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Diabetes & its Complications

Nutrient Composition and Microbiological Status of Nutrient-Dense Flour Produced from Indigenous Crops

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ABSTRACT

Consuming diets with low glycemic index may reduce postprandial glycemia and triglyceridemia. The flour which is high in fibre and low in fat (produced from maize, cowpea and Moringa seeds) is a good component for diabetes patient. The objective of the study was to increase the nutrient content of the cereal and improve the nutritional intake of the consumers. Flour blends were produced from maize, cowpea and moringa seed flours in the following ratios of maize: cowpea: moringa seed; 100:0:0; 80:10:10, 75:15:10, 75:10:15, 75:20:05 respectively. Determination of the Proximate and mineral composition were carried out on the flour blends using standard methods. The result for the proximate composition shows that the moisture, ash, fat and fibre contents ranged from 8.75% to 11.23%, 2.00% to 3.10%, 8.15 to 15.00 and 2.00 to 3.94% respectively, while protein and carbohydrate contents ranged from 10.05% to 15.10% and 57.40 to 66.57% respectively. The result of the mineral composition of the flour blends showed that Calcium content of the sample ranged from 6.00 to 29.30 mg/100 g, magnesium 53.10 to 81.40 mg/100 g, potassium 192.35 to 451.15 mg/100 g, sodium 17.90 to 366.25 mg/100 g, manganese 1.15 to 1.60 mg/100 g, iron and zinc content ranged from 4.20 to 7.70 and 2.40 to 3.10 mg/100 g respectively. Sample C (75:15:10) was higher in calcium, magnesium and potassium than the rest blends. The microbiological status of the flour blends from maize/cowpea/moringa seeds showed that the Total Viable Count (TVC) and Yeast and Mould count (YMC) were significantly higher in sample A, C, D, E than in sample B, while Coliform was not detected in any of the samples, an indication that there was no faecal contamination. The microbial count ranged from 29.00 x 10^3 to 51.00 x 10^3 and 21.50 x 10^3 to 46.6 x 10^3 for TVC and YMC respectively with the lowest value recorded for sample B in both cases. Little information exist for the microbiological status of flour, but the presence of yeast and mould in the flour samples could be attributed to the surrounding air and packaging materials. Conclusively, enrichment of maize flour with cowpea and moringa seeds flours will improve the protein, magnesium, potassium, iron and zinc and make a suitable meal which is high in fibre for people including diabetes patients.

Keywords

Indigenous crops, Diabetes, Nutrient, Moringa seeds, Cowpea and maize.

Introduction

The cause of malnutrition in Nigeria is not only due to decreased food production but largely due to ignorance of what food combinations to eat and how best to process each food. However, the nutritional quality of cereal protein is poor because they are deficient in the essential amino acid Lysine, tryptophan though rich in methionine. The level of protein-energy malnutrition in Nigeria particularly Ondo State calls for urgent attention, for instance, according to a research work carried out by Ijarotimi and Odeyemi [1], about 37% of the children in public schools in Ondo State

(Nigeria) were food secure while the remaining 63% were food insecure, hence, the need for a complementary which can serve as infant meal as well as a composite flour for adult's pudding.

Maize (*Zea mays*) or corn is a cereal crop that is grown widely throughout the World in a range of agro ecological environments [2]. It is one of the main staple cereals in the world. It can be cultivated in areas with rainfall higher than 760 mm a year and adequate sunshine. In many tropics, corn is a vital staple food particularly in rural areas [3]. Maize is a major source of starch. Cornstarch (maize flour) is a major ingredient in home cooking and in many industrialized food products. Maize is also a major source of cooking oil (corn oil) and of maize gluten. Maize starch can be hydrolyzed and enzymatically treated to produce syrups, particularly high-fructose corn syrup, a sweetener; and also fermented and distilled to produce grain alcohol. Grain alcohol from maize is traditionally the source of Bourbon whiskey. Maize is sometimes used as the starch source for beer. Within the United States, the usage of maize for human consumption constitutes about 1/40th of the amount grown in the country. In the United States and Canada, maize is mostly grown to feed for livestock, as forage, silage (made by fermentation of chopped green cornstalks), or grain. Maize meal is also a significant ingredient of some commercial animal food products, such as dog food. Maize is also used as a fish bait, called "dough balls". It is particularly popular in Europe for coarse fishing.

