



## **Antinutrients and Bioavailability of Nutrients in Maize, Cassava and Soybeans Composite Flour**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author FZI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SOA and NPI managed the analyses of the study. Author AAD managed the literature searches. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Composite flour production and utilization in Nigeria and globally is on the increase. This is to take advantage of its nutritional and health benefits at the same time increase the food value of certain cereals and tubers. This study was designed to evaluate the antinutrients and bioavailability of nutrients in maize, cassava and soybeans composite flour. Maize, cassava and soybean flours were composite at 70:25:5, 70:20:10, 70:15:15 and 70:10:20 blends while whole maize (100:0:0) and maize/cassava (70:30:0) blends served as controls. The result showed that substitution of cassava flour with dehulled soybeans elevated the mineral contents of maize, cassava and soybeans composite flour. It, however, decreased the anti-nutritional contents of the flour. Phytate, tannins, oxalates and cyanide contents of the composite flour were within the recommended safe ranges. The molar ratios of the phytate/Ca, phytate/Fe, phytate/Zn and Oxalate/Ca were far below their critical values. This implies that the bioavailability of these critical mineral elements of this composite flour is high. It was, therefore, recommended that the use of soybeans in composite flour production should be encouraged to boost the nutritional and health quality of our local flours.

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## 1. INTRODUCTION

Minerals and vitamins deficiencies in diet are common in Nigeria, resulting to micronutrients malnutrition. Globally, an estimated two billion people suffer from a chronic deficiency of micro nutrients resulting into most health problems [1, 2]. The total micronutrients is not important in foods, their bioavailability is more effective factor than their level. Improving the nutritional value of foods will improve the nutritional status of the population [3]. Anti-nutritional factor; most especially phytate has been recognized affecting the bioavailability of minerals like calcium, iron, copper, zinc and manganese [4]. Other antinutritional factors in foods are tannins, polyphenol, oxalate and trypsin. These antinutrients limit the bioavailability of food minerals [4].

Reducing the anti-nutrients in food is necessary, due to their influences on nutrition. Many anti-nutrients, in spite of their effects on bioavailability of nutrients may also be toxic beyond a certain dose, for instance oxalate or cyanogenic acids, therefore interest has been drawn to reduce anti-nutritional factor in foods [5]. Most of the anti-nutritional factors in food become ineffective or reduced by simple treatment or pretreatment such as soaking, dehulling, heating, germination and fermentation [6]. Germination and fermentation enrich the nutritional value of cereals, legumes, roots and tubers by causing significant changes in chemical composition and elimination of anti-nutritional factors [6]. The study was aimed at determination of antinutrients and bioavailability of nutrients in maize, cassava and soybeans composite flour formulated.

## 2. MATERIALS AND METHODS

### 2.1 Sources of Materials

Maize (*Zea mays*), Soybean (*Glycine max*) and fresh cassava roots for the study were purchased from a local farmer in Mbabuande, Gwer-west Local Government Area of Benue State, Nigeria. White varieties of maize were chosen because; it is commonly and widely grown in Benue State for human consumption.

### 2.2 Production of Maize Flour

The maize flour was prepared according to the method of Houssou and Ayemor [7]. The maize

grains (free from dirt, damaged and contaminated grains) were cleaned and soaked in potable water for 30 minutes with occasional change of the soak water. The soaked grains were drained, rinsed and sun dried. The dried grains were milled in chemistry Laboratory, Benue State University. The powder obtained was sieved through a muslin cloth (250  $\mu$ m) into a clean plastic bowl. The maize flour produced was packaged in an airtight plastic container for further use.

### 2.3 Production of Cassava Flour

The cassava flour was produced according to the method of Adekunle et al. [8]. The cassava roots were peeled manually, cut into slices with a sharp knife, washed and soaked in water for 72 hours. The soaked cassava roots were packed in a sack and dewatered by pressing with hands. The dewatered cassava was molded and sundried to constant weight. The dried cassava was milled in attrition mill and sieved through muslin cloth (250  $\mu$ m).

### 2.4 Production of Soybean Flour

The soybean flour was produced according to the method described by Edema et al. [9]. The soybeans were sorted to remove pebbles, stones and other extraneous materials. The seeds wet cleaned and steeped in water for 2 hours. The steeped soybeans were drained and boiled for 30 minutes at 100°C, dehulled manually by rubbing in between the palms and the hulls were removed by rinsing with clean water. The dehulled soybeans were sun dried to constant weight and milled in attrition mill. The soybean powder was sieved through a sieve of 250  $\mu$ m aperture size to obtain smooth flour. The soybean flour was packaged in a low density polyethylene bag until used.

