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Promoting the use of locally produced crops in making cereal-legume-based composite flours: An assessment of nutrient, antinutrient, mineral molar ratios, and aflatoxin content

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A B S T R A C T

Cassava, rice, and banana flours were used individually to replace wheat flour in cereal-legume-based composite flours. The proximate composition, mineral content, antinutritional effect, mineral molar ratios, and aflatoxin level were investigated. Replacing wheat flour with rice flour significantly ($P < 0.05$) improved protein, fat, potassium, and phosphorus content in samples. The molar ratios of phytate or oxalate to minerals (calcium and zinc) in all composite flours were lower than the reported critical values, except phytate to iron. However, all samples, except full replacement by rice flour, might not provide adequate zinc bioavailability when the effect of calcium and phytate on zinc absorption was collectively considered. Although all composite flours were contaminated with aflatoxins, only the control composed of wheat flour did not meet the EU regulatory threshold (4.0 µg/kg) for total aflatoxins. The findings showed that nutritional properties and aflatoxin content of composite flours can be improved by replacement with local crops.

1. Introduction

Cereal–legume-based flour is a basic staple food for many African populations and is commonly consumed as porridge for breakfast, as an ingredient for fufu, or used as a child-weaning food. Most cereals, such as maize, wheat, and sorghum, are a source of calories and complex carbohydrates but have a low protein and lysine content. On the other hand, legumes like soybean and other beans are relatively richer in proteins and dietary fiber, as well as a significant number of vitamins and minerals (Tharanathan and Mahadevamma, 2003). Today, many countries in Sub-Saharan Africa (SSA), except Sudan and Ethiopia, have become completely dependent on imported wheat resulting in a debt burden (Eleazu et al., 2014). Another challenge associated with wheat consumption is the development of celiac disease (gluten-sensitive enteropathy) (Elijah, 2014). To counteract such problems, the use of composite flour consisting of several flours obtained from local crops has been emphasized in many low-income countries, with or without the addition of wheat flour (Shittu, Raji, & Sanni, 2007; Renzetti, Dal Bello, & Arendt, 2008).

One of the major problems of plant-based flours is the presence of antinutrients such as phytate, oxalate, and tannin which limit their utilization (Kathirvel & Kumudha, 2011). Although, some health benefits from tannin and phytate have been shown, such as reductions in blood glucose and insulin response to starchy foods, and/or in plasma cholesterol and triglycerides (Thompson, 1993), and oxalate is rather a pro-oxidant with reports of toxicity due to a build-up of free radicals (Kumar, Sinha, Makkar, & Becker, 2010), these antinutrients can adversely interfere with the absorption of minerals, such as calcium, zinc, and iron (Kruger et al., 2013) and prevent efficient utilization of proteins (Gibson, Bailey, Gibbs, & Ferguson, 2010). The processing method either by soaking and/or heating have been found to reduce those anti-nutritional factors (Patterson, Curran, & Der, 2017), however it could still pose a significant health impact especially in cases where the original levels of the anti-nutritional factors are high (Gibson, Perlas, &

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Holz, 2006). Shi, Arntfield, and Nickerson (2018) reported that the soaking and cooking processes were found to be more effective in reducing lectins and oxalate in common beans and soybean, except phytic acid. The optimal exploitation of ingested nutrients is therefore hampered, rendering to decreasing their nutritive value (Fekadu, Beyene, & Desse, 2013; Gemede, Haki, Beyene, Woldegiorgis, & Rakshit, 2015). Low mineral availability in staple foods can lead to malnutrition and to physiological pathology, such as osteoporosis, impairment of child growth, and anemia (Akhter, Saeed, Irfan, & Malik, 2012). In addition, cereals and legumes are susceptible to aflatoxin contamination during production and storage which causes serious health complications in human and animals, such as stunting in children, liver damage, and cancer (Ismail et al., 2018).

To achieve efficient utilization and promotion of cereal–legume-based composite flours among the poor, especially in rural areas, a study of their functional properties and aflatoxin contamination is necessary. Therefore, the objective of this study aims at evaluating the nutrient, antinutrient, mineral molar ratios, and aflatoxin contents in cereal-legume-based composite flours.

2. Materials and methods

2.1. Composite flour preparation

Cereal-legume-based flours from a mixture of maize, wheat, sorghum, soybean, and sesame flours were prepared as the control, whereas flours obtained from local crops, including cassava, rice, and banana flours, were used to partly and fully replace wheat flour in the formula. All grain and seed samples were procured from local markets in Bujumbura, Burundi, except wheat flour which was obtained "as it is". Maize, sorghum, soybean, and sesame samples were separately cleaned, sorted, sun-dried for 1–2 days, and roasted at 150 °C for 20 min. The roasted maize and soybean seeds were dehulled and allowed to cool at room temperature; rice seeds were sorted and roasted at 150 °C for 5 min. Fresh cassava was peeled and cleaned, subsequently soaked in water for 12–16 h, then grated and pressed to remove water. The pressed mash obtained was sun-dried for 3–4 days. Fresh green and firm banana was also peeled, cleaned, and then cut into small pieces before being sun-dried for 2 days. All samples were dried/roasted to a moisture content of 7.5 ± 0.5 g water/100 g, ground to obtain a fine powder, and passed through a 750 μm mesh sieve.