Cowpea (*Vigna unguiculata*) is a dicotyledonous plant belonging to the family *fabaceae* and sub-family *fabiodeae*. It is grown extensively in the low land and mid-altitude region of Africa (particularly in the dry Savannah) sometimes as sole crop but more often intercropped with cereals such as sorghum or millet [4]. Beans (Cowpea) provides a notable combination of plant protein and soluble fibre that can help boost feeling of fullness and manage blood sugar levels [5]. Protein also helps with the uptake of carbohydrates so that the body can process it more easily [6]. World production of cowpea was estimated to be 2.27 million tonnes of which Nigeria produces about 850,000 tonnes [7,8]. Cowpea is of major importance to millions of relatively poor people in less developed countries. Among the legumes, cowpea is the most extensively grown, distributed and traded food [9,10]. It is an extremely resilient crop and cultivated under some of the most extreme agricultural condition in the world [11]. In Nigeria, cowpea is majorly produced in the North in the savanna belt. Its yield in the south is affected by some environmental factors including rainfall hence it is seasonal. It does well and most popular in the semiarid of the tropics where other legumes crop do not perform well [12]. In Nigeria, cowpea is consumed in the form of bean pudding, bean cake, baked beans, fried beans, bean soup amongst other. The main advantage of cowpea over many other crops apart from being the most practical source of storable and transportable protein is due to the fact that it is a cheap source of protein.

Moringa seed can also serve as a food fortification agent. *Moringa* seed is from *Moringa oleifera* which is the most widely cultivated species of the genus *Moringa*, which is a genus in the family *Moringaceae*. The seeds sometimes remove from more mature pods and eaten like *peas* or roasted like nuts [13]. *Moringa* pods, bark, flower, fruits, leaves, roots and seeds are all useful. All of them contain various valuable nutrients, antioxidants as well as amino acids. Because of that, this plant has various benefits to humans and animals. These *moringa* seeds are full of nutrients and also can be easily digested. *Moringa* seeds contain a good amount of vitamin A, vitamin B, Vitamin C, vitamin D, vitamin E as well as iron. Actually *moringa* seeds have more amount of Vitamin compared with various food that is claimed a prime source of them such as oranges, carrot and milk. These vitamins provide a great benefit for us. [14]. *Moringa* seeds have numerous benefits for us since it is full of nutrient and antioxidants. It is able to eaten and supplement substitute as it is really full of nutrients and completely natural. It is also able to make minor injuries like bruises, cuts, or even burns heals faster because of the nutrients it has. *Moringa* seed also serve as a good source of protein [13]. *Moringa* seeds do not only serve as protein source but can also be used as medicine and food commodity which has received enormous attention as the natural nutrition of the tropics. Moringa seeds had been reported to be a good source of fat, crude fibre and proteins (especially rich in cysteine and methionine amino acids which are lacking in cowpea).

There is prevalence of Protein-Energy Malnutrition (PEM) in Nigeria in spite of the abundant food crops. Inappropriate production method of indigenous foods, coupled with inadequate information on the composition of naturally enriched meal also contributes to malnutrition and contamination. Cowpea and maize, in spite of their great nutritional and economic importance are highly susceptible to many diseases and pests right from growing stage up to storage [15] and final consumption. Therefore, there is need to reduce post-harvest losses of these valuable grains via processing them into nutrient-rich flour. Food has a direct effect on blood glucose, some foods raise blood glucose more than others. Eating a variety of nutrient-rich foods in each meal is pivotal to healthy living [16].