### 2.5 Blends Formulation

Maize, cassava and soybeans flours were blended on percentage basis as shown in Table 1. The quantity of maize flour was kept constant while that of cassava and soybeans flour were varied by 5%.

### 2.6 Mineral Determination

The mineral contents (calcium, magnesium, phosphorus, potassium, iron and zinc) of the composite flour were determined using AOAC [10].

**Table 1. Blend formulation for maize/cassava/soybean composite flour (%)**

Sample	Maize flour	Cassava flour	Soybean flour
MCS1	100	0	0
MCS2	70	30	0
MCS3	70	25	5
MCS4	70	20	10
MCS5	70	15	15
MCS6	70	10	20

MCS1 100% Maize + 0% Cassava flours+0% Soybeans flour; MCS2: 70% Maize flour+30% Cassava flour + 0% soy flours; MCS3: 70% Maize flour+25% Cassava flour + 5% soy flours; MCS4: 70% Maize flour+20% Cassava flour + 10% soy flours; MCS5: 70% Maize flour+15% Cassava flour + 15% soy flour; MCS6 :70% Maize + 10% Cassava flours+20% Soybeans flour

## 2.7 Determination of Antinutrient

The phytate determination was done as described by Thompson, and Erdman [11] method; The Folin-Denis colorimetric method as described by Kirk and Sawyer [12] was used for the determination of tannin content; Oxalate were determined according to AOAC [10] and Hydrogen Cyanide (HCN) was determined by the alkaline picrate colorimetric method of Bradbury et al. [13].

## 2.8 Mineral Bioavailability

The molar ratios of phytate to Calcium (phy: Ca), phytate to iron (phy:Fe), phytate to zinc (phy:Zn) and oxalate to calcium (Oxa:Ca) were calculated to estimate the relative bioavailability of calcium, iron and zinc in the presence of anti-nutrients. Molar ratios of antinutrient/minerals were used to predict the mineral bioavailability [14]. The molar ratios were calculated as:

$$\text{Molar ratio} = \frac{\text{Mole of Antinutrient}}{\text{Mole of Mineral}} \quad (1)$$

$$\text{Mole of antinutrient} = \frac{\text{Antinutrient (mg)}}{\text{Atomic weight (g/mol)}} \quad (2)$$

$$\text{Mole of mineral} = \frac{\text{Mineral (mg)}}{\text{Atomic weight (g/mol)}} \quad (3)$$

The molar mass of phytate used was 660 g/mol and that of oxalate was 88 g/mol while the molar mass of calcium was 40 g/mol, Iron (56 g/mol) and Zinc (65 g/mol). The recommended critical values used to predict the bioavailability were phytate: calcium >0.24 [15], phytate: zinc > 15 [14], phytate: iron > 1 [16] and Oxalate: calcium > 1 [17].

## 2.9 Statistical Analysis

Data obtained were subjected to one-way analysis of variance (ANOVA) and mean separation was done by Duncan multiple range

test ( $p=0.05$ ), using Statistical Package for Social Sciences (SPSS, Version 20).

## 3. RESULTS AND DISCUSSION

### 3.1 Mineral Composition Maize-cassava-soybeans Composite Flour

The mineral composition of maize-cassava-soybeans composite flour is shown in Table 2. These values were reported alongside the corresponding values from the United States Recommended Dietary Allowance (USRDA). Generally, significant difference ( $p<0.05$ ) existed among the samples in all the samples except iron in sample MCS3 and MCS4. The mineral content increased with addition of soybeans flour in the formulation.