The composition and ratio of the control (CON) was selected, based on a commercial product. The ratio of maize, sorghum, sesame, and soybean flours were fixed in all treatments (Table 1); the composition of wheat flour was partly (P) and fully (F) replaced by cassava (C), rice (R), and banana (B) flours. In each treatment, all flours were accurately weighed and mixed for 10 min with a double cone solids blender which was locally fabricated in Burundi. All composite flour samples were stored in airtight containers prior to biochemical analyses.

Table 1
Seven different treatments of composite flours.

Composition (g/100 g)	CON[1]	P-C	F-C	P-R	F-R	P-B	F-B
Maize flour	30	30	30	30	30	30	30
Wheat flour	25	10	–	10	–	10	–
Sorghum flour	15	15	15	15	15	15	15
Sesame flour	10	10	10	10	10	10	10
Soybean flour	20	20	20	20	20	20	20
Cassava flour	–	15	25	–	–	–	–
Rice flour	–	–	–	15	25	–	–
Banana flour	–	–	–	–	–	15	25
Total	100	100	100	100	100	100	100

[1] CON = control with 25 g wheat flour /100 g composite flour.
P-C = partial substitution of wheat flour with 15 g/100 g of cassava flour.
F-C = full substitution of wheat flour with 25 g/100 g of cassava flour.
P-R = partial substitution of wheat flour with 15 g/100 g of rice flour.
F-R = full substitution of wheat flour with 25 g/100 g of rice flour.
P-B = partial substitution of wheat flour with 15 g/100 g of banana flour.
F-B = full substitution of wheat flour with 25 g/100 g of banana flour.

2.2. Biochemical analyses

2.2.1. Proximate analysis

Moisture content was determined using the method of AOAC (1990). The sample was dried at 105 °C for 16 h in a draft of air (model UF55, Memmert Oven, Germany). The loss in weight was recorded as the moisture content. Protein content was investigated by the Kjeldahl method. A conversion factor of 6.25 was used to convert from total nitrogen to percentage crude protein. Ash content was determined by the method of AOAC (1990) that involved burning off moisture and all organic constituents at 600 °C in a VULCAN™ furnace (model 3-1750, Cole-Parmer, Illinoi, USA). The weight of the residue after incineration was recorded as the ash content. Fat content of the samples was also determined by the method of AOAC (1990), using the Soxhlet extraction technique (model FOSS Soxtec™ extraction, Sweden). The crude fiber content was determined using fiber extraction equipment (model FOSS Fibertec™ 2010, Sweden). The percentage of carbohydrate content was calculated by subtraction of the percentages of moisture, crude protein, ash, fat, and crude fiber from 100. The caloric value (kcal/100 g) of each loaf was calculated using Atwater's conversion factors, based on the caloric coefficients corresponding to the contents of protein (4 kcal/g), carbohydrate (4 kcal/g), and fat (9 kcal/g). All measurements were carried out in triplicate.

2.2.2. Determination of total sugar content

Total sugar content was determined by the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956). About 0.02 g of the sample was weighed into a centrifuge tube; 1 mL ethanol, 2 mL distilled water, and 10 mL hot ethanol were added. The mixture was vortexed and centrifuged at 2000 rpm for 10 min. The supernatant was decanted and used for determining sugar content. Phenol sulfuric acid reagent was used for color development, and glucose standards were used for estimation of sugar. The absorbance was read with a spectrophotometer (model Genesys G10S, USA) at 490 nm.

2.2.3. Mineral analysis

Potassium (K) in composite flour samples was determined using a Flame photometer (model 410, Sherwood Scientific Ltd., UK); analysis of phosphorus (P) was performed using the UV spectrophotometer (model 7305, Jenway, UK). Contents of calcium (Ca), iron (Fe), magnesium (Mg), and zinc (Zn) were analyzed using the Atomic Absorption Spectrophotometer (AAS) (model 205, Buck Scientific, USA). All measurements were carried out in triplicate.

2.2.4. Determination of antinutrients

The method of Latta and Eskin (1980) was modified to determine the phytate content. Phytate was extracted from 1 g of composite flour with 20 mL of 2.4% (v/v) hydrochloric acid (HCl) by shaking at room temperature for 2 h, followed by high speed centrifugation of the suspension for 15 min. The supernatant decanted and filtered through Whatman No. 1 filter paper. A 3 mL aliquot of filtrate was diluted to 18 mL with distilled water and the diluted sample was passed through a 200–400 mesh AG1-X8 chloride anion exchange resin (Bio-Rad Laboratories GmbH, München, Germany). Inorganic phosphorus was eluted with 0.07 M sodium chloride (NaCl) followed by elution of phytate with 0.7 M NaCl. Phytate was determined colormetrically based on pink color of Wade reagent, which is formed upon the reaction of the ferric ion and sulfosalicyclic acid. One milliliter of Wade reagent (0.03% solution of $FeCl_3 \cdot 6H_2O$ containing 0.3% (v/v) sulfosalicyclic acid in water) was added to 3 mL of the clear supernatant sample and then centrifuged at 2000 rpm for 15 min. The absorbance was measured by a spectrophotometer (model Genesys G10S, USA) at 500 nm. The phytate content was calculated using a standard phytic acid curve and results were expressed as mg/100 g.

