the extracts and mixed well. After keeping for 1 h at room temperature, the absorbance at 420 nm was measured. A yellow color indicates the presence of flavonoids. The total flavonoids content were expressed as mg quercetin equivalent (QE) per 100 gdw.

Determination of total phenolic compounds

The Folin-Ciocalteu assay, adapted from Ramful *et al.* (2011) was used for the determination of total phenolics present in the citrus fruit extracts. To 0.25 mL of diluted extract, 3.5 mL of distilled water was added followed by 0.25 mL of Folin-Ciocalteureagent (Merck). A blank was prepared using 0.25 mL of 80% methanol instead of plant extract. After 3 min, 1 mL of 20% sodium carbonate was added. Tube contents were vortexed before being incubated for 40 min in a water-bath set at 40ºC. The absorbance of the blue coloration formed was read at 685 nm against the blank standard. Total phenolic were calculated with respect to gallic acid standard curve (concentration range: 0-12 µg mLG1). Results are expressed in mg of gallic acid 100 gG1 of plant material.

Determination of starchyose and raffinnose

The high performance liquid chromatography (HPLC) method for Sugar analysis was used for the determination of raffinose and starchyose (BabuValliyodan *et al*., 2015)

Mineral determination

AOAC (2005) methods were used to determine the mineral compositions of the samples. One gram of sample was digested with nitric/perchloric/sulfuric acid mixture in the ratio 9:2:1, respectively, and filtered. The filtrate was made up to mark in a 5-mL volumetric flask. The standard curve for respective mineral was prepared from known standards and corresponding values of minerals in the samples estimated. Values of sodium and potassium were determined using a Flame photometer (Sherwood Flame Photometer 410; Sherwood Scientific Ltd., Cambridge, U.K.) using NaCl and KCl as the standard (AOAC 2005), while phosphorus was determined using the Vanodo-molybdate method.

Determination of starch/protein digestibility of the blends

The in vitro protein digestibility of the samples were determined using the procedure described by Mertz *et al*. (1984) and Aboubacar *et al*., (2001) while in vitro starch digestibility were determined as described by Shekib *et al*. (1988) and Chinma *et al*. (2012)

Detrmination of oligosaccharides (raffinose and stachyose)

Raffinose and starchyose were determined by the methods of Matella *et al*. (2005) and Siddiq*et al*. (2006).

Quantitative analysis of the vitamins

To measure the Vitamin C contents of the samples Gholamreza *et al*. (2015) method was used. The experimental methods described by Gholamreza *et al*. (2015) were used to measure the Vitamin A contents of the sample.

Determination of hydrogen cyanide

Titrimetric method (AOAC, 2005) was used. The titration procedure using silver nitrate with p-dimethylamino-benzal-rhodanine indicator was used.

Statistical analysis

Data was analyzed using analysis of variance. Duncan multiple range test was used to determine significant difference among the various samples in triplicate. Data were analyzed using the software, statistical package for social science (SPSS) version 11.00 SPSS inc., Chicago, IL, USA at the 0.05 confidence level.

Results and Discussion

Phytochemical composition of acha-moringa flour blend

The results of thephytochemical composition of moringa seed-acha flour blend are shown in Table 1. The result showed increase in phenol (2.67 to 3.15 mg/100g), steroids (0.35 to 0.52 mg/100g) and flavonoid (0.83 to 1.01 mg/100g), but decrease in saponin (2.96-1.98 mg/100g) with increase in the added moringa paste (0-25%). The effects were significant, $p< 0.05$. The increase in the value of the phytochemical with addition of moringa could be due to the relative high value of the same in the moringa seed flour (Barakat and Ghazal 2016; Melesse *et al*., 2013). Moringa has been found to be a good source of polyphenols and antioxidants (Melesse *et al*., 2013). Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol and quercetin have been reported in its flowers, roots, fruits, and seeds (Mishra *et al*., 2011; Kowalski 2010). The increase in the phenolic values of acha-moringa flour blend could be due to the relatively high phenolic values (10.179 ± 2.894 mg/100g) as earlier reported (Sulaiman and Fazilah, 2015; Singh *et al*., 2009; GovardhanSingh, 2013). However, the relatively low value observed in the work could be due environmental factors such as light, germination, degree of ripeness, variety, processing and storage, genetic factors can influence levels (Ahn *et al*., 1989). The trace quantities of flavnoid compounds indicate that the sample could act as immune enhancers, hormone modulators, antioxidant, anti-clothing and anti-inflammatory Okwu and Omodamoro (2005). They have been reported to be a potential contender to combat free radicals, which are harmful to our body and food systems (Nagai *et al.*, 2003).

The saponin values (1.98-2.94 mg/100g) observed in the work is relatively higher than the value (0.5 mg/100g) observed by Price *et al*. (1987), however, the decrease in the saponin values with increase in the added moringa flour could be due to its low saponin content of moringa. Saponins are generally characterized by their bitter taste, their ability to foam in aqueous solution, causing nausea, vomiting and their ability to hemolyse red blood cells (Jansman *et al*., 1998). Similarly, the saponin content of acha-moringa flour blends (1.98-2.94 mg/100g) were lower than that (5.20 mg/100 g) observed by Seena (2008). The lowering of the saponin value with addition of moringa flour could be an advantage over the deleterious effects of the same.

