

Microbiological, Nutrient and Aflatoxin Content in Maize Flour Fortified with Cassava Flour

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ABSTRACT

The maize meal (*nni oka* in ibo or *tuwo* and *agidi* (eko) in Yoruba, *mielie* in South Africa, *ukpo oka* in Ibo Delta State. Maize (*Zea mays* spp) is a staple food which plays important roles in the diets of people especially in Latin America and Africa and also used as food as well as feed in Asia. Maize is the most important cereal crop in the world with respect to cultivation area and total production while it is second most important in Nigeria follow by sorghum which is mostly consumed by the populace, apart from being delicious and wholesome, maize provides a level of protection against a number of illnesses and chronic diseases. The microbiology, proximate content, mineral composition, aflatoxin level and the sensory properties of maize blend fortified with cassava flour meal in ratio 3:1 (maize: cassava flour) were determined and compared to the commercially available flour meals. Bacteria isolated includes were isolated. The fungi isolated include *Aspergillus flavus*, *Aspergillus niger* and *Penicillium chrysogenum*. The fortified maize samples had less protein content 7.92% and 8.74% respectively, while the crude fat content was also lower compared to the commercial flour meal 2.88% and 2.95%. The crude fibre and Ash content were higher in the commercial flour meal, while lower in the fortified maize meal 2.43% and 2.34% (fibre) then 3.68% and 3.76% (ash content). Furthermore, the energy level of the fortified maize flour (4.136 and 4.131) had higher calories than the unfortified maize flour (4.114 and 4.108) except for Dangote semovita (4.156) and Golden penny semovita (4.163). However, the carbohydrate content varied among the flour meals. The moisture content of the fortified maize flour was higher than those of the unfortified maize meal and the commercial flour meal. Aflatoxins were present in all the samples though within acceptable standard except for the big kernel maize with aflatoxin content of 70ppb. This study revealed that fortification of maize meal with cassava flour had effect on the nutritional and sensory characteristic of the meal.

Keywords: Fortification, aflatoxin, unfortified, ash content, moisture content

Introduction

The deficiency in micronutrient required for development and growth also known as 'hidden hunger' affects a high proportion of the world population (Ranum *et al.*, 2004). This problem is more prevalent in developing countries compared

to developed countries it is then inevitable to fortify food especially with public health significant micronutrients. Biofortification of most commonly consumed food provides cheaper cost with minute nutrients made available to the total

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populace. Maize meal known as *Nni oka* in Igbo, *agidi (eko)* or *Tuwo* in Yoruba *mielie* in South Africa, *Ukpo oka* in Ibo (Cindy, 2012) and Cassava known as *fufu* which is one of the major flour meals eaten in Nigeria. Maize has a high nutritional composition and this makes it a good substrate for fungi development which causes nutritional losses and leads to production of toxic substances known as mycotoxin.

Maize (*Zea mays* spp) is a basic staple food and an important ingredient in the diets of people especially in Latin America and Africa. It is also used as food as well as feed in Asia (Kumar *et al.*, 2014). Maize is ranked as the third most important cereal crop in the world with respect to cultivation area and total production while it is second most important in Nigeria follow by sorghum which is mostly consumed by the populace (Ifemeje *et al.*, 2015). Besides being delicious and wholesome, maize provides a level of protection against a number of illnesses and chronic diseases. Maize flour has a calorific content of 342 calories 100/g, which is among the highest for cereals. Presence of phenolic phytochemicals can regulate the absorption and release of insulin in the body thus, reducing the chance of spikes and drops for diabetic patients and help maintain a more normal lifestyle (Bhaskarachary *et al.*, 2017). A cup of maize is equivalent to 18.4% amount of fibre daily required by the body, and this helps to reduce risk to haemorrhoid and colorectal cancer (Nagdeve, 2019). Maize flour provides some minerals for body function and wellbeing such as phosphorus, magnesium, manganese, Zinc, iron, copper and selenium (Ranum *et al.*, 2004). A good percentage of this micronutrient is destroyed during corn flour production. The conversion of corn into flour meal causes reduction in the minute nutrient contents, compared to the whole corn (Ranum *et al.*, 2004). Cassava (*Manihot esculenta* crantz) belongs to the family *Euphorbiaceae* is another staple food in Nigeria, and can be used in fortifying corn meal as it is also rich in micronutrient as well as cost effective. Nigeria currently is the largest producer