Tannin content was determined by weighing 0.5 g of a composite flour sample and then adding 5 mL of 1% (v/v) of HCl in methanol. The sample was allowed to stand at room temperature for 15 min before vortex mixing and centrifugation at 3000 rpm for 10 min. The supernatant of 2.5 mL was transferred to a 10 mL flask containing 7.5 mL of water, then 0.5 mL of Folin-Denis regent and 1 mL of sodium carbonate were added. The final volume was adjusted to 10 mL with water, and the absorbance was determined after 30 min of incubation at room temperature using a UV/Vis spectrophotometer (model Genesys G10S, USA) at 760 nm. The content of tannin was calculated from the standard curve of tannic acid solution.

Total oxalate content was assayed using the method of Savage, Vanhanen, Mason, and Rose (2000) with a slight modification. Total oxalates were extracted from 0.5 g of composite flour samples with 50 mL of 0.2 M HCl and then incubated in a water bath at 80 °C for 15 min. The extracts were allowed to cool to room temperature before being transferred to a 100 mL volumetric flask; the final volume was adjusted with water. Subsequently, the extracted samples were centrifuged at 2800 rpm for 15 min. The supernatant was filtered through a 0.45 μm cellulose acetate filter (Sartorious, Göttingen, Germany) prior to analysis with High-Performance Liquid Chromatography (HPLC). The HPLC system used consisted of a binary pump (model ProStar 210, Varian, USA), auto-sampler (model Triathlon, Spark, Holland) with a UV detector set at 210 nm (model Waters 2487 Dual λ, absorbance detector, Waters Corporation, USA). Separation was conducted in a Supelco reversed phase column (4.6 mm I.D. × 250 mm length). The mobile phase was 0.25 g/100 mL dehydrogenate phosphate and 0.0025 mol/L tetrabutyl ammonium hydrogen sulphate buffered at pH 2.0 with ortho-phosphoric acid. The column temperature was set at room temperature and the total running time was 20 min at a flow rate of 1 mL/min. The injection volume was 5 μl. Identification of oxalic acid was carried out by comparing the HPLC retention time of unknown peaks to the chromatogram with added standard.

2.2.5. *Determination of molar ratio of antinutrients to minerals*

The predicted bioavailability of dietary minerals was determined based on the molar ratio (Gemede et al., 2015). The molar ratio of phytate and oxalate to calcium, iron, or zinc was obtained after dividing the mole of the antinutrient with the mole of the mineral.

2.2.6. *Aflatoxin analysis*

Composite flour samples were extracted according to the procedure described by ISO (2003) method number 16,050, with minor modifications. Briefly, 12.5 g of sample was weighed into a 100 mL amber glass conical flask, and 3 g of sodium chloride was added, followed by 50 mL of methanol and purified water (30 mL: 20 mL). The mixture was shaken (model HS 501 D Shaker, IKA, Germany) at 200 rpm for 1 h. The suspension was allowed to settle before being filtered through Whatman No. 1 paper. Fifty milliliters of the filtrate were collected into a conical flask, then 10 mL of the filtrate was placed in a beaker and 30 mL of phosphate buffer solution was added. The pH value was then adjusted to 7.4 using 0.1 mol/L sodium hydroxide (NaOH). The sample was passed through an immune-affinity column (AflaStar TM R-IAC, Romer Labs Inc., Missouri, USA), followed by a 20 mL rinse with ultrapure water. Total aflatoxins were eluted with 1.5 mL of water-free methanol (HPLC grade).

A HPLC system (Agilent 1260, Agilent Technologies Inc., USA) consisting of a binary pump, auto sampler, fluorescence detector, reverse phase column C18 (Hypersil ODS, 4.6 × 100 mm, 5 μm), and column heating chamber was used for the separation and quantification of aflatoxins. The mobile phase consisted of an isocratic mixture of water/acetonitrile (75 mL: 25 mL) and flow rate was set at 1.0 mL/min. The fluorescence detector was set at an excitation wavelength of 360 nm and an emission wavelength of 423 nm. The retention times (mins) were aflatoxin-B_1 7.62; aflatoxin-G_1 4.01; aflatoxin-B_2 5.21; and aflatoxin-G_2 2.53. To assess the reliability of the method, standards for aflatoxin-B_1, aflatoxin-G_1, aflatoxin-B_2, and aflatoxin-G_2 were spiked into blank flour samples at three concentration levels: 2.25, 3.75, and 5.25 mg/kg. The selection of these concentration levels was based on the level of background contamination of collected samples. On average, 95.5% of aflatoxin-B_1 was recovered; 101.2% of aflatoxin-G_1; 98.5% of aflatoxin-B_2; and 99.7% of aflatoxin-$G_{2.}$

2.3. *Statistical analyses*

Statistical analysis was performed using the general linear model. Least significant difference (LSD) was used to estimate the significant differences among the means for each treatment at 5% probability level using the SAS program (Ver. 9.4, SAS Inst., Cary, NC, USA).

3. Results and discussion

3.1. *Nutritional composition*

The result showed that the moisture content of all composite flours was in the range of 7.2–7.7 g water/100 g, with no statistically significant difference between the flours (Table 2). A significant increase ($P < 0.05$) of protein (17.5–18.5 g/100 g) and fat content (10.1–11.0 g/100 g) was found in partial (P) and full (F) replacement with rice flours (P-R and F-R) when compared with the corresponding values of other samples. The higher level of protein observed in replacement with rice could be an advantage to those who suffer from protein deficiency, assisting them to regain loss of functions, including growth and maintenance of enzymes, hormones, neurotransmitters, and body tissues. An increase of dietary fat would be able to compensate for reduced carbohydrate-derived energy and facilitate the absorption of fat-soluble vitamins as well as increasing palatability by absorbing and retaining flavors. However, it is a concern that cereal-legume-based foods are known to be susceptible not only to deterioration by microbial growth, especially mycotoxins but also prone to lipid oxidation (Temba, Njobeh, & Kayitesi, 2017). Thus, more attention needs to be paid to the storage conditions for these composite flours.