Materials and Methods

Materials

Dried yellow maize (*Zea mays*), cowpea (*Vigna unguiculata*) and other ingredients (onion, fresh pepper, palm oil, vegetable oil) used for this research were sourced from Obada market in Emure-ile, Owo L.G.A of Ondo State, Nigeria. While the AAS (Model 210 VGP Buck Atomic Absorption Spectrophotometer) and flame photometer used for mineral determination were from the Chemistry Laboratory of Science Laboratory Technology department, Hot air oven (Model Memmert 854, Gallenkamp, UK), water bath (Model HH - 6), kenwood mixer (Model A 907 D, Kenwood Ltd, England) and other equipment used for this research were from the Food Chemistry Laboratory of the Department of Food Science and Technology, Rufus Giwa Polytechnic, Owo, Ondo State. All chemicals used for the proximate analysis were of analytical grade and were supplied by Pascal Scientific Limited, Akure.

Methods

Production of maize flour

Maize flour was produced following the procedure adopted by Barber *et al*., (2010) with slight modification. The maize grains were sorted to remove extraneous matter and washed with potable water. The drying was achieved with the aid of hot air oven (Memmert 854, Gallenkamp, UK) at 55°C for 8 hours. The dried maize was milled into flour using attrition mill and was stored in air tight container until needed for further analysis (Figure 1).

Production of cowpea flour

Matured and dried cowpea seeds were carefully cleaned, sorted to remove defective ones, stones and other extraneous matters. The cleaned seeds were soaked in potable water for just 10 minutes to soften the seed coat which was manually dehulled. The dehulled cowpea was dried in cabinet dryer at 60°C for 24 hours and milled into flour, the flour was stored in air-tight polythene until needed (Figure 1).

Preparation of *moringa* seeds flour

Moringa seed flour was produced by removing the dried and mature seeds from the pods and was dried at 50°C in a hot air oven (Model Memmert 854, Gallenkamp, UK) for 5 hours in order to give room for easy breaking. After breaking, the dried seeds were size reduced in laboratory mortar and pestle before milling into flour with the aid of Kenwood blender and the flour was stored in air tight container for further analysis (Figure 1).

Formulation

The maize:cowpea:moringa seeds flour blend (Table 1) were formulated into five ratios: 100:0:0; 80:10:10, 75:15: 10, 75:10:15, 75:20:05 respectively, these were kept in air tight container until needed to be used for analysis.

Analysis

Determination of proximate composition

This includes moisture, fat, total ash, crude fibre and crude protein contents and were determined according to the methods of AOAC [17].

Determination of moisture content

Sample (2 g) was weighed and put into a dried and cooled Petri dish of a known weight, the weight of the Petri-dish and the sample was taken and recorded as W_2. It was then dried in the oven at 105 °C for 3 hours. The sample was removed and placed in the desiccators to cool for 30 minutes. After cooling, the sample together with the Petri-dish was weighed and recorded as W_3; subsequent weighing was done at 30 minutes interval until a constant weight was obtained. The result was then calculated as follows:

$$\%\ \text{Moisture content} = \frac{\text{weight loss}}{\text{weight of sample}} \times \frac{100}{1}$$

Or

$$\%\ \text{Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = weight of Petri dish