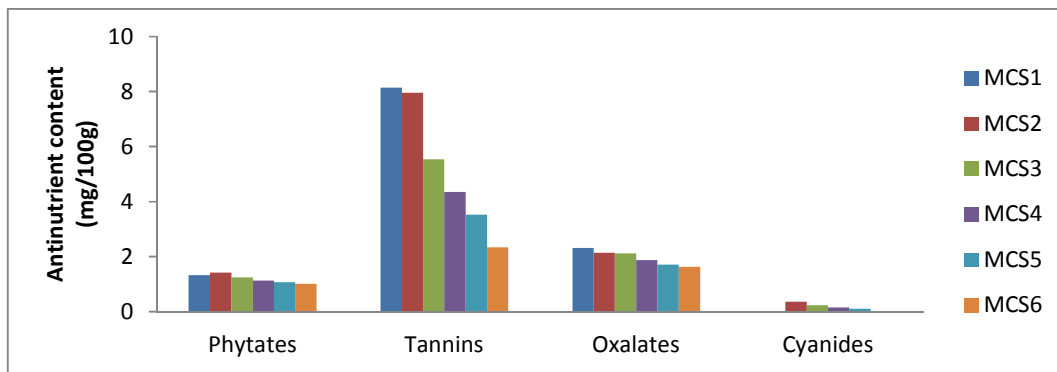
The calcium content obtained from the samples ranged between 162.13mg/100g to 193.46 mg/100g. The highest value was found in the sample containing 70:10:20(MCS6) formulations while the least value occurred in the sample with 100% maize. These values were less than the USRDA (1000 mg) for calcium. This result was higher than the calcium content of 0.062, 0.052 and 0.060 mg/100g reported for three Sudanese sorghum-soy flour [18]. The calcium content was also higher than 9.32 to 14.02 mg/100g of maize-soybeans flour blends. Calcium plays significant roles in blood clotting, muscle contractions, optimal growth and development of infant and children [19]. The combination of calcium, phosphorus and vitamin D eliminate rickets in children and osteomalacia (in adult) as well as osteoporosis (bone thinning) among elderly people [20].

The magnesium content obtained for the samples ranged from 31.26 to 46.63mg/100g. The highest value was recorded for sample MCS6 containing (70:10:20) formulation. These values were however; lower than the USRDA,

**Table 2. Mineral composition of maize-cassava-soybeans composite flour (mg/100 g)**

Sample	Calcium	Magnesium	Potassium	Phosphorus	Iron	Zinc
MCS1	162.13 <sup>d</sup> ±0.70	33.16 <sup>e</sup> ±0.01	325.63 <sup>f</sup> ±0.01	87.63 <sup>e</sup> ±1.40	9.35 <sup>d</sup> ±0.00	3.33 <sup>e</sup> ±0.01
MCS2	165.35 <sup>e</sup> ±0.01	31.26 <sup>f</sup> ±0.01	386.35 <sup>e</sup> ±0.01	77.30 <sup>f</sup> ±0.02	9.12 <sup>e</sup> ±0.01	3.25 <sup>f</sup> ±0.01
MCS3	167.43 <sup>d</sup> ±1.40	35.82 <sup>d</sup> ±0.72	394.54 <sup>d</sup> ±0.01	95.53 <sup>d</sup> ±0.01	9.62 <sup>c</sup> ±0.00	4.46 <sup>d</sup> ±0.01
MSC4	178.63 <sup>c</sup> ±2.14	38.65 <sup>c</sup> ±0.02	412.39 <sup>c</sup> ±0.01	98.55 <sup>c</sup> ±0.01	9.66 <sup>c</sup> ±0.01	5.52 <sup>c</sup> ±0.01
MSC5	187.64 <sup>b</sup> ±1.45	42.92 <sup>b</sup> ±1.41	444.36 <sup>b</sup> ±0.02	103.77 <sup>b</sup> ±1.41	9.83 <sup>b</sup> ±0.01	6.53 <sup>b</sup> ±0.01
MSC6	193.46 <sup>a</sup> ±1.43	46.63 <sup>a</sup> ±1.43	454.05 <sup>a</sup> ±0.21	107.15 <sup>a</sup> ±1.41	10.02 <sup>a</sup> ±0.01	6.66 <sup>a</sup> ±0.01
USRDA	1000	350	3500	800-700	10-15	12-15

Values are means +Standard deviation (n=2). USRDA= United States Recommended Dietary Allowance. MCS1 100% Maize + 0% Cassava flours+0% Soybeans flour; MCS2: 70% Maize flour+30%Cassava flour + 0% soy flours; MCS3: 70% Maize flour+25%Cassava flour + 5% soy flours; MCS4: 70% Maize flour+20%Cassava flour+ 10% soy flours; MCS5: 70% Maize flour+15%Cassava flour + 15% soy flour; MCS6 :70% Maize + 10% Cassava flours+20% Soybeans flour