The values of phenol of the ach-moringa flour blend increased from 2.73-3.15 mg/100g with increase in the added moringa flour (5-25%), and the increase was significant, $p<0.5$. The increase could be due to the relatively high level of phenol (2.900 ± 0.002 mg) inherent in moringa flour as observed by Sulaimanand Fazilah (2015). Phenol have been observed as antioxidant suppress ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation, scavenging ROS; and protection of antioxidant defenses, anti-inflammatory, and antihypertensive properties (Singh *et al.*, 2009; Ayinde *et al*., 2007; Li-Weber 2009; Shashank and Abhay, 2013).

Table 1: Phytochemical composition of acha- moringa flour blend

Moringa (%)	Acha flour (%)	Saponin (%)	Phenol (mg/100g)	Steroid (mg/100g)	Flavonoid (mg/100g)
0	100	2.96±.01a	2.67± 03e	0.35±.01d	0.83±.01e
5	95	2.84±.05a	2.73±.04de	0.4±.02cd	.84 ± .02e
10	90	2.52±.06b	2.87±.04cd	0.41±.04cd	.89 ±.01d
15	85	2.53±.08b	2.95±.07bc	0.44±.00bc	.95 ±.01c
20	80	2.3 ± .06c	3.06±.08ab	0.47±.01ab	.20 ±.02b
25	75	1.98±.12d	3.15±.08a	0.52±.04a	1.01±.02a

Plant sterols, also called phytosterols, found in plants, are clinically shown to lower LDL cholesterol as part of a heart-healthy diet. Clinical studies suggest that plant sterols can reduce cholesterol by 8–15% (Kowalski, 2010). Plant sterols

have been observed to be Generally Recognized as Safe in a variety of food and beverage applications (Kowalski, 2010; Harborne, 1998).

Anti-nutrient composition of acha –moringa flour blend

The result of the anti-nutrient compounds is shown in Table 2. The results showed increase in tannin ranging from (0.86 to 1.28 mg/100g), cyanogenic glycoside (HCN) (2.72 to 3.03 mg/100g), alkaloid (1.23 to 1.49 mg/100g), alkaloid (1.23-1.43 mg/100g), but decrease in phytate (1.63 - 1.06 mg/100g) with increase in the added moringa paste. The increase in the tannin value of the blends agreed with findings of Sulaiman and Fazilah (2015) that moringa seed contain 0.890 ± 0.020 mg/100g of tannin. Satinder *et al.* (2011), reported lower value of tannin for wheat bran, rice bran, oat bran and the value reported by Okwu and Ndu (2006) is lower than the value reported for this work. Tannin contents of the acha-moringa flour blends (0.98- 1.28 mg/100g) were lower than those reported for groundnut seeds (450.00 mg/100 g; Fasoyiro *et al.*, 2008), sorghum grains (280.00 mg/100 g; Elemo *et al*., 2001), and Cajanuscajan (550.00 mg/100 g; Ayodele and Kigbu, 2005).

Tannins have been reported to speed up the rate of healing in enlarged mucous membrane, to be quick in curing of wounds and to possess astringent properties. The presence of tannin in the flour will support their use in treating hemorrhoid, varicose ulcers, frostbite, burns in herbal medicine and wound (Okwu and Okwu, 2004).

Phytic acid has a strong ability to chelate multivalent metal ions, specially zinc, calcium, iron and as with protein residue. The binding can result in very insoluble salts with poor bioavailability of minerals (Zhou and Erdman, 1995).They reduce the bioavailability and digestibility of nutrients by forming complexes with minerals, protein, digestive enzymes and amino acids mainly lysine, methionine, arginine and histidine (Bird 1991; Saunders *et al*., 1986).

The hydrogen cyanide value of the acha-moringa flour blend were very low (2.9 -3.03 mg/100g) and insignificant to the upper safe level 50-200 mg/100g or 100-200 ppm) (Public Health England, 2014). It could therefore be said that the blends are safe for consumption and free from ill effects of hydrogen cyanides such as: non-specific CNS symptoms, muscular and neurological effects, tachyponea and tachycardia, include seizures, a rapid loss of consciousness, cardio respiratory depression and collapse, pulmonary oedema and death (Public Health England, 2014; Shanthakumari *et al.*, 2009)

The increase in the phytochemical values as observed in this work agreed with observation of Soetan (2008) that addition of moringa seed flour influenced the phytochemical compositions of the blend flour and subsequently that of the processed food materials. Comparatively, the alkaloid content of acha-moringa seed flour blends (1.29-1.49 mg/100g) were lower than that of the upper limit of 60 mg/100 g recommended for a safe feed (McDonald *et al*., 1995).

It is evident that anti-nutrients and phytochemicals have both adverse and beneficial effects in humans (Soladoye and Chukwuma, 2012). For example, phytic acid, lectins, phenolic compounds and tannins, saponins, enzyme inhibitors, cyanogenic glycosides, and glucosinolates reduce the bioavailability of certain nutrients and impair growth in children (Elemo *et al*., 2001; Dingynan *et al*., 2003). On the contrary, when phytic acid, lectins, and phenolic compounds and saponins were used at low levels, they exhibited hypoglycemic, hypocholesterolemic and anticancer properties (Yoon *et al*., 1983; Sidhu and Oakenful 1986; Thompson *et al*., 1988; Jariwalla *et al*., 1990; Oakenfull and Sidhu, 1990).