W_2 = weight of Petri dish + sample

W_3 = weight of Petri dish + sample after drying

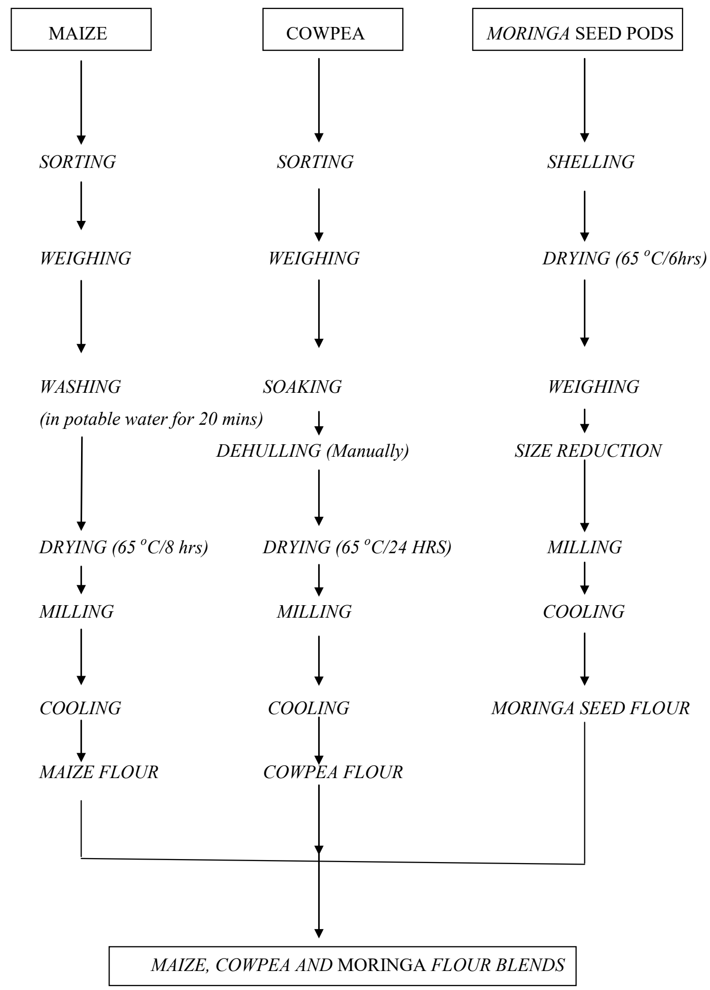

Figure 1: Unit operations involved in the production of maize, cowpea and *moringa* flour blend.

Determination of fat content

This was determined using Soxhlet apparatus, in which 2 g of sample was weighed into fat free and pre-weighed filter paper. The filter paper containing the sample was placed in the thimble of the Soxhlet apparatus. Normal hexane of boiling point range of 60 – 68°C was used as solvent for the extraction; a 500 ml round bottom flask was filled to ¾ with the solvent. The flask was fitted to the Soxhlet apparatus with a reflux condenser and placed in an electric heating mantle. Extraction began as the solvent refluxed several times and continued for over 4-5 hours until the condenser was detached. The filter paper containing the defatted sample was removed and dried to a constant weight in an oven at 50°C. The difference in weight before and after extraction was recorded in order to obtain the value of the fat extracted and the percentage fat content was calculated as follows:

$$\% \text{ Fat content } = \frac{\text{weight of fat extracted}}{\text{weight if sample}} \times \frac{100}{1}$$

Determination of crude fibre

Each of the defatted samples (4 g) was weighed into a 500 ml conical flask where 200 ml of 1.25% H_2SO_4 was added and this was boiled for 30 minutes using cooling fingers to maintain constant volume. After boiling, the mixture was poured into filter paper under gentle suction using a butcher funnel, rinsed well with hot distilled water until the filtrate was neutral. The residue was then transferred from the filter paper to another conical flask containing 200 ml of 1.25% boiling NaOH and then allowed to boil and reflux for 30 minutes shaking gently to avoid spillage. The solution was filtered again and the residue was washed with hot distilled water and 10% HCl respectively until the filtrate again was neutral. The washing was repeated twice with ethanol and thrice with petroleum ether to remove any remaining fat. The residue was scrapped and then transferred into already cleaned, dried crucible and oven dried at 110°C for 30 minutes, allowed to cool in desiccator and weighed (W_2). The dried sample was further put into furnace at 600°C for 30 minutes. Cooled in a desiccator and re-weighed (W_3). Therefore, crude fibre was calculated as:

$$\% \text{ Crude fibre } = \frac{(\text{weight of crucible} + \text{sample}) - (\text{weight of crucible} + \text{ash})}{\text{weight of defatted sample}} \times \frac{100}{1}$$