The content of crude fiber (2.8–2.9 g/100 g) and ash (2.9–3.0 g/100 g) was significantly higher ($P < 0.05$) in both partial and full substitution by banana flours (P-B and F-B). Total carbohydrate content accounted for more than 60 g/100 g in control (CON), partial replacement with cassava (P-C), full replacement with cassava (F-C), P-B, and F-B samples. The carbohydrate content was significantly lower ($P < 0.05$) in P-R and F-R. Even though the lower carbohydrate content is statistically significant, it is less of a concern as the total carbohydrate content is still high. There was no significant difference in total sugar content (2.4–2.9 g/100 g) and gross energy value (396.1–402.2 kcal/100 g) among all composite flours, suggesting that they can serve as a good source of energy. Additionally, the energy values obtained from composite flours were in accordance with the recommendations of WHO/FAO (2003) which specify energy-supplying micronutrients, for example, protein (10–15% of total energy), total fat (15–30% of total energy), and total carbohydrate (55–75% of total energy), could prevent chronic non-deficiency diseases.

Minerals are essential in metabolism and homeostasis of the human body. Deficiency of minerals can lead to symptoms of common disorders and diseases (Gharibzahedi & Jafari, 2017). In this study, significantly higher ($P < 0.05$) amounts of potassium (600.8–1182.1 mg/100 g) and phosphorus (215.2–257.8 mg/100 g) were observed in all samples, particularly in F-R and F-B, when compared with CON (Table 2). The contents of calcium (1580.3–1623.6 mg/100 g) and iron (13.9–14.5 mg/100 g) were highest ($P < 0.05$) in P-C and F-C samples. Partial and full replacement of wheat flour with banana flour (P-B and F-B) led to a significant reduction ($P < 0.05$) of iron content. Among composite flours, the lowest amount of magnesium (191.1–210.4 mg/

Table 2
Proximate and mineral concentrations of composite flours.

Quality^{2}	CON^{1}	P-C	F-C	P-R	F-R	P-B	F-B
Proximate (g/100 g)							
Moisture	7.4^{a} (1.0)	7.2^{a} (1.2)	7.5^{a} (0.8)	7.7^{a} (0.5)	7.5^{a} (0.8)	7.6^{a} (1.0)	7.7^{a} (0.9)
Protein	15.2^{b} (1.2)	15.5^{b} (0.9)	15.8^{b} (1.3)	17.5^{a} (1.4)	18.5^{a} (1.1)	12.1^{c} (1.2)	13.8^{c} (1.5)
Fat	8.8^{b} (0.6)	7.7^{b} (1.1)	7.9^{b} (0.8)	10.1^{a} (1.0)	11.0^{a} (0.8)	8.1^{b} (0.8)	8.3^{b} (1.1)
Crude fiber	1.8^{b} (0.2)	2.1^{b} (0.3)	2.2^{b} (0.2)	2.2^{b} (0.1)	2.3^{b} (0.3)	2.8^{a} (0.2)	2.9^{a} (0.2)
Ash	1.9^{b} (0.1)	2.2^{b} (0.3)	2.2^{b} (0.1)	2.2^{b} (0.0)	2.4^{b} (0.2)	2.9^{a} (0.1)	3.0^{a} (0.2)
Carbohydrate	64.9^{a} (0.8)	65.3^{a} (1.2)	64.4^{a} (1.1)	60.3^{b} (1.0)	58.3^{b} (1.1)	66.5^{a} (0.9)	64.3^{a} (1.2)
Total sugar	2.6^{a} (0.0)	2.4^{a} (0.1)	2.5^{a} (0.1)	2.8^{a} (0.2)	2.9^{a} (0.1)	2.5^{a} (0.2)	2.7^{a} (0.1)
Gross energy (kcal/100 g)	399.7^{a} (1.3)	398.8^{a} (1.3)	396.8^{a} (1.5)	402.1^{a} (1.8)	402.2^{a} (1.0)	396.3^{a} (1.5)	396.1^{a} (1.2)
Minerals (mg/100 g)							
Potassium (K)	582.2^{f} (2.5)	600.8^{e} (2.2)	650.3^{d} (3.1)	959.2^{b} (2.5)	1182.1^{a} (2.4)	878.9^{c} (2.0)	1008.5^{b} (1.9)
Phosphorus (P)	193.5^{d} (1.9)	241.9^{b} (2.2)	252.2^{a} (1.2)	240.2^{b} (3.0)	257.8^{a} (2.6)	215.2^{c} (2.5)	248.7^{ab} (2.7)
Calcium (Ca)	817.8^{c} (5.3)	1580.3^{a} (4.8)	1623.6^{a} (3.2)	778.0^{cd} (2.7)	719.2^{d} (4.1)	858.7^{bc} (2.3)	958.4^{b} (3.0)
Iron (Fe)	10.4^{c} (0.1)	13.9^{b} (0.2)	14.5^{a} (0.3)	9.8^{c} (0.1)	10.6^{c} (0.1)	5.8^{d} (0.0)	4.1^{d} (0.2)
Magnesium (Mg)	224.3^{bc} (3.4)	210.4^{c} (2.4)	191.1^{d} (2.0)	219.8^{c} (2.8)	230.7^{b} (2.5)	225.6^{bc} (3.2)	244.0^{a} (1.1)
Zinc (Zn)	5.2^{c} (0.3)	4.1^{d} (0.3)	3.4^{e} (0.2)	6.2^{b} (0.2)	8.3^{a} (0.1)	5.2^{c} (0.1)	4.8^{d} (0.1)