of cassava in the whole world with an annual output of over 34 million tons of tuberous root and it play a dominant role in the rural economy in the southern agro-ecological zone. In Nigeria, cassava has been used in confectionary for fortification of flour products such as bread, cakes etc (Odetunde *et al.*, 2014). Cassava is an essential carbohydrate source. Its composition shows 60-65 percent moisture, 20-31 percent carbohydrate, 1-2 percent crude and a comparatively low content of vitamins and minerals (Falade and Akingbala, 2011), it also has moderate source of some of the valuable B-complex group of vitamins, compared to other roots and tubers, cassava is free from gluten. Gluten-free starch is used in special food preparations for celiac disease patients (Kuethe, 2014). Cassava has lower fats and protein content than in cereal and pulses, but has more protein than that of other tropical food sources like yam, potato, plantains among others. Cassava roots have moderate amount of essential amino acids (Longe, 1980). Besides, fortifying maize meal, their health safety is to be considered. Nutritional composition present in maize makes it a good substrate for fungi development which can result to nutritional losses and production of toxic substances known as mycotoxin (Lancey, 1998). Most toxic of the mycotoxin is aflatoxin commonly found in cereals and other foods, according to IARC (2002) aflatoxins are group one carcinogenic agents to humans.

This work aims at determining the microbiological profile and aflatoxigenic content of industrially processed flour meals and processed samples (maize fortified with cassava flour), its proximate composition along with the elements and minerals of health value.

Materials and Methods

Sample Collection

Two varieties of white corn (big and small kernel size) *Zea mays* Linn UIH – 22828 were purchased at Bodija market and cassava tubers were purchased

at Ijokodo market along Sango-Eleyele road Ibadan, Oyo State Nigeria. Three commercial semovita (Dangote, Honeywell and Golden penny) were purchased at Apete market, Ibadan. The samples were collected in clean and properly labelled containers and transported immediately to the laboratory for further analysis and were also identified as the Botany Department of University of Ibadan by a known Botanist.

Sample Preparation

The maize was sorted, handpicked, de-shelled, milled into flour and packed into containers. Cassava tubers were peeled, cut into pieces, rinsed and steeped in water for 5 days, it was packed into sack and dewatered, oven dry at 80°C for 3 days until stable moisture content was reached. The dried cassava was milled into flour. The different corn flour samples (big and small kernel flour) and cassava flour were mixed in the ratio 3:1 respectively while other portions were not mixed with the cassava flour which serves as the control.

Isolation and Determination of Microbial Load of Flour Meals

Ten grams of each of the flour meals were suspended in 90 ml of sterile distilled water and appropriate serial dilutions of each sample using tenfold dilution. An aliquot of 1 ml of 10⁻⁵ was pour plated in duplicate Nutrient agar (NA) and Potato dextrose agar Oxoid (PDA) containing fluconazole and streptomycin respectively. The plates were gently swirled for uniform distribution and allowed to set, incubated at 37°C for 24 to 48 hours while the PDA Oxoid plates were 37°C for 3-5 days. At expiration of incubation period, distinct and characteristic colonies were picked and purified by repeated sub-culturing to obtain pure cultures for further identification. The bacteria identification was based on cultural characteristics, Gram staining reaction, and biochemical tests such as; catalase, oxidase, IMVIC and TSI agar according to the method of Olutiola *et al.*, 2000 and the identification was done using Bergeys manual of

systemic bacteriology (Sneath *et al.*, 1986). Fungi isolates were identified based on their microscopic and macroscopic characteristics according to the method of Watanbe (2002).