Or

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W_1} \times \frac{100}{1}$$

Where:

W_1 = weight of defatted sample

W_2 = weight of crucible + sample after oven drying

W_3 = weight of crucible + sample after ashing

Determination of ash content

The crucible was ignited at 550 °C for 2 hours; it was removed and put inside desiccators to cool for about an hour. After cooling, it was weighed and the weight recorded as W_1. Sample (2 g) was weighed into the crucible, the weight of the crucible and sample was taken as W_2. The crucible with the sample was ignited in the muffle furnace at 550°C for about 3 hours to ensure complete ashing, cooled in a desiccator and weighed as W_3.

$$\% \text{ Ash content } = \frac{\text{weight of ash}}{\text{weight of sample}} \times \frac{100}{1}$$

Or

$$\% \text{ Ash content } = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = weight of empty crucible

W_2 = weight of crucible + sample

W_3 = weight of crucible + ashed sample

Determination of crude protein

The crude protein in the sample was determined by the routine semi - micro Kjeldahl method. This consists of three stages of analysis namely, digestion, distillation and titration.

Digestion

Sample (1 g) was measured carefully into the kjeldahl digestion flask and was ensured that the sample got to the bottom of the flask which contains the kjeldahl catalyst tablet and 10 ml of conc. H_2SO_4 was added. This was set in the appropriate hole of digestion block heater in fume cupboard. The digestion was left on to heat for an hour, after which a clear colourless solution was obtained in the flask. The digest was cooled and carefully transferred into 100 ml volumetric flask thoroughly rinsing the digestion tube with distilled water.

Distillation

A portion (10 ml) of the digest was pipetted into the body of the apparatus through the small funnel apparatus 10 ml of 40% NaOH was added through same opening with 10 ml pipette. The mixture was steam distilled for 2 minutes into a 50 ml conical flask containing 5 ml of 2% boric acid and 3 drops of mixed indicator, placed at the receiving tip to the condenser. The boric acid plus indicator solution change from red to green showing that all the ammonia liberated have been trapped.

Titration

The light green colour solution obtained was then titrated against 0.0l M HCL contained in a 50 cm^3 burette. At the end point, the green colour change to wine colour which indicates that all the nitrogen trapped as Ammonium borate (NH_4BO_3) has been removed as ammonium chloride (NH_4CL). The value of protein was calculated from the equation below:

$$\% \text{Nitrogen } = \frac{\text{titre value x molarity of acid x } 0.014}{\text{weight of sample}} \times \frac{\text{vol. of digest}}{\text{vol. of digest used}} \times \frac{100}{1}$$

% Protein = % Nitrogen x 6.25

Determination of carbohydrate by difference

This is the summation of the result obtained from moisture, fat, crude fibre, ash and protein contents determination, all subtracted from 100.

$$\% \text{CHO content } = 100 - (\text{Protein} + \text{Ash} + \text{Moisture} + \text{crude fibre} + \text{fat}$$

Determination of mineral content

The mineral contents of the samples were determined after acid digestion of the ashed samples as follows: 2 ml of aqua regia (mixture of HCl and HNO_3 in ratio 3:1) was added to each ashed sample in 100 ml flask and made up to the mark with distilled water. The solution was then filtered through N0.4 Whatman filter paper and the clear solution was kept in plastic bottle with lid. Calcium, Zinc and Iron were determined using atomic absorption spectrophotometer (210 VGP Buck Atomic Absorption Spectrophotometer) while sodium and potassium were determined using flame photometer.

Microbiological analysis

This was carried out on both the flour blends and the nutrient-dense snack samples. The media used for microbiological analysis include Nutrient agar (NA), EMB and Potato Dextrose Agar (PDA) for total viable count, coliform test and yeast and mould count respectively. These media were obtained from the Microbiology Laboratory of the Department of Food Science and Technology, Rufus Giwa Polytechnic, Owo and were prepared according to the manufacturer's instruction. The procedure described by Collins *et al*. (1989) as reported by Otunola *et al*., 2012 was used to evaluate the microbiological characteristics of the samples. The sample (10 g) was added to 100 ml of distilled water and mixed thoroughly after which 1ml of the mixture was serially diluted for estimating the number of microorganisms.