^{1}CON = control with 25 g wheat flour /100 g composite flour.
P-C = partial substitution of wheat flour with 15 g/100 g of cassava flour.
F-C = full substitution of wheat flour with 25 g/100 g of cassava flour.
P-R = partial substitution of wheat flour with 15 g/100 g of rice flour.
F-R = full substitution of wheat flour with 25 g/100 g of rice flour.
P-B = partial substitution of wheat flour with 15 g/100 g of banana flour.
F-B = full substitution of wheat flour with 25 g/100 g of banana flour.
^{2}All values are expressed as mean (standard deviation) (N = 3).
$^{a\text{-}f}$, values within a row with different superscript letters are significantly different ($P < 0.05$).

100 g) was found in P-C and F-C samples, whereas P-R and F-R contained the highest amount of zinc (6.2–8.3 mg/100 g).

3.2. Antinutrient composition

Phytic acid, known as myo-inositol hexakisphosphate (IP6), has been found to be effective in chelating metal ions. These health benefits of phytic acid have been identified as having anti-carcinogenic and antioxidant properties (Campos-Vega, Loarca-Piña, & Oomah, 2010). On the other hand, phytic acid can form very stable, insoluble complexes with minerals such as calcium, zinc, and iron (Bohn, Meyer, & Rasmussen, 2008) as well as the chelation of amino acids, thereby decreasing mineral and amino acid bioavailability (Kruger et al., 2013). In this study, the replacement of rice flour in P-R and F-R samples showed the significantly highest ($P < 0.05$) content of phytic acid (198.4–211.6 mg/100 g) when compared to other flours, while the content was significantly lower in composite flour with cassava flour as a replacement (Table 3). This is in an agreement with a finding of Svanberg, Lorri, and Sandberg (1993) who report that phytases, which are special phosphatase enzymes, can catalyze the hydrolysis of phytate in crops into lower inositol phosphates and inorganic phosphorus during the fermentation process. The low content of phytate in cassava flour might therefore be a result of the fermentation process as Marfo, Simpson, Idowu, and Oke (1990) observed a substantial reduction of phytate in cassava after 72 h of fermentation.

Nissar, Ahad, Naik, and Hussain (2017) stated that phytate content should be lowered as much as possible, ideally ≤ 25 mg/100 g or 3% of the phytate-containing food should be consumed to minimize the micronutrient losses. The Reference Daily Intake (RDI) value of phytate varied by country; for example, 180 mg RDI/ day has been specified for Sweden, whereas UK and USA accept 631–746 mg RDI/ day (Nissar et al., 2017). As phytate is heat stable, heat treatment is not able to degrade it substantially owing to the formation of either insoluble complexes between phytate and other macro- and micronutrients, such as phytate–protein and phytate–protein–mineral complexes, or the penta- and tetra-phosphate hydrolyzed products of inositol hexaphosphate (Siddhuraju & Becker, 2001). However, some thermal degradation could have occurred during pressurized steam cooking as presented by Wu, Ashton, Simic, Fang, and Johnson (2018) in sorghum flaked breakfast cereals.

Tannins are water-soluble polyphenols found in plant-based foods (Sehrawat, Sharma, & Sultana, 2006). Though tannins have many

Table 3
Antinutritional factor contents of composite flours.

Antinutrient (mg/100 g)^{2}	CON^{1}	P-C	F-C	P-R	F-R	P-B	F-B
Phytate	170.8^{c} (2.3)	155.5^{d} (4.2)	142.4^{e} (3.1)	211.6^{a} (2.8)	198.4^{b} (2.2)	168.2^{c} (3.1)	153.7^{d} (4.5)
Tannin	161.2^{c} (2.3)	122.3^{e} (3.1)	95.6^{f} (2.0)	142.5^{d} (4.2)	120.8^{e} (1.8)	222.5^{b} (3.7)	250.8^{a} (4.1)
Oxalate	32.2^{a} (1.9)	29.9^{a} (2.1)	27.4^{a} (2.4)	31.4^{a} (1.5)	29.9^{a} (2.2)	29.2^{a} (1.2)	27.1^{a} (1.7)

^{1}CON = control with 25 g wheat flour /100 g composite flour.
P-C = partial substitution of wheat flour with 15 g/100 g of cassava flour.
F-C = full substitution of wheat flour with 25 g/100 g of cassava flour.
P-R = partial substitution of wheat flour with 15 g/100 g of rice flour.
F-R = full substitution of wheat flour with 25 g/100 g of rice flour.
P-B = partial substitution of wheat flour with 15 g/100 g of banana flour.
F-B = full substitution of wheat flour with 25 g/100 g of banana flour.
^{2}All values are expressed as mean (standard deviation) (N = 3).
$^{a\text{-}f}$, values within a row with different superscript letters are significantly different ($P < 0.05$).