**Fig. 1. Anti-nutrients composition of maize-cassava-soybeans composite flour (mg/100g)**

MCS1: 100% Maize + 0% Cassava flours+0% Soy flour; MCS2: 70% Maize flour+30%Cassava flour + 0% soy flours; MCS3: 70% Maize flour+25%Cassava flour + 5% soy flours; MCS4: 70% Maize flour+20%Cassava flour+ 10% soy flours; MCS5: 70% Maize flour+15%Cassava flour + 15% soy flour; MCS6 :70% Maize + 10% Cassava flours+20% Soybeans flour

**Table 3. Molar ratios of anti-nutrients/mineral bioavailability of maize, cassava and soybeans composite flour**

Sample	Phytate: Ca	Phytate: Fe	Phytate: Zn	Oxalate: Ca
MCS1	0.00049	0.01198	0.03922	0.00641
MCS2	0.00048	0.01227	0.04000	0.00580
MCS3	0.00048	0.01163	0.02941	0.00573
MCS4	0.00045	0.01156	0.02381	0.00470
MCS5	0.00034	0.01136	0.02000	0.00405
MCS6	0.00031	0.01117	0.01961	0.00394
Critical value	0.24	1.00	15	1.00

MCS1: 100% Maize + 0% Cassava flours+0% Soy flour; MCS2: 70% Maize flour+30%Cassava flour + 0% soy flours; MCS3: 70% Maize flour+25%Cassava flour + 5% soy flours; MCS4: 70% Maize flour+20%Cassava flour+ 10% soy flours; MCS5: 70% Maize flour+15%Cassava flour + 15% soy flour; MCS6 :70% Maize + 10% Cassava flours+20% Soybeans flour

which is 350 mg for men and 280 mg for women. Magnesium helps in the maintenance of electrical potentials in nerves, activator of many enzymes system, assist in muscle contraction, blood clotting, regulation of blood pressure and lungs function [21].

As can be seen in (Table 2) the composite flour has potassium ranging from 325.63 mg/100g to 454.05 mg/100g. The highest value occurred in the sample with ratio 70:10:20. This range is lower than the USRDA for both men and women (350 mg). The potassium values are higher than 2.18-3.82 mg/100g reported for maize-soybeans

flour blends [9]. The blends have potassium content that increased significantly ( $p < 0.05$ ) with increasing level of soybeans flour in the blends. The result indicates that a soybean is a good source of potassium [22]. Potassium is very essential in blood clotting, muscle contraction. Potassium is primarily an intercellular cation, in large part this cation is bound to protein and with sodium influence osmotic pressure and contributed to normal pH equilibrium [22].

It was observed that phosphorus content of the composite flour ranged from 77.30 to 107.15 mg/100g. The phosphorus content of 30% cassava flour was lower (77.30mg/100g) while that of other blends increased with increasing level of soybeans flour. This is an indication that soybeans flour has higher phosphorus content. The results of this study are lower than (800 mg) USRDA and higher than 2.84 to 4.24 mg/100g reported [9]. The result is in agreement with the report that soybean is a good source of phosphorus [22]. Phosphorus helps in the formation of Adenosine Triphosphate (ATP) in the body [21].

The iron content of the samples ranged from 9.12mg/100g to 10.02 mg/100g with the blends containing 70:30:0% of cassava and 70:10:20% soybeans flours having the least and highest values, respectively. This indicates that soybean has a good source of iron. The value obtained from sample MCS6 (10.02 mg) was within the recommended range of the USRDA (10-15 mg) while other samples MCS1, MCS2, MCS3, MCS4 and MCS5 were below the USRDA recommended value (10-15 mg). The values of this study are higher than 1.98 to 3.08 mg/100g as reported [9]. Iron is an important component of hemoglobin which is an oxygen-carrying pigment in the blood [21]. When foods with iron are eaten, it is absorbed into proteins and these proteins take in, carry and release oxygen throughout the body. An iron deficiency called iron-deficiency anemia is very common around the world, especially for women and children in developing nations. Symptoms of iron deficiency take years to develop and include fatigue, weakness and shortness of breath [23].

The zinc content of the composite flour varied between 3.33 mg/100g to 6.66 mg/100g. The amount of zinc increased significantly ( $p < 0.05$ ) with increasing level of soybeans flour. These values are lower than the USRDA (15 mg for men, 12 mg for women). The zinc content of the study is higher than 2.25 to 3.22 mg/100g as

reported [9]. The increase in the amount of zinc is an indication that the products from the flour will be good for pregnant women. Zinc is a component of every living cell and plays a role in hundreds of bodily functions from assisting in enzyme reactions to blood clotting and is essential to taste vision and wound healing [23].