Table 2: Anti nutrient composition of acha- moringa flour blend

Moringa (%)	Acha flour (%)	Tannin (mg/100g)	HCN (mg/100g)	Phytate (mg/100g)	Alkaloid (mg/100g)
0	100	0.86± .01c	2.72±06d	1.63±.03a	1.23± .04d
5	95	0.98±.012bc	2.80±.14cd	1.53±.08ab	1.29±.04cd
10	90	1.00±.03abc	2.90±.06bc	1.47±.02bc	1.34±.06bc
15	85	1.06±.03ab	2.94±.06abc	1.41±.04cd	1.38±.03abc
20	80	1.15±.04a	3.00±.03ab	1.36±.06cd	1.43±.01ab
25	75	1.28± .03a	3.0 ±.02a	1.34±.02d	1.49± .07a

In-vitro protein and In-vitro carbohydrate digestibility of Acha-moringa flour blend

The result of the *in-vitro* protein and *in-vitro* starch digestibility are shown in Fig. 1. The in-vitro protein digestibility increased from 75.70±0.14 – 82.90±0.18% while the in-vitro starch digestibility decreases from 68.60±0.14 – 62.03±0.04% with increase in the added moringa flour (0-25%). The in-vitro starch digestibility values obtained in this study is relatively higher than 26.43-57.25% for tigernut-pigeon pea blend reported by Chinma *et al*. (2011), 32.68-53.12% for unripen plantain-defatted sesame flour blend biscuits (Chinma *et al*., 2012; Zebib *et al*., 2015) and 36.08-52.36% reported by Jishaand (2011) for whey-protein concentrate-cassava flour biscuits. The decrease in the in-vitro starch digestibility of the flour blend as the level of moringa seed paste increased in the blend may be attributed to the increased crude fiber content which could caused a reduction in the starch digestibility by trapping starch granules within a viscous protein-fiber-starch net work (Chinma *et al*., 2011; Chinma *et al*., 2012). The presence of protein bodies around starch granules (due tin increased protein content) as observed by Chinma *et al*. (2012) may restrict granule swelling and starch gelatinization and hence, reduce the susceptibility to enzymatic attack (Aarath *et al*., 2003), thereby reducing in-vitro starch digestibility of acha-moringa flour blend.

The poor starch digestibility values of the flour blend as observed by Chinma *et al*. (2012) and Kin-Kabari and Giami (2015) may be an indication that the blend flour could serve as a functional food for groups with special calorie and glycemic requirements such as obesity or diabetic people. One of the major developments in the understanding of the importance of carbohydrates for health in the past twenty years has been the discovery of resistant starch. Resistant starch is defined as "starch and starch degradation products not absorbed in the small intestine of healthy humans" (Mishra *et al*., 2011). The main forms of resistant starch are physically enclosed starch, e.g. within intact cell structures (RS_1), some raw starch granules (RS_2) and retrograded amylose (RS_3) (Mbikay, 2012).

The in-vitro starch digestibility and glycemic property of acha, iburu and maize porridge has been investigated (Jideani and Podgorski, 2009; Jideani *et al*., 1996) and showing that the total starch (TS) of their respective flours to be 45.3, 43.6 and 41.5%, respectively. The resistant starch (RS) was 2.9, 2.1 and 1.2, respectively for maize, acha and iburu flours and the digestible starches (DS) 43.7, 41.4 and 40.0%. The authors conclude that acha and iburu may have potential in a low GI food as porridge from both grains had low estimated value of 40 (Jideani and Podgorski, 2009). A relatively higher value of starch digestibility (68.60±0.14%) values for 100% acha was observed in this work.

In-vitro protein digestibility is an important criterion for evaluation of protein quality as well as an indicator for protein bioavailability in foods (Chinma *et al*., 2012, Ayo *et al*., 2007). The *in-vitro*-protein digestibility of the acha-moringa flour blend increased from 75.70±0.14 to 82.90±0.18% with

increase in the added moringa paste (5-25%). The effect of the added moringa paste on the in-vitro protein digestibility of the flour blend is significant, $p<0.05$. The increase in the in-vitro protein digestibility of the blend flour could be due to the increase in the protein content inherent in the added moringa seed paste.

The addition of moringag flour to the flour blend improve the protein digestibility over the control which confirm that flour blend have better nutritional value than the 100% acha flour. The in-vitro protein digestibility obtained in this work(75.70±0.14 – 82.90±0.18%) is in close agreement with the value (71.20 to 80.0%) reported by El-Adawy (1997) and the value (72.05 to 80.12%) reported by Chinma *et al*. (2015) for wheat-sesame flour blend and for unripe-plantain- sesame flour blend, but slightly higher than the value (60.20 to 71.57%) reported by Chinma *et al*., (2011) for tigernut-pigeon pea flour blend.

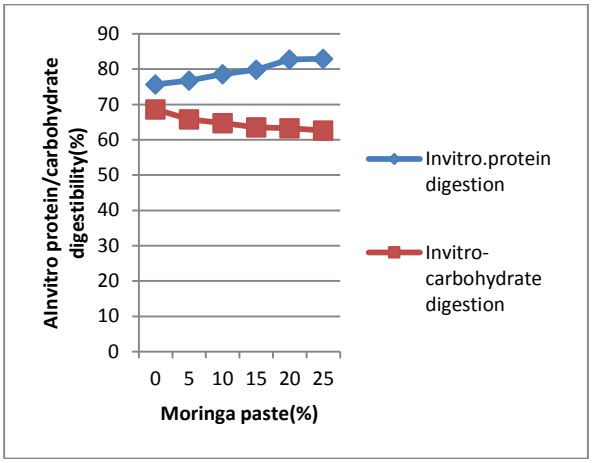

Fig. 1: Invitro protein and carbohydrate digestibility of acaha-moringa flour blend