Proximate Analysis of Flour Meal Samples

The proximate composition of the flour meal samples was determined which includes crude protein (AOAC, 2010), crude fat (Aremu *et al.*, 2015), moisture content (Enyisi *et al.*, 2015), ash content (AOAC, 2010), fibre content (AOAC, 2010) and total carbohydrate by differential method. The carbohydrate was achieved by subtracting the total protein, lipid, moisture and ash content from 100 thus:

% Carbohydrate (100 – (% moisture + % ash + % fat + % protein + % fibre))

Aflatoxin content

The level of aflatoxin contents in the flour meal were determined using standard AgraQuant Elisa test kit according to the manufacturer instructions (Adesokan *et al.*, 2016).

Minerals Composition

The mineral contents of the maize flour samples (Ca, Mg, Fe, Zn, K and Na) were determined according to AOAC (2005) using atomic absorption spectrophotometer, while the Phosphorus was determined using spectrophotometer at 430nm.

Sample Production for Sensory Analysis

One cup of water was boiled in cooking pot. Some flour meal of each sample was mixed in clean ordinary water to form a paste. The mixture was poured into the boiling water, stirred and more flour was added to it until mixture was smooth. The pot was covered and allowed to steam. Water was added when needed. The pot was removed and mixture was stirred and package into clean nylon bags. This was done for all the samples respectively. The samples were allowed to cool

down at ambient temperature (modified method of Adeniyi, 2017).

Sensory Evaluation

The sensory evaluation of the flour meals was carried out by 12 groups of panellists made up of students who are familiar with the product. The evaluation was rated using 7-point hedonic scales, whereby 1 was extremely dislike and 7 was extremely liked.

Results

The total viable count of bacteria was shown in table 1. Small kernel corn blend mixed with cassava flour and Big kernel corn blend mixed with cassava flour has total viable count of 3.0×10^5 cfu/ml and 3.1×10^5 cfu/ml while Golden penny Semovita (G) has the least viable count of 1.0×10^5 cfu/ml. The frequency of occurrence of the bacteria isolates were illustrated in figure 2. Where the *Bacillus* spp has the largest frequency of occurrence (35.89 %) while the *Salmonella* spp has the least occurrence (5.12%) which may be attributed to the processing, packaging and storage processes. A total of seven different species of bacteria were identified based on their Gram reaction, motility, oxidase, spore formation, catalase and reaction on TSI agar and IMVIC test which were illustrated in table 2. The following organisms were isolated *Lactobacillus* spp, *Bacillus* spp, *Salmonella* spp, *E. coli* spp, and *Staphylococcus* spp. The characteristics of the fungi isolates were illustrated in table 3, and the following fungi were isolated *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Sclerotium rolfsu*. All the samples contain various proximate nutrient compositions which varied from one sample to another which were illustrated in table 4. The mineral compositions of the different samples were also related in table 5 which includes potassium, calcium, phosphorous, magnesium, iron and zinc.

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Table I: Total viable count of flour meals

Sample (n=10 ⁵)	cfu/ml
A	2.4 x10 ⁵
B	1.4 x10 ⁵
C	3.0 x10 ⁵
D	3.1 x10 ⁵
E	2.8 x10 ⁵
F	1.4 x10 ⁵
G	1.0 x10 ⁵

Key: A-Small kernel corn powder, B- Big kernel corn powder, C-Small kernel corn powder blend with cassava powder, D-Big kernel corn powder blend with cassava powder, E- Dangote Semovita, F- Honeywell Semovita, G- Golden penny Semovita