Statistical analysis

The SPSS for windows programme version 15.0 was used to analyze the results obtained, means and standard deviation of all the samples were calculated and compared. The results obtained were in triplicate and subjected to analysis of variance ANOVA and the means were separated by New Duncan Multiple Range Test (NDMRT).

Results and Discussion

Proximate composition of the flour blends

The result for the proximate composition of maize/cowpea/moringa seeds flour blends is as presented in Table 1. The moisture content (MC) which was significantly different from one sample to another ranged from 8.75% to 11.23% with sample A (100:0:0 maize/cowpea/moringa seed) having the highest value and sample D (75:10:15) had the least. These values were significantly higher than those obtained by Shakpo and Osundahunsi [18] in the study of "Effect of Cowpea Enrichment on some Properties of Maize Flour" but similar to those obtained by Abegunde et al. [19]. The 100% maize retained the highest value of moisture compared to the blends, there was no significant difference between the 100% maize and 80:10:10 maize/cowpea/moringa seeds blend whereas sample E (75:20:05 maize/cowpea/moringa seeds) was significantly difference from the rest blends. The MC of samples C and D were similar and lowest, indicating that they will have longer shelf life than other samples. It has been shown that moisture content in food products facilitates the growth of microorganisms, which in turns causes spoilage and low nutritional qualities of the food products. The ash content ranged from 2.00% to 3.10% the least value was recorded for the 100:0:0 maize/cowpea/moringa seeds and was significantly different from the blends which had values ranging from 2.5 to 3.10%, the ash content of sample B was not significantly different from sample C but different significantly from sample D and E with 3.00 and 3.10% respectively, thus moringa seeds and cowpea inclusion to maize flour significantly improved the ash content of the flour which gives an idea of the inorganic content of the sample from where the mineral content could be determined [20]. Fats are essential because they provide the body with maximum energy, approximately twice that for an equal amount of protein and carbohydrate [21]. Lipids facilitate intestinal absorption and transport of fat-soluble vitamins A, D, E and K and also provide varying quantities of essential nutrients such as linoneic acid [22]. Fat and fibre contents which ranged between 8.15 to 15.00 and 2.00 to 3.94% respectively varied significantly among the 100:0:0 maize/cowpea/moringa seed and the blends. The 100:0:0 maize/cowpea/moringa seed having the least value in each case. Sample C, D and E were not significantly different from one another in term of fibre but significantly different in fat, whereas the sample containing 100:0:0 maize/cowpea/moringa seed was similar in fibre content to the sample 80:10:10 maize/cowpea/moringa seed but significantly different in fat content. The lower fibre content recorded for samples A and B (with higher proportion of maize) must have been due to the intensity of the processing of the maize to flour causing great loss of fibre. Increase in the fat content of samples B to E with addition of moringa seed flour is a reflection of the high fat content (41.58 to 45.84%) of moringa seed [23,24]. The protein content of the samples ranged from 10.05% to 15.10%, the blends had higher values than the 100:0:0 and these values were significantly difference among the blends and the control. Sample D (with 75:10:15 maize/cowpea/moringa seed) had the highest protein value while the 100:0:0 had the least. The findings of this research support the claim of Makkar and Becker [25] that the appreciable protein content of moringa seeds (40.31%) establishes it as a supplement to other plant protein. Sample D had lower moisture content than Cerelac (Commercial maize/soya complementary food (11.3%), similar to ash and protein content. The fat, fibre and energy contents were within ranges recommended for infant complementary foods (FAO/WHO, 1991) Conversely, carbohydrate was higher in 100:0:0 than in the blends and the highest energy value was recorded for sample D (75:10:15).