Starchyose and raffinose composition of acha –moringa flour blend

The starchyose and raffinnose values of the acha-moringa seed flour blends ranges from 0.51± 0 .01 - 0.66± 0.04 and 0.36± .03-0.41 ± .01%, respectively, as shown in Fig. 2. The values of thestarchyose and refinnose in the blend flours and the control (100% achaflour) were relatively low. The presences of starchyose and rsfinnose could be advantageous or discomfort depending on the level or concentration (Siddiq *et al*., 2006). Raffinose and stachyose are non-digestible short-chain carbohydrates or oligosaccharides. Humans do not have enzymes to digest them, so they pass unchanged to the colon where the normal intestinal bacteria ferment them to gases (methane, carbon dioxide, hydrogen-gases that are responsible for the characteristic features of flatulence, namely nausea, cramps, diarrhea, and the social discomfort associated with the release of rectal gases.), which can cause abdominal bloating (Storey *et al*., 1998; Matella *et al*., 2005 and Siddiq *et al*., 2006). In the large intestine, raffinose and stachyose could act as a soluble dietary fiber, which means they can make stools softer (Matella *et al*., 2005; Nakakuki, 2002). They could also be used as bulk sweeteners (Siddiq *et al*., 2006).

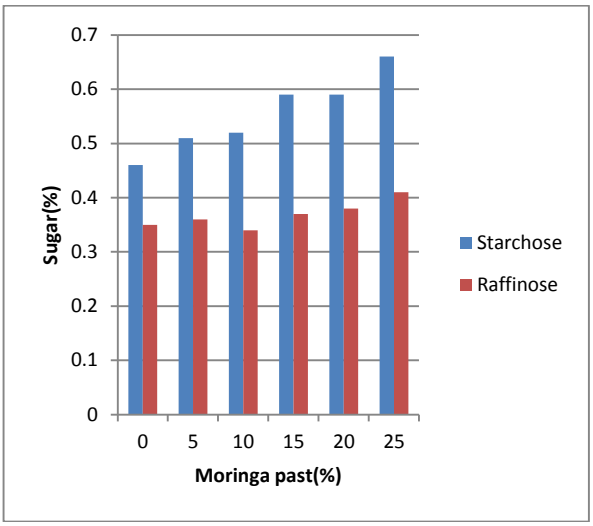

Fig. 2: Starchose and rafffinose content of acha-moringa flour blend

Minerals and vitamin composition of acha-moringa flour blend

The minerals content as macro-elements (calcium, phosphorus and magnesium) and microelements (iron and zinc) in mg 100 g−1 of acha-moringa flour blend were given in Table 3. The calcium, magnesium, phosphorous, zinc and iron content of the acha–moringa flour blend ranged from 18.62±.08-20.29 ±.19, 28.94 ± .09-30.95± .08, 35.29 ± .08- 42.34± .2, 0.72± .03-1.08 ± .06 and 0.84± .08b-1.39 ± .05 mg/100g, respectively with addition of moringa flour at 5, 10, 15, 20 and 25%. The increase were significantly higher ($p<0.05$) than the control (100%). The increase in the elemental content with increase in added moringa agreed with findings of Barakat and Ghazal (2016) that the moringa seed flour contain calcium (2016 to 2620 mg/100g), magnesium (322 to 340.6 mg/100g) and phosphorious (1817 to 1845 mg/100g), while Zn was 1.0 mg/100g (w/wt).

The minerals found in M. oleifera could play both a curative and preventive role in combating human disease. For example, Ca is a multifunctional nutrient essential to the body metabolism (Sizer and Whitney, 1999; Freire *et al*., 2005), and a natural cure for osteoporosis (Howard, 2014.). Furthermore, there is strong biological plausibility for the direct impact of Mg intake on cardiovascular disease prevention, insulin sensitivity, and diabetes (Bo and Pisu 2008.), increasing the rate of pregnant female milk production and healing of wounds and functions as an antioxidant as a result of the high zinc content (Rathi *et al*., 2006), Fe has several essential functions in the body, such as its roles in oxygen transport and oxidative metabolism (Bothwell *et al*., 1979).

The phosphorous ratio (Ca/P) ratios is an indices for bone formation and the values (0.479-0.527) were relative low and within the recommended (<1) for diets, particularly for hypertensive patients. Therefore, the observed for the acha-moringa seed flour blend in this study is suitable for people who have the risk of high blood pressure and could also be of nutritional benefit, particularly for children and the aged who need higher intakes of calcium and phosphorus for bone formation and maintenance. It is well known that diets with high value of Ca/P ratio are considered "good," particularly for growing children who require high intake of calcium and phosphorus for bone and teeth formation (Nieman *et al*., 1992). Zinc is also important in the healing of wounds and functions as an antioxidant.