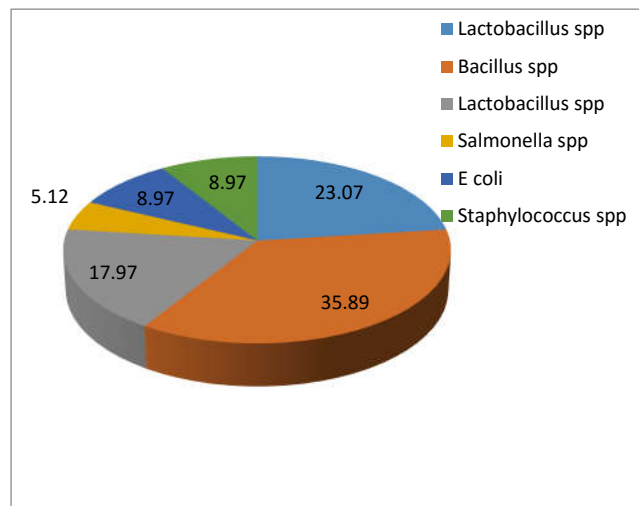


Figure 1: Frequency of occurrence of Bacteria Isolates (Percentage)

Table 2: Identification of Bacteria isolates from maize flour meal

Probable organisms	Grams staining reaction					TSI agar				IMVIC			
		Motility	Oxidase	Spore formation	Catalase reaction	slant	Gas	H ₂ S	Urease	Indole	MR	VP	Citrate
<i>Lactobacillus</i> spp	(+) long rod	-	-	-	-	A/A	+	-	-	-	+	-	-
<i>Bacillus</i> spp	(+) long rod	+	+	+	+	K/A	+	-	-	-	-	+	+
<i>Bacillus</i> spp	(+) short rod	+	+	+	+	A/A	+	-	-	-	-	+	+
<i>Lactobacillus</i> spp	(+) short rod	-	-	-	-	A/A	+	-	-	-	+	-	-
<i>Salmonella</i> spp	(-) rod	-	-	-	+	K/A	-	+	-	-	+	-	+
<i>E. coli</i>	(-) rod	-	-	-	+	A/A	+	-	-	+	+	-	-
<i>Staphylococcus</i> spp	(+) cocci	-	-	-	+	A/A	-	-	+	-	+	+	+

Table 3: Fungi Identification

Samples	Isolated Organisms
A	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> and <i>Penicillium chrysogenum</i>
B	<i>Aspergillus niger</i> , <i>Penicillium chrysogenum</i> and <i>Sclerotium rolfsii</i>
C	<i>Penicillium chrysogenum</i> , <i>Aspergillus niger</i> and <i>Aspergillus flavus</i>
D	<i>Penicillium chrysogenum</i> , <i>Aspergillus niger</i> and <i>Aspergillus flavus</i>
E	<i>Penicillium chrysogenum</i> and <i>Sclerotium rolfsii</i>
F	<i>Rhizopus microspores</i>
G	<i>Penicillium chrysogenum</i> , <i>Aspergillus niger</i>

Key: A-Small kernel corn powder, B- Big kernel corn powder, C-Small kernel corn powder blend with cassava powder, D-Big kernel corn powder blend with cassava powder, E- Dangote Semovita, F- Honeywell Semovita, G- Golden penny Semovita

Table 4: Proximate Composition of samples

Sample	%Crude protein	%Crude fat	%Crude fibre	%Ash	%Moisture	%CHO	Gross Energy (Kcal/g)
A	9.13	3.10	2.25	3.61	14.10	67.81	4.114
B	9.76	3.26	2.30	3.53	14.21	66.94	4.108
C	7.92	2.88	2.43	3.68	14.73	68.36	4.136
D	8.74	2.95	2.34	3.76	14.67	67.54	4.131
E	12.84	3.57	2.66	3.91	14.33	62.69	4.156
F	11.93	3.53	2.73	3.86	14.27	63.68	4.127
G	12.33	3.63	2.61	3.94	14.27	63.22	4.163

Key: A-Small kernel corn powder, B- Big kernel corn powder, C-Small kernel corn powder blend with cassava powder, D-Big kernel corn powder blend with cassava powder, E- Dangote Semovita, F- Honeywell Semovita, G- Golden penny Semovita