health-promoting properties such as antioxidant, anti-carcinogenic, anti-inflammatory, anti-arteriosclerotic, and anti-microbial activities (Kumar et al., 2010; Mazni, Ho, Azizul, & Nurdin, 2016), their anti-nutritional properties have been reported, including reduction of protein digestibility, leading to poor absorption of iron (Chung, Wong, Wei, Huang, & Lin, 1998). The result in this study showed that the replacement of banana flour in P-B and F-B samples significantly increased ($P < 0.05$) tannin content (310.4–332.1 mg/100 g) in composite flours when compared with CON; the content was significantly lower in flours in which rice (120.8–142.5 mg/100 g) and cassava (95.6–122.3 mg/100 g) were the replacements (Table 3). Fekadu et al. (2013) indicated that the anti-nutritional effect of tannins depends on chemical structure and dosage and the acceptable total daily intake of tannic acid for a man is 560 mg/100 g. Therefore, the content of tannin in composite flours might not cause a toxic effect on ingestion.

Oxalate is a metabolic end-product of ascorbate, glyxylate, and glycine metabolism in humans. Oxalate content tends to be highest in the leafy part of vegetables rather than in the seeds and stems (Savage, 2000). There are two major forms of oxalates, soluble and insoluble. Soluble oxalates are mostly bound to sodium, calcium, and ammonium ions, while insoluble oxalates are bound to calcium, magnesium, and iron ions (Savage et al., 2000). Insoluble oxalates are less absorbed in the digestive tract and are excreted in the feces (Massey, 2003). High intake of soluble oxalate can cause calcium oxalate crystallization and the formation of kidney stones (nephrolithiasis) in the urinary tract (Morozumi & Ogawa, 2000).

There was no significant difference in oxalate content (27.1–32.2 mg/100 g) among composite flours; however, a reduction was found at full replacement by cassava, rice, and banana flours (Table 3). By the presence of oxalate content in composite flours, patients with kidney stone should be carefully considered as they are advised to limit total oxalate intake to not more than 50–60 mg/day (Massey, Palmer, & Horner, 2001). However, the adverse effect of consuming food that are high oxalate-containing has been shown to be considerably reduced by forming insoluble oxalate with calcium-rich foods (Faudon & Savage, 2014). Also, processing methods such as soaking and cooking may cause considerable skin rupture and facilitate the leakage of soluble oxalate, resulting in low available oxalate for absorption in the digestive tract (Savage et al., 2000).

3.3. Molar ratios and predicted mineral bioavailability

As the concentrations of phytate and oxalate were high in studied cereal–legume-based composite flours, it is important to consider their effects on the bioavailability of critical minerals, calcium, iron, and zinc. The molar ratios of antinutrients to minerals are important for predicting the potential mineral bioavailability, with lower molar ratios indicative of higher mineral bioavailability. From the molar ratio of the antinutritional factor to minerals in composite flours, it could be seen that the [phytate]: [calcium] molar ratio ranged from 0.005 to 0.017; [phytate]: [iron], 0.833–3.181; [phytate]: [zinc], 2.354–4.125; [oxalate]: [calcium], 0.017–0.042; and [phytate][calcium]/[zinc], 0.423–1.674 mol/kg (Table 4). In addition, the molar ratio of [phytate]: [calcium] was significantly lower ($P < 0.05$) in P-C and F-C samples, while the molar ratio of [phytate]: [iron] was significantly affected ($P < 0.05$) by the replacement with banana flour. A significant increase ($P < 0.05$) of [phytate]: [zinc] molar ratios was observed in P-C and F-C samples when compared with CON, while F-R showed the lowest values. The molar ratio of [oxalate]: [calcium] was significantly decreased ($P < 0.05$) by the replacement with cassava and banana flours.

Generally, the suggested critical value of the [phytate]: [calcium] molar ratio is < 0.17 (Umeta, West, & Fufa, 2005). However, Morris and Ellis (1985) mentioned that a [phytate]: [calcium] molar ratio > 0.24 will diminish calcium absorption. In addition, phytate was found to increase available soluble oxalate, which can bind calcium, resulting in a reduction of calcium absorption (Brogren & Savage, 2003). The molar ratio of [oxalate]: [calcium] has been reported as hazardous when it is > 2.0 (Israr, Frazier, & Gordon, 2013); the inhibitory effect of phytate on iron absorption is dose-dependent (Gibson et al., 2010) and can occur at a very low phytate concentration. Although Hurrell et al. (1992) indicated that a strong inhibitory effect of phytate on iron exists even when the [phytate]: [iron] molar ratio is as low as 0.2, the critical value of the [phytate]: [iron] molar ratio of < 1.0 is accepted as phytate begins to lose its inhibitory effect on iron absorption at this level (Hurrell, 2004). Zinc absorption is also a dose-dependent response (Nävert & Sandström, 1985) and poor availability of zinc is encountered when the molar ratio of [phytate]: [zinc] is > 15 (Sandberg, Anderson, Carlesson, & Sandström, 1987). In a study by Hunt and Beiseigel (2009), the amount of zinc absorbed from a 1-day diet was reduced by 25% (1 mg) when the [phytate]: [zinc] molar ratio was increased from 4 to 15. Ellis et al. (1987) recommended a ≤10 [phytate]: [zinc] molar ratio for adequate zinc bioavailability. It could be seen that the phytate or oxalate to mineral (calcium and zinc) molar ratios of all composite flours were lower than the reported critical values, while phytate to iron was significantly higher. Therefore, a significant adverse effect of the composite flours, if any was indicated on the absorption of iron was indicated.