Table 3: Effect of added moringa on the minerals composition of acha-moringa flour blend

Acha: Moringa flour(%)	Ca (mg/100g)	Mg (mg/100g)	P (mg/100g)	Zn (mg/100g)	Fe (mg/100g)
100:0	14.61± .29c	28.67± .16f	30.61± 58c	0.66± .04d	0.76± .01c
95:5	18.62± .08b	28.94± .09e	35.29± .08b	0.72± .03d	0.84± .08bc
90:10	18.92± .06b	29.43± .04d	35.98± .42b	0.87± .02c	0.87± .03bc
85:15	19.76± .36a	30.09 ± 05c	41.47± 1.44a	0.96± .01b	0.97± .09b
80:20	20.13± .11a	30.69± .01b	40.38± 1.31a	1.01± .02ab	1.30± .11a
75:25	20.29± 19a	30.95± .08c	42.34± .2a	1.08± .06a	1.39± .05a

Table 4: Effect added of moringa on the vitamin composition of acha-moringa flour blend

Acha: Moringa flour	Vit A (mg/100g)	Vit. C (mg/100g)	Vit. B_{12} (mg/100g)
100:0	2.51 ± .04e	3.61 ± .16d	0.14 ±.02c
95:5	2.65 ± .01de	3.78 ± .13d	0.17 ± .01c
90:10	2.76 ± .02d	4.37 ± .09c	0.2 ± .04c
85:15	2.94 ± .12c	4.78± .11b	0.23 ± .04bc
80:20	3.31± .1b	5.29 ± .02a	0.31± .05ab
75:25	3.56 ± .06a	5.50 ± .06a	0.39± .05a

The results of the vitamin content of the acha-moringa flour blend and the 100% acha flour is shown in Table 4. The vitamins values for the blends were vitamins A (2.65 ± .01- 3.56 ± .06), vitamin C (3.78 ± .13- 5.50 ± .06) and vitamin B_{12} (0.17 ± .01- 0.31± .05). The vitamins values of the blends were significantly ($p<0.05$) higher than that of the 100% acha flour (2.51, 3.61 and 0.14 for vit.A, vit.C and vit.C, respectively). The increase could be due to the relatively high content of the respective vitamins inherent in moringa seed flour as observed by Gholamreza *et al*. (2015) that mornga seed contain Vitamin C (14 ± 0.6 mg/100 g) and Vitamin A (24.8 ± 0.7 mg/100g).

Conclusion

The moringa seed flour was observed to improve the phytochemical (phenol) and nutritional(minerals- Fe, Zn, P),and vitamins- Vits A, B_{12} and C) potential with improved *in-vitro* protein digestibility of the acha-moringa flour blend. The relatively low concentrations of anti-nutritional factors (phytate) on addition of moringa flour could be an added advantage in reducing the chelating of minerals such as calcium in the acha-moringa flour blend.

Acknowledgments

The authors thank the TETFUND, Nigeria for financial support and also the Department of Food Science and Technology, Federal University for allowing the use of their laboratory facilities.

References

Aboubacar A, Axiel JD, Huagng CF & Hamaker BR 2001. A rapid protein digestibility assay for identificatifying highly digestible sorghum lines. *Cereal Chem*., 78: 160-165.

Alain MM, Israel MP & Rene MS 2007. Improving the nutritional quality of cowpea and bambara bean flours use in infant feeding. *Pak. J. Nutr*., 6: 660-664.

Alamu A 2001. The Effect of Cooking on Proteins from Acha (*Digitariaexilis*) and Durum Wheat. *J. Sci. Food Agric.*, 65: 465–476.

Anwar F, Sajid L, Muhammad A & Anwarul HG 2007. *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother. Res.*, 21:m17–25.

Arabshahi DS, Devi DV & Urooj A 2007. Evaluation of antioxidant activity of some plant extracts and their heat, pH and storage stability. *Food Chem.*, 100: 1100–1105.

Association of Official Analytical Chemists (AOAC) 2005. Official methods of analysis of the Association of Official Analytical Chemists. 14th edition. Washington DC, USA, pp. 52-162.

AOAC 2005. Association of Analytical Chemists Official Methods of Analysis. In: Horowitz W, editor. Official Methods of Analysis. 18th ed. Gaithersburg, MD: AOAC; 2005.

Ayinde BA, Onwukaeme DN & Omogbai EKI 2007. Isolation and characterization of two phenolic compounds from the stem bark of *Musangacecropioides* R. Brown (Muraceae). *Acta Pol. Pharm.*, 64: 183–185.

Ayo JA & Nkama I 2003. Effects of acha (*Digitariaexilis* Staph) grain flour on the physical sensory quality of biscuit. *J. Nutr. & Food Sci.*, 33(3): 125-135.

Ayo JA, Ibrahim A & Nkama I 2003. Effect of Acha (*Digitariaexilis*) on the Body Weight, Glucose Blood Level, Haemoglobin and Packed Column Cell of Rabbit. Proceedings, National Conference of the Application of science for National Development, Mubi, Adamawa state. 25-28 June, p. 56.

Ayo JA, Ayo VA, Nkama I & Adewori R 2007. Physicochemical, in-vitro digestibility and organoleptic evaluation of acha wheat biscuit supplementation with soybean flour. *Nigeria Food Journal*, 25(1): 77-89.

Ayo JA & Nkama I 2006. Acha (*Digitariaexilis*) in West Africa. *Int J Food Agric*, 1: 129–144.

Ayodele JT & Kigbu PE 2005. Some antinutritional factors in *Cajanus cajan*, *Sterculia setigera* and *Vignadekindtiana*. *Biol. Environ. Sci. J. Tropics*, 2: 43–45.

BabuValliyodan, Haiying Shi, & Nguyen HT 2015. *A Simple Analytical Method for High-Throughput Screening of Major Sugars from Soybean by Normal-Phase HPLC with Evaporative Light Scattering Detection.* Hindawi Publishing Corporation Chromatography Research International Volume, Article ID 757649, 8.

Barakat H & Ghazal GA 2016. physicochemical properties of *Moringa oleifera* seeds and their edible oil cultivated at different regions in Egypt. *Food and Nutrition Sciences*, 7: 472-484.