Table 5: Mineral Composition of samples

Sample	%Na	%K	%Ca	%P	%Mg	Fe(mg/kg)	Zn(mg/Kg)
A	0.103	0.149	0.097	0.131	0.168	6.6	11.4
B	0.080	0.161	0.114	0.141	0.177	8.4	13.6
C	0.051	0.111	0.037	0.105	0.126	4.0	6.8
D	0.057	0.115	0.074	0.118	0.156	4.6	9.7
E	0.087	0.170	0.124	0.157	0.191	12.7	15.0
F	0.091	0.160	0.131	0.163	0.196	12.0	15.5
G	0.084	0.157	0.120	0.154	0.185	11.6	14.4

Key: A-Small kernel corn powder, B- Big kernel corn powder, C-Small kernel corn powder blend with cassava powder, D-Big kernel corn powder blend with cassava powder, E- Dangote Semovita, F- Honeywell Semovita, G- Golden penny Semovita

Table 6: Aflatoxin Content of the Samples

Sample	Aflatoxin concentration value	Value in PPB
A	0.176	40
B	0.753	70
C	0.220	30
D	0.177	40
E	1.809	0.5
F	1.742	1
G	1.921	0.2

Standard 0: 1.946 Standard 4: 1.045, Standard 10: 0.633 Standard 20: 0.337 Standard 40: 0.176

Key: A-Small kernel corn powder, B- Big kernel corn powder, C-Small kernel corn powder blend with cassava powder, D-Big kernel corn powder blend with cassava powder, E- Dangote Semovita, F- Honeywell Semovita, G- Golden penny Semovita

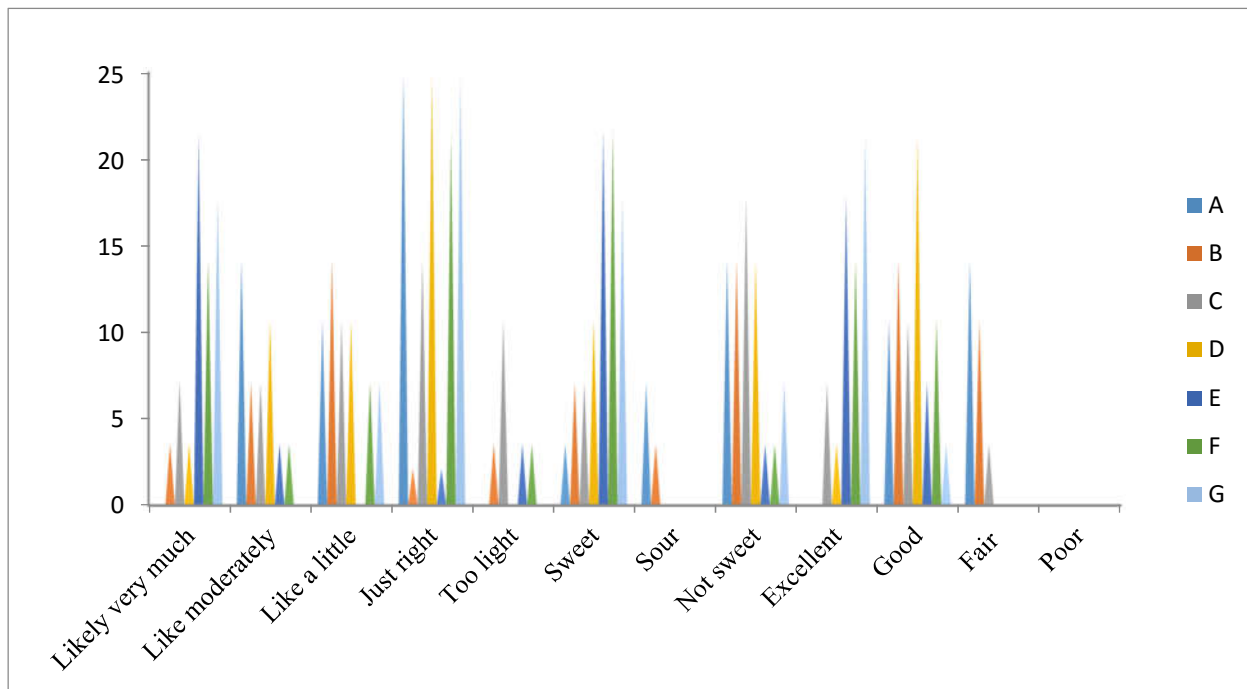


Figure 2: Sensory Evaluation in Percentage (%)