Interestingly, the effect of phytate on the bioavailability of minerals generally depends not only on the phytate content in the staple food but also on the interaction between phytate and minerals (Ma, Li, Zhai, Kok, & Yang, 2007). Some studies have reported that high dietary calcium impairs zinc absorption in the presence of a high intake of phytate (Gibson et al., 2010; Gemede et al., 2015). Therefore, the molar ratio of [phytate][calcium]/[zinc] might be better used as an indicator of zinc bioavailability than the molar ratio of [phytate]: [zinc] alone (Obah & Amusan, 2009). The molar ratio of [phytate][calcium]/[zinc] of < 0.5 mol/kg has been positively associated with zinc absorption (Adetuyi, Osagie, & Adekunle, 2011). From the [phytate][calcium]/[zinc] molar ratio of cereal-legume based composite flours, a significantly higher amount ($P < 0.05$) of [phytate][calcium]/[zinc] was found in composite flour where cassava flour had been the replacement, while only full replacement with rice flour (F-R) had a value less than the critical level.

To lower the inhibitory effect of phytate on minerals in plant-based complementary foods, some methods such as food preparation and processing as well as fortification with micronutrients including zinc have been suggested (Gibson & Holz, 2001; Gibson et al., 2006). A study by Hurrell, Reddy, Juillerat, and Cook (2003) exhibited that complete phytate degradation can improve the absorption of iron in cereal porridge when being prepared with water but not with milk. This finding indicates that it is difficult to predict the fraction of mineral absorption since it can be varied by several factors such as the composition and preparation methods of ingested food, and age and nutritional status of the target group (Mazariegos et al., 2006; Gibson et al., 2010).

3.4. Aflatoxin contamination

All composite flours in this study were contaminated with aflatoxins and levels ranged from 2.6 to 8.8 μg/kg (Table 5). Among the samples, a high concentration of total aflatoxins was significantly detected ($P < 0.05$) in CON. High levels of aflatoxin-B_1 were found in all composite flours, followed by aflatoxin-G_1, and aflatoxin-B_2. However, aflatoxin-G_1 was not detected in P-B and F-B samples, whereas all samples were not contaminated by aflatoxin-G_2. Although all samples met the proposed East Africa regulatory threshold of 10 μg/kg (Udomkun et al., 2017), CON, P-C, P-R, and P-B did not meet the EU regulatory threshold for total aflatoxins of 4.0 μg/kg (EC, 2007,2010).

In general, aflatoxin contamination in agricultural commodities can be compounded by a wide range of pre- and post-harvest factors such as high temperature and drought conditions (Paterson & Lima, 2010;

Table 4
Molar ratios of antinutritional factor to mineral of composite flours.

Molar ratio[2]	CON[1]	P-C	F-C	P-R	F-R	P-B	F-B
Phytate: Calcium	0.013^{b} (0.003)	0.006^{c} (0.002)	0.005^{c} (0.002)	0.016^{a} (0.004)	0.017^{a} (0.003)	0.012^{b} (0.002)	0.010^{b} (0.001)
Phytate: Iron	1.393^{e} (0.011)	0.949^{f} (0.008)	0.833^{f} (0.005)	1.832^{c} (0.012)	1.588^{d} (0.014)	2.460^{b} (0.010)	3.181^{a} (0.018)
Phytate: Zinc	3.235^{c} (0.038)	3.735^{b} (0.031)	4.125^{a} (0.028)	3.361^{c} (0.057)	2.354^{e} (0.021)	3.185^{d} (0.046)	3.153^{d} (0.032)
Oxalate: Calcium	0.039^{a} (0.003)	0.019^{d} (0.002)	0.017^{d} (0.003)	0.040^{a} (0.002)	0.042^{a} (0.001)	0.034^{b} (0.005)	0.028^{c} (0.002)
Phytate × Calcium: Zinc[3]	0.661^{d} (0.011)	1.476^{b} (0.009)	1.674^{a} (0.018)	0.654^{d} (0.022)	0.423^{e} (0.018)	0.684^{d} (0.019)	0.756^{c} (0.017)

[1] CON = control with 25 g wheat flour /100 g composite flour.
P-C = partial substitution of wheat flour with 15 g/100 g of cassava flour.
F-C = full substitution of wheat flour with 25 g/100 g of cassava flour.
P-R = partial substitution of wheat flour with 15 g/100 g of rice flour.
F-R = full substitution of wheat flour with 25 g/100 g of rice flour.
P-B = partial substitution of wheat flour with 15 g/100 g of banana flour.
F-B = full substitution of wheat flour with 25 g/100 g of banana flour.
[2] All values are expressed as mean (standard deviation) (N = 3).
[3] The unit of [Phytate][Calcium]/[Zinc] is mol/kg.
[a-g], values within a row with different superscript letters are significantly different ($P < 0.05$).

Bandyopadhyay et al., 2016; Kamika, Ngbolua, & Tekere, 2016), poor farm practices (Hell, Mutegi, & Fandohan, 2010; Kang'ethe et al., 2017), and a lack of drying facilities and inappropriate storage practices (Matumba et al., 2017). Interestingly, the highest aflatoxin contamination in this study was found in all samples composed of wheat flour. This might be attributed to poor storage of wheat flour in the markets as well as poor governmental regulations and legislations on aflatoxin contamination in the country.