Bhara R, Tabassum J & Azad MR 2003. Chemomodulatory effect of *Moringa oleifera*, Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice. *Asian Pacific J. Cancer Prevention*, 4: 131-139.

Bothwell TH, Charlton RV, Cook JD & Finch CA 1979. Iron Metabolism in Man, Blackwell Scientific Publications, Oxford, UK

Bo S & Pisu E 2008. Roleofdietary magnesium in cardiovascular disease prevention, insulin sensitivity and diabetes. *Current Opinionin Lipidology*, 19(1): 50–56.

Budda, S, Butryee C, Tuntipopipat S, Rungsipipat A, Wangnaithum S, Lee JS & Kupradinun P. 2011. Suppressive effects of *Moringa oleifera* Lam pod against mouse colon carcinogenesis induced by azoxymethane and dextran sodium sulfate. *Asian Pacific J. Cancer Prevention*, 12: 3221-3228.

Brunelli D, Tavecchio M, Falcioni C, Frapolli R, Erba E, Iori R, Rollin P, Barillari J, Manzotti C, Morazzoni P & D'Incalci M 2015. kB and Reduces Myeloma Growth in Nude Mice in Vivo. *Biochemical Pharmacology*, 79: 1141-1148.

Chinma CE, James S, Imam H, Ocheme OB & Anuonye JC 2011. Biscuit making potentials of tigernut (*Cyperusesculentus*) and pigeon pea (*Cajanuscajan*) flour blends. *Nig. J. Nutr. Sci*., 32: 55-62.

Chinma CE, Igbabul BD & Omotayo OO 2012. Quality characteristics of cookies prepared from unripe plantain and defatted sesame flour blends. *Am. J. Food Techn.*, 7(7): 398-408.

Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales, NP, Phivthong-ngam L, Ratanachamnong P, Srisawat S & Pongrapeeporn KUS 2008. The in Vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. *Journal of Ethnopharmacology*, 116: 439-446.

Elemo BO, Elemo GN, Agboola OO & Oyedun AB 2001. Studies on some anti-nutritive factors and in-vitro protein digestibility of *Thaumatococcusdanielli* (Benth) wastes. *Nig. J. Biochem. Mol. Biol*., 16: 43–46.

Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K & Gilani AH 1994. Isolation and structure elucidation of new nitrile and mustard oil glycosides from *Moringa oleifera* and their effect on blood pressure. *Journal of Natural Products*, 57: 1256-1261.

Freire JE, Vasconcelos IM, Moreno FB, Batista AB, Lobo MD, Pereira ML, Lima JP, Almeida RV, Sousa AJ, Monteiro-Moreira AC, Oliveira JT & Grangeiro TB 2015. Mo-CBP3, an antifungal chitin-bind- ing protein from *Moringa oleifera* seed.

Gholamreza A, Abbasali P & Behnosh B 2015. Quantitative analysis of the nutritional components in leaves and seeds of the Persian *Moringaperegrina* (Forssk.) fiori. *Pharmacognosy Res.*, 7(3): 242–248.

Ghasi S, Nwobodo E & Ofili JO 2000. Hypocholesterolemic effects of crude extract of leaf of Moringaoleifera Lam in high-fat diet fed Wistar rats. *J. Ethnopharmacol.*, 69: 21–5.

Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T, Kozuka M, Ito Y, Tokuda H & Nishino H 1999. An antitumor promoter from *Moringa oleifera* Lam. *Mutat. Res./Genet. Toxicol. Environ. Mutagen*., 440: 181–188.

GovardhanSingh RS, Negi PS, Radha C 201). Phenolic composition,antioxidant and antimicrobial activities of free and bound phenolic extracts of Moringaoleifera seed flour. J.Funct. Foods , 5, 1883–1891.

Gassenschmidt U, Jany KD, Tauscher B & Niebergall H 1995. Isolation and Characterization of a flocculating protein from *Moringa oleifera* Lam. *Biochimicaet Biophysica Acta*, 1243: 477-481.

Harborne JB 1998. Phytochemical Methods. A Guide to Modern Technology of Plant HN Graham. Wiley Encyclopedia of Food Science and Technology, John Wiley and Sons, New Jersey, 1999, pp. 1–4.

Howard A 2014. Moringa – The Natural Cure for Osteoporosis. Free Press Release 5.0, Free Press.

Kumaran A & Joel Karunakaran R 2007. In vitro antioxidant activities of methanol extracts of five phyllanthus species from India. *LWT-Food Science and Technology*, 40: 344-352.

Khalafalla MM, Abdellatef E, Dafalla HM, Nassrallah A, aboul-Enein A & Lightfoot DA 2010. Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma. *African J. Biotech.*, 9: 8467-8471.

Kumar V, Pandey N, Mohan V & Singh RP 2012. Antibacterial and antioxidant activity of extract of *Moringa oleifera* leaves—An in-vitro study. *Int. J. Pharmac. Sci. Review & Res.*, 12: 89-94.

Kumar, G.S., Kumar, B.F.B.P., Srinivasan, B.F.T.C., Nag, T.F.S., Srivastava, S.F.R., Saxena RFA & Aggarwal A 2013. Retinoprotective effects of *Moringa oleifera* via antioxidant, anti-inflammatory, and anti-angiogenic mechanisms in streptozotocin-induced diabetic rats. *J. Ocular Pharmacology and Therapeutics*, 29: 419-426.

Kin-Kabari DB & Giami SY 2015. Production and quality assessment of enriched cookies from plantain flour and bambara groundnut protein concentrate. *Eur. J. Food Sc. & Techn.*, 3(4): 32-40.