Mineral composition of the flour blends

Table 2 depicted the result of the mineral composition of Maize/cowpea/moringa seeds flour blends. Calcium, an essential micronutrient for teeth and bone health was found to be higher in the sample with 100:0:0 Maize/cowpea/moringa seeds, than in the blends. Magnesium content which ranged from 53.10 to 81.40 mg/100 g was highest in sample C (75:15:10) followed by sample D (75:10:15), E (75:20:05), B (80:10:10) and A (100:0:0). The significant difference among the samples revealed that cowpea and moringa enrichment enhanced the magnesium content of the flour blend as the least value was recorded for the sample A (100:0:0). The potassium content of the flour blends which was significantly different among the samples ranged from 389.45 to 451.15 mg/100 g and much higher than that of 100% maize (192.35 mg/100 g). There were significant differences in sodium, iron and zinc contents of the samples with values ranging from 17.90 to 366.25, 4.20 to 7.70 and 2.40 to 3.10 respectively. Manganese content of sample A was not significantly different from that of sample C but significantly different samples B, D, E. Chikwendu [26] opined that addition of legumes such cowpea to cereals like maize increases the mineral content of the blend, the result obtained from this research upholds such fact as all the elements examined (magnesium, manganese, iron, zinc, potassium) were higher in the blends than in the 100% maize flour with the exception of calcium and sodium. This trend concurred with the findings of Shakpo and Osundahunsi [18].

Table 1: Proximate Composition of Maize/Cowpea/Moringa Seeds Flour Blends (%).

Samples	Moisture	Ash	Fat	Fibre	Protein	Carbohydrate	Energy (Kcal)
A (100:0:0)	11.23 ± 1.33a	2.00 ± 0.80c	8.15 ± 0.65e	2.00 ± 1.50b	10.05 ± 1.22d	66.57 ± 1.80a	379.83 ± 2.23e
B (80:10:10)	10.50 ± 0.00a	2.50 ± 0.50b	9.14 ± 0.13d	2.20 ± 1.00b	11.25 ± 0.57c	64.41 ± 1.50b	384.90 ± 1.88d
C (75:15:10)	8.93 ± 0.25c	2.88 ± 0.50b	10.58 ± 0.20c	3.80 ± 0.05a	11.50 ± 0.70b	62.31 ± 2.20c	390.46 ± 0.50c
D (75:10:15)	8.75 ± 0.58c	3.00 ± 0.50a	15.00 ± 1.60a	3.94 ± 0.50a	15.10 ± 2.55a	54.21 ± 0.33e	412.24 ± 2.00a
E (75:20:05)	9.50 ± 2.30b	3.10 ± 1.05a	14.10 ± 3.05b	3.90 ± 0.50a	12.00 ± 0.55b	57.40 ± 0.55d	404.50 ± 2.50b

Values with the same letters along the column are not significantly different ($p \leq 0.05$) A(100:0:0): Ratio of Maize (M) :Cowpea (C) :Moringa seeds flour (MSF); B (80:10:10): Ratio of M:C:MSF; C (75:15:10): Ratio of M:C:MSF; D (75:10:15) Ratio of M:C:MSF; E (75:20:05): Ratio of M:C:MSF. Letters a-e shows the degree of significant difference.

Table 2: Mineral Composition of Maize/Cowpea/Moringa Seed Flour Blends (mg/100 g).

Samples	Calcium	Magnesium	Potassium	Sodium	Manganese	Iron	Zinc
A (100:0:0)	29.30 ± 0.20a	53.10 ± 0.10e	192.35 ± 0.25e	32.65 ± 0.15c	1.15 ± 0.05b	4.20 ± 0.20e	2.40 ± 0.20b
B (80:10:10)	6.00 ± 0.05d	77.40 ± 0.40d	393.45 ± 0.25c	27.60 ± 0.50d	1.55 ± 0.06a	7.70 ± 0.150a	2.70 ± 0.10ab
C (75:15:10)	7.00 ± 0.15b	81.40 ± 0.12a	451.15 ± 0.15a	17.90 ± 0.22e	1.15 ± 0.03b	4.65 ± 0.14d	2.80 ± 0.35a
D (75:10:15)	6.00 ± 0.01d	80.15 ± 0.15b	416.80 ± 0.30b	35.55 ± 0.40b	1.60 ± 0.20a	6.05 ± 0.05c	2.90 ± 0.20a
E (75:20:05)	6.55 ± 0.07c	77.90 ± 0.15c	389.30 ± 0.20d	366.25 ± 0.25a	1.60 ± 0.01a	7.35 ± 0.25b	3.10 ± 0.01c