### **3.2 Antinutrient Contents of Maize, Cassava and Soybeans Composite Flour**

The anti-nutrient contents of maize, cassava and soybeans composite flour is shown Fig. 1. The result obtained from the phytate ranged from 1.02 to 1.43 mg/100g. There was a gradual decrease in phytate with addition of soybeans flour. Maize-cassava (70:30) had the highest phytate content (1.43 mg/100g) while the lowest value was observed in the sample with 20% soybeans flour. The phytate content of maize-cassava-soybeans (1.02-1.43 mg/100g) reported in this study is lower than the phytate content 39.4% of raw oat cereal [24]. The result is also lower than 36.6% and 25.7-39.4% for malted sorghum-soy flour [25]. Many dietary fibres contain phytic acid which binds minerals in the digestive tract, which eventually expels the minerals from the body. Some of these minerals are essential for good health, including Zinc, Iron and calcium. Although health experts recommended increasing intake of dietary fibre, eating too much fibre containing phytic acid can cause mineral deficiencies [26].

The result for tannin ranged from 2.34 to 8.15 mg/100g. 100% maize flour had the highest tannin content (8.15 mg/100g) while substitution with 20% soybeans flour had the lowest value (2.34 mg/100g). There was significant ( $p < 0.05$ ) decrease in the level of tannin in with increase in soybeans flour. The tannin content (2.34-8.25 mg/100g) of the maize-cassava-soybeans flour obtained in this study is lower than the tannin content of 23.8 to 26.7% as reported in sorghum cultivars [27] and sorghum-soy-plantain flour 23.8-27.4% as reported in literature [28]. The result is also lower than tannin content of 35.8% as reported in malted sorghum flour [29]. Lower tannin content observed in this study could be due to degradation of tannin during soaking, boiling and fermentation process [6]. Tannins are located in the seed coat of the grains and are known to have deleterious effects due to their strong interactions with proteins, with the resulting complexes which are not readily

digested by monogastrics. This lowers the protein digestibility [29].

The oxalates contents ranged from 1.63-2.32 mg/100g. The oxalates value of 100% maize flour had the highest value 2.32 mg/100g while that of the blends decreased significantly ( $p < 0.05$ ) as the proportion of soybeans flour increased. The decrease in the oxalates could be due to the boiling process of soybeans which reduced the contents of the oxalates [6]. Usman [30] reported the oxalate content of the african yam bean (*Sphenostylis stenocarpa*), maize (*zea mays*) and defatted coconut (*cocosnucifera*) to range from 0.076 to 0.302mg/100g.

The cyanides contents of the composite flour ranged between 0.06 mg/100g - 0.36 mg/100g. The sample with 70:30% maize-cassava flour had the highest cyanides content with 0.36% while 20% soybeans substitution had the lowest value. However, sample with 100% maize had 0.00 mg/100g. The cyanides contents of the composite flour decreased with increase in the substitution level of soybeans. Cyanides is said to cause acute intoxication, goiters, partial paralysis or even death [31].

### 3.3 Mineral Bioavailability

The molar ratios along with the suggested critical values for predicting the bioavailability of calcium, iron, and zinc are presented in Table 3. The phytate/calcium molar ratios in all the samples were  $< 0.24$ , which is regarded as favourable for calcium absorption [15], predicting that a good bioavailability could be achieved from maize, cassava and soybeans flours. The molar ratio of phytate/zinc is within the range of 0.01961-0.04000 which is lower than the range of the suggested critical level  $< 15$  regarded as favourable for zinc absorption [14]. The ratio  $\geq 15$  are associated with low zinc bioavailability. The phytate/iron molar ratios in all the samples were  $< 1$ , which is regarded as favourable for iron absorption [16], predicting that a good bioavailability could be achieved from maize, cassava and soybeans flours. Oxalate/calcium molar ratios of all the samples were below the critical level of 2.5 known to significantly impair bioavailability suggesting that they are good calcium bio resource [17].

## 4. CONCLUSION

The substitution of cassava flour with dehulled soybeans flour elevated the mineral contents of

maize, cassava and soybeans composite flour. It, however, decreased the anti-nutrient contents of the flour. Phytate, tannins, oxalates and cyanide content of the composite flour were within the recommended safe ranges. The molar ratios of the phytate/Ca, phytate/Fe, phytate/Zn and Oxalate/Ca were far below their critical values. This implies that the bioavailabilities of these critical mineral elements of this composite flour are high.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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