Kwaambwa HM, Hellsing MS, Rennie AR & Barker R 2015. Interaction of *Moringa oleifera* seed protein with a mineral surface and the influence of surfactants. *J. Colloid and Interface Sci*., 448: 339-346.

Jansman AJ & Hill GD, Huisman J & Vander Poel AF 1998. Recent advances of research in anti-nutritional factors in legumes seeds. Wageningen. The Netherlands: *WageningenPers*, pp. 76- 81

Jideani VA & Podgorski SC 2009. In-vitro starch digestibility and glycemic property of acha (Digitariaexilis) porridge. *Cereal Foods World Suppl.*, 54: 48.

Jideani AI & Akingbala JO 1993. Some physiochemical properties of acha (*Digitariaexilis*Staph) and Ibuns (*Digitariainunia*Staph) Grains. *J. Food Sci. Agric.*, 63: 369-374.

Jideani IA & Ibrahim ER 2005. Some food potential of acha (*Digitariaexilis*) and iburu (*Digitariaiburua*) grains emanating from *Current Research*. In: Okoli EC (ed) Proceedings of the 29th AnnualNigerian Institute of Food Science and Technology Conference/AGM, 11–13 October at the Women Development Centre Abakaliki, Nigeria, pp. 60–61.

Jideani I & Jideani VA 2011. Developments on the cereal grains *Digitariaexilis* (acha) and *Digitariaiburua* (iburu). *J. Food Sci. Techn.*, 48(3): 251–259.

Jideani IA, Takeda Y & Hizukuri S 1996. Structures and physico-chemical properties of starches from acha (*Digitariaexilis*), iburu (*Digitariaiburua*) and tamba (*Eleusinecoracana*). *Cereal Chem.*, 73: 677–685.

Kowalski RE 2010. Plant sterols: a natural way to lower cholesterol. http://www.corowise.com/pdf/PlantSterols.pdf/. Accessed 13 December 2010.

Lister E & Wilson P 2001. Measurement of total phenolics and ABTS assay for antioxidant activity (personal communica-tion). Crop Research Institute, Lincoln, New Zealand.

Li-Weber M 2009. Therapeutic aspects of flavones: The anticancer properties of Scutellaria and its main active constituents Wogonin, Biacalum and Bacalin. *Cancer Treat. Rev.*, 35: 57–68.

Matella NJ, Dolan KD, Stoeckle AW, Bennink MR, Lee YS & Uebersax MA 2015. Use of hydration. Germination, and a-galactosidase to reduce oligosaccharides in dry beans. *J. Food Sci.*, 70: 203 - 207.

McDonald P, Edwards RA & Greenhalgh JFD 1995. Morgan CA. Animal nutrition. 5th ed. Singapore: Longman Singapore Publishers (Pte) Lt.

Mehta LK, Balaraman R, Amin AH, Bafna PA & Gulati OD 2003. Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits. *J. Ethnopharmacol* 86: 191–5.

Melesse A, Steingass H, Boguhn J & Rodehutscord M 2013. In–vitro fermentation characteristics and effective utilisable crude protein in leaves and green pods of *Moringa stenopetala* and *Moringa oleifera* cultivated at low and mid-altitudes. *J. Animal Physio. & Animal Nutr.*, 97: 537-546.

Mertz ET, Hassen MM, Cairns-Whittern C, Kireis AW, Tu T & Axtell JD 1984. Pepsin digestibility of proteins in sorhum and major ereals. *Proc. Natl. Acad. Sci. USA*, 81: 1-2.

Mishra G Singh PR & Verma R 2011. Traditional uses, phytochemistry and pharmacological properties of Moringaoleifera plant: An overview. *Der Pharmacia Lettre*, 3(2): 141– 164.

Miyoshi N, Takabayashi S, Osawa T & Nakamura Y 2004. Benzyl Isothiocyanate inhibits excessive superoxide generation in inflammatory leukocytes: Implication for

prevention against inflammation-related carcinogenesis. *Carcinogenesis*, 25: 567-575.

Mohdaly AAA, Hassanien MFR, Mahmoud A, Sarhan MA & Smetanska I 2012. Phenolics Extracted from Potato, Sugar Beet, and Sesame Processing By-Products. *Int. J. Food Properties*, 16: 1148-1168.

Nakakuki T 2002. Present status and future of functional oligosaccharide development in Japan (PDF). *Pure and Applied Chemistry*. 74(7): 1245–1251.

Nieman DC, Butterworth DE & Nieman CN 1992. Nutrition. Dubuque, IA: WmC. Brown, pp. 237 – 312.

Ogbonna C & Abdulkadir AJ 2008. Proximate chemical composition of acha (*Digitariaexilis* and *Digitariaiburua*) grains. *J. Food Techn.*, 6 (5):214-216.

Okeke CU & Elekwa I 2003. Phytochemical study of the extract of *Gongronema*.

Okwu DE & Omodamiro OD 2005. Effects of hexane extract and phytochemical content of xylopiaaethiopila and ocimumgratissimum on the uterus of guinea pig. *Biological Research*, 4(2): 45-51.

Okwu DE & Ndu CU 2006. Evaluation of the phytonutrients, mineral and vitamin contents of some varieties of yam (*Dioscoreasp*). *Int. J. Molec. Medicine & Advance Sci.*, 2(2): 199-203.