Key: A-Small kernel corn powder, B- Big kernel corn powder, C-Small kernel corn powder blend with cassava powder, D-Big kernel corn powder blend with cassava powder, E- Dangote Semovita, F- Honeywell Semovita, G- Golden penny Semovita

Discussion

Corn flour is known to be a safe commodity due to its low water activity, but microbial isolation and enumeration in this study revealed presence of important population of microorganisms which is in relation to the work of Akpe *et al.* (2010) and Aziz *et al.* (2006) confirming that cereals can be carrier of different types of microflora such as yeast, mould, *Bacillus cereus* and *Staphylococci*. The low number of various bacteria may be attributed to the moisture content as bacteria require high moisture content or water activity than that of the flour (Bullerman and Bianchin, 2009). In addition, Berghofer *et al.* (2003) noted that microflora concentrate on the outer layer of grains; hence the insignificant presence of bacteria may also be attributed to the removal of the outer layer of maize during milling leaving the end product relatively safe. In reference to Codex Alimentarius of (193-195) issued in 2013, the colony forming units are higher than the maximum standards. In accordance to Saleemi *et al.* (2012) about 75% of maize meal are contaminated with fungi especially *Aspergillus flavus*, while other main contaminants include; *A. niger*, *A. ochraceous*, *P. verrucosum* and *P. chysogenum*. Moisture is a very important parameter when taking into account the quality of flour and acceptability of flour product. Result of the present study shows that the moisture content was between 14.73 ± 14.10 with sample C having the highest content (14.73) and sample A with the lowest content (14.10). The presence of the highest moisture content level shows that fortification of cassava powder with sample A increase the level of its moisture. Protein content

also plays a very important role especially in the texture and palatability of the final product of corn flour. Sample E with the highest level of protein (12.84) and sample C with the lowest level of protein (7.92). Ash content of the samples are within the recommended limits with sample G having the highest content (3.94) and sample B having the lower content (3.53). Sample A has the lowest fibre content (2.25) and sample F with the highest fibre content (2.73). The carbohydrate content of the samples shows that all the samples contains the required percentage recommended limits with sample C having the highest (68.36) and sample G having the lowest content (63.22). The proximate analysis result shows that sample C (corn powder with cassava powder) contains all the necessary nutrients together with sample G (Golden penny) which was also in accordance to the manufacturer composition.

The mineral composition of all the samples is satisfactory because all the samples contain necessary mineral composition of sodium, potassium, calcium, phosphorus, magnesium, iron and zinc.

All the samples contain aflatoxin which is of health risk to the body. The aflatoxin in sample B (70ppb) was quite higher than the recommended limit of aflatoxin content of 20ppb. Samples E, F and G meet the recommended level with 0.5ppb, 1ppb and 0.2ppb respectively. Sample G has the lowest level of aflatoxin. The high level of aflatoxin in the samples may be due to processing and storage.

Sensory evaluation analysis indicated significant different in colour, texture, taste and general

acceptability result shows that sample G was like very much, samples D was like moderately and sample A and B was like a little. According to the result of the colour, all the samples have the right colour except sample B with too light colour. Sample F has a sweet taste while sample C is not sweet. The result shows that sample G has excellent odour with sample A, C, D, with poor odour.

Conclusion

The data indicated that all the samples contained necessary nutrient and mineral content needed by the body. This study reveals that fortification of corn powder with cassava powder is cheaper than the commercially made ones and can compete nutritionally with the commercially made counterpart. In reference to aflatoxin contamination based on this study, sample G (Golden penny semovita) is the safest as it has the least aflatoxin content.

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