Values with the same letters along the column are not significantly different ($p \leq 0.05$) A(100:0:0): Ratio of Maize (M) :Cowpea (C) :Moringa seeds flour (MSF); B (80:10:10): Ratio of M:C:MSF; C (75:15:10): Ratio of M:C:MSF; D (75:10:15) Ratio of M:C:MSF; E (75:20:05): Ratio of M:C:MSF. Letters a-e shows the degree of significant difference.

Table 3: Microbiological Status of Maize/Cowpea/Moringa Seeds Flour Blends.

Samples	Total Viable Count (x 10^3 cfu/ml)	Yeast and Mould Count (x 10^3 sfu/ml)	Coliform Count (x 10^3 cfu/ml)
A (100:0:0)	46.50 ± 7.50a	35.50 ± 3.80a	ND
B (80:10:10)	29.00 ± 2.50b	21.50 ± 10.00b	ND
C (75:15:10)	48.00 ± 4.60a	34.80 ± 2.00a	ND
D (75:10:15)	51.00 ± 3.00a	38.00 ± 0.00a	ND
E (75:20:05)	49.50 ± 6.00a	46.60 ± 8.40a	ND

Values with the same letters along the column are not significantly different ($p \leq 0.05$) A(100:0:0): Ratio of Maize (M) :Cowpea (C) :Moringa seeds flour (MSF); B (80:10:10): Ratio of M:C:MSF; C (75:15:10): Ratio of M:C:MSF; D (75:10:15) Ratio of M:C:MSF; E (75:20:05): Ratio of M:C:MSF. Letters a-e shows the degree of significant difference. Cfu/ml – Colony forming unit per millilitre; sfu/ml – spore forming unit per milliliter; ND means Not Detected Standards: Total aerobic bacteria count (TABC) = $< 10^5$/g, Fungal count (FC) = $< 10^4$/g, Coliform count (FC) = < 100/g (FSANZ, 2001, PHLS, 2000, CFS, 2007).

Microbiological status of the flour blends

Table 3 depicted the result of the microbiological status of the flour blends from maize/cowpea/moringa seeds. It shows that the Total Viable Count (TVC) and Yeast and Mould count (YMC) were significantly higher in sample A, C, D, E than in sample B, while Coliform was not detected in any of the samples, an indication that there was no faecal contamination. The microbial count ranged from 29.00 x 10^3 to 51.00 x 10^3 and 21.50 x 10^3 to 46.6 x 10^3 for TVC and YMC respectively with the lowest value recorded for sample B in both cases. Little information exists for the microbiological status of flour, but the presence of yeast and mould in the flour samples could be attributed to the surrounding air and packaging materials.

Conclusion

One of the goals of the Medical Nutrition Therapy for diabetes is to improve health through healthy food choices and activity. The trends observed from the results of this research revealed that cowpea and moringa seeds inclusion to maize flour had no adverse effect on the keeping and nutritional qualities of the flour as it enhanced the nutrient content with respect to protein, macro and micro elements, the microbiological status of these flour blends revealed good manufacturing practices as the values obtained were within the tolerable limit of $\leq 10^5$. The coliform count showed there was no faecal contamination. Therefore, it can be concluded that nutritious and affordable flour blends which can serve as nutrient-rich meal can be produced from our indigenous crops like maize, cowpea and moringa seeds.

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