Oluwole S Ijarotimi, Oluwole A Adeoti & Oluwaseun Ariyo 2013. Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented Moringaoleifera seed flour , *Food Science & Nutrition*, 1(6): 452– 463.

Pavankumar AR, Kayathri R, Murugan NA, Zhang Q, Srivastava V, Okoli C, Bulone V, Rajarao GK & Agren H 2014. Dimerization of a flocculent protein from *Moringa oleifera*: Experimental evidence and in silico interpretation. *J. Biomol. Structure & Dynamics*, 32: 406-415.

Pinto CE, Farias DF, Carvalho AF, Oliveira JT, Pereira ML, Grangeiro TB, Freire JE, Viana DA & Vasconcelos IM 2015. Food safety assessment of an antifungal protein from *Moringa oleifera* seeds in an agricultural biotechnology perspective. *Food and Chemical Toxicology*, 83: 1-9.

Price KR, Johnson IT & Fenwick GR 1987. The chemistry and biological significance of saponins in foods and feeding stuffs. *Crit. Rev. Food Sci. Nutrit*., 26(1): 27-135

Public Health England 2014. Hydrogen cyanide health effectIn: Chemical harzards compendium and healthemergency planning, Public health England.

Ramful D, Tarnus E, Aruoma OI, Bourdon E & Bahorun T 2011. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. *Food Res. Int*., 44: 2088-2099.

Rathi BS, Bodhankar SL & Baheti AM 2006. Evaluation of aqueous leaves extract of *Moringa oleifera* Linn for wound healing in albino rats. *Indian J. Experimental Bio.*, 44(11): 898–901.

Saini RK, Shetty NP, Prakash M & Giridhar P 2014. Effect of dehydration methods on retention of carotenoids, tocopherols, ascorbic acid and antioxidant activity in *Moringa oleifera* leaves and preparation of a RTE product. *J. Food Sci. and Techn.*, 51: 2176-2182.

Santos AFS, Luciana A, Adriana CCA, Teixeira JA, Paiva PMG & Coelho LCBB 2009. Isolation of a seed coagulant *Moringa oleifera* lectin. *Process Biochemistry*, 44: 504–508.

Satinder K, Savita S & Nagi HPS 2011. Functional properties and anti-nutritional factors in cereal bran. *Asian J. Food Agric. Industry*, 4(2): 122-131.

Seena LP 2006. Functional properties of great northern beans (*Phaseolus vulgaris* L) protein. *J. Food Sci*. 46: 71–72.

Shekib AL, El-Iraqui SM & Abo-Bakr MT 1988. Stuies on amylse inhibitors in some Egyptian legumes seeds. *Plant Foods Hum. Nutr*., 38: 325-352.

Shashank Kumar & Abhay K Pandey 2013. Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, 16.

Siddiq M, Nyombaire G, Dolan KD, Matella NJ & Harte JB 2006. Processing of sugar-coated red kidney beans *{Phaseolus vulgaris):* Fate of oligosaccharides and phytohemagglutinin (PHA), and evaluation of sensory quality. *J. Food Sci*., 71: C521-6.

Sizer F & Whitney E 1999. Nutrition:Concepts and Controversies. 8th edition Wadsworth, Belmont, Calif, USA.

Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G & Singh HB 2009. Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*. *Food Chem. Toxicol.,* 47: 1109–1116.

Sulaiman M & Fazilah AM 2015. Analysis of total phenolics, tannins and flavonoids from *Moringa oleifera*seed extract. *J. Chem. & Pharmaceutical Res.*, 7(1): 132-135.

Soetan KO & Oyewole OE 2009. The need for adequate processing to reduce the antinutritional factors in plants used as human foods and animal feeds: A review. *Afr. J. Food Sci.*, 3(9): 223-232.

Soladoye MO & Chukwuma EC 2012. Quantitative phytochemical profile of the leaves of *Cissuspopulnea* Guill. and Perr. (Vitaceae) – an important medicinal plant in central Nigeria. A*rch. Appl. Sci. Res.*, 4: 200–206.

Storey B, Noiles E & Thompson K 1998. Comparison of glycerol, other polyols, trehalose, and raffinose to provide a defined cryoprotectant medium for mouse sperm cryopreservation. *Cryobiology*, 37 (1): 46–58.

Swain T Tannins 1979. Lignins. In: Rosenthal GA, Janzen DH, editors. Herbivores: their interactions with plant metabolites. New York, NY: Academic Press, pp. 657–682.

Teixeira EM, Carvalho MR, Neves VA, Silva MA & Arantes-Pereira L 2014. Chemical Characteristics and Fractionation of Proteins from *Moringa oleifera* Lam. leaves. *Food Chemistry*, 147, 51-54.

Voet Donald, Voet Judith & Pratt Charlotte 2013. *Fundamentals of Biochemistry: Life at the Molecular Level* (4th ed.). Hoboken, NJ: John Wiley & Sons, Inc.

Yoon JH, Thompson LU, Jenkins DJ 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *Am. J. Clin. Nutr*., 38: 835–842.

Yuan GF, Sun J, Yuan Q & Wang QM 2009. Effects of different cooking methods on health-promoting compounds of broccoli. *Journal of Zhejiang University-Science*, B(10): 580-588.

Zebib H, Bultosa G & Abera S 2015. Physico-chemical and Sensory properties of banana flour-sesame paste blends. *Int. J. Scientific & Res. Publica.*, 5: 1-9.

Zhou RJ & Erdman JW Jr. 1995. Phytic acid in health and disease. *Crit. Rev. Food Sci. Nutr*., 35: 495-508.