In addition, the low level of aflatoxin contamination in composite flour with full replacement by cassava flour could be explained by the fact that the lactic acid bacteria and/or some microorganisms, such as *Saccharomyces cerevisiae* strains obtained from the fermentation process during the 12–16 h of soaking in cassava flour production, have the ability to bind or degrade aflatoxins (Shetty, Hald, & Jespersen, 2007). Moreover, Rodriguez, Buschmann, Iglesias, and Beeching (2000) mentioned that scopoletin—a thermostable coumarin phytoalexin with fungicidal properties—may also contribute to reducing aflatoxin-B_1 in cassava. In the case of rice flour, Pitt, Taniwaki, and Cole (2013) reported that rice cultivated with an irrigation system leads to low levels of aflatoxin in the final product but the contamination becomes more probable when rice seeds are collected wet and then dried. Likewise, the milling process during polished rice production has been shown to decrease the level of aflatoxin in rice (Katsurayama et al., 2018). Sales and Yoshizawa (2005) showed that the level of aflatoxins in brown rice was reduced by 78% after being processed to polished rice, while a study by Trucksess, Abbas, Weaver, and Shier (2011) indicated that aflatoxin was reduced from 158 µg/kg in brown rice to 56 µg/kg in polished rice. Although there is no study on aflatoxin contamination in banana flour, the result might be related to the presence of polyphenols in banana. Several polyphenols, such as gallic acid, catechins, epicatechins, anthocynins, and other flavonoid derivatives, are reported to be essentially found in banana (Bennett et al., 2010; Pereira & Maraschin, 2015). Scaglioni, Souza, Schmidt, and Badiale-Furlong (2014) suggested that the polyphenol compounds have an ability to inhibit α-amylase enzyme—an important enzyme for microbial growth—from the fungal species *Aspergillus oryzae*.

4. Conclusions

This study provides data on nutrients, antinutrients, molar ratios of the anti-nutritional factor to minerals (mineral availability), and aflatoxin content in cereal-legume-based composite flours with partial and full replacement of wheat flour by local crops such as cassava, rice, or banana flour. Although the modified composite flours were observed to be rich in some macro- and micro-nutrients and low in aflatoxin contamination, the level of antinutrients should be addressed as it was found to influence the bioavailability of minerals, especially in diets that are high in phytate and calcium but low in zinc as well as iron.

At the household level, food preparation (soaking, germination, or fermentation) and processing methods (boiling or roasting) can be used to reduce some antinutrients, while fortification with micronutrients, including minerals, at appropriate levels can be an alternative option for the commercial level. In conclusion, these composite flours are recommended as a practical and sustainable approach for improving

Table 5
Aflatoxin concentration in composite flours.

Aflatoxin (µg/kg)[2]	CON[1]	P-C	F-C	P-R	F-R	P-B	F-B
Aflatoxin-B_1	5.6^{a} (0.2)	4.4^{b} (0.1)	1.5^{c} (0.0)	3.4^{b} (0.1)	2.2^{c} (0.1)	4.3^{b} (0.2)	2.6^{bc} (0.1)
Aflatoxin-G_1	3.1^{a} (0.1)	0.2^{b} (0.0)	0.8^{b} (0.0)	0.7^{b} (0.1)	0.5^{b} (0.0)	ND	ND
Aflatoxin-B_2	0.1^{a} (0.0)	0.1^{a} (0.0)	0.3^{a} (0.0)	0.1^{a} (0.0)	0.2^{a} (0.0)	0.5^{a} (0.0)	0.2^{a} (0.0)
Aflatoxin-G_2	ND[3]	ND	ND	ND	ND	ND	ND
Total aflatoxins	8.8^{a} (0.2)	4.7^{b} (0.1)	2.6^{c} (0.0)	4.2^{b} (0.1)	2.9^{c} (0.0)	4.8^{b} (0.1)	2.8^{c} (0.1)

[1] CON = control with 25 g wheat flour /100 g composite flour.
P-C = partial substitution of wheat flour with 15 g/100 g of cassava flour.
F-C = full substitution of wheat flour with 25 g/100 g of cassava flour.
P-R = partial substitution of wheat flour with 15 g/100 g of rice flour.
F-R = full substitution of wheat flour with 25 g/100 g of rice flour.
P-B = partial substitution of wheat flour with 15 g/100 g of banana flour.
F-B = full substitution of wheat flour with 25 g/100 g of banana flour.
[2] All values are expressed as mean (standard deviation) (N = 3).
[3] ND = non-detected.
[a-d], values within a row with different superscript letters are significantly different ($P < 0.05$).

macro- and micronutrients and mineral availability for the resource-poor in low-income countries. In addition, the result of this finding will play an integral role in making savings in wheat costs, promoting utilization of local crops, encouraging local farmers, and enhancing agricultural systems. However, a consumer study is still required to represent trade-offs in low-income contexts and the promotion of different compositions needs to be considered, as a one-size-for-all approach might not be acceptable. Finally, it is worth noting that all composite flours are still prone to deterioration by aflatoxin and multiple mycotoxins during storage owing to their physical and chemical constituents. Therefore, there is a critical need to raise awareness on the dangers of aflatoxin and other mycotoxins and to develop strategies to manage mycotoxin contamination.

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Ethical statement

This study does not involve any human or animal testing.

Conflict of interest

The authors have no conflict of interests.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.02.055.

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