European Journal of Agriculture and Food Sciences
Vol 6 | Issue 2 | April 2024
ISSN 2684-1827

RESEARCH ARTICLE

Microbiological Safety and Nutritional Adequacy of Composite Porridge Flour Used in a Selected School-Feeding Program in Kamuli District, Uganda

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ABSTRACT

By providing nutrient-enriched meals, the Iowa State University-Uganda Program plays a crucial role in combating hunger and improving school attendance by pupils in Kamuli district, Uganda. The lunchtime meals for pupils in primary grades 3 to 7 consist of a blend of maize grains and beans cooked together with vegetables and eggs to enhance their nutritional value. The program introduced a new composite porridge flour (44% maize, 26% millet, and 18% amaranth grain) for feeding pupils from kindergarten to primary grade 2 at break time. However, the microbiological safety of the flour for making the porridge had yet to be assessed. Additionally, though the proportions of the flour ingredients were known, its proximate composition and energy density were unknown. Hence, the porridge's contribution to each pupils recommended dietary allowance had never been evaluated for adequacy. This study, therefore, assessed the microbiological safety and nutritional adequacy of the composite flour (n = 5 batches). Fungal contamination was determined by spread plating on acidified Potato Dextrose agar. Aflatoxin concentrations were determined by Liquid Chromatography Tandem Mass Spectrometry. Proximate composition was determined following standard methods. Gross energy was estimated using the energy conversion factors. To evaluate nutritional adequacy, the contribution of the porridge flour to pupils' RDA was compared with the 1998 FAO/WHO RDA guidelines. The mean fungal count (4.4 ± 0.1 log cfu/g) slightly exceeded the acceptable maximum limit (4 log cfu/g), although no aflatoxins were detected, thus indicating satisfactory microbial safety. The flour predominantly contained carbohydrates (81.0% ± 0.7), moderate amounts of protein (8.2% ± 0.1), and crude fat (7.6% ± 0.5) alongside minimal levels of total ash (1.8% ± 0.1) and crude fiber (1.7% ± 0.6). Its contribution to the pupils' RDA was very minimal owing to the low porridge intake (about 10 g dry matter per pupil per day). Therefore, there is an urgent need to enhance the nutrient profile of the composite flour by incorporating more protein and fiber rich ingredients and increasing the serving size to meet the dietary needs of the pupils. The flour could also be extruded to ensure a higher bulk density of the porridge.

Keywords: Composite porridge flour, Food safety, Nutritional composition, School-feeding program.

Submitted: March 03, 2024

Published: April 21, 2024

10.24018/ejfood.2024.6.2.794

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1. Introduction

Three hundred eighty-eight million learners worldwide are estimated to receive meals daily from school feeding programs (SFPs) in at least 161 countries [1]. SFPs mainly aim to combat hunger and improve child health and school performance [2]. They effectively reduce child malnutrition and improve anthropometric indicators [3]. They also significantly facilitate children's academic achievement and cognitive skills [4].

Iowa State University-Uganda program (ISU-UP) is part of the Center for Sustainable Rural Livelihood (CSRL) in the College of Agriculture and Life Sciences (CALS) of Iowa State University [5]. The program operates in the Kamuli district in Eastern Uganda with a mission of supporting resilient, sustainable rural livelihoods by discovering and applying science-based indigenous knowledge [5]. Kamuli has one of the country's highest food insecurity and poverty levels [6]. The poverty and food insecurity levels stood at 24.3% by 2012 and were close to the then national average of 24.5% [7]. The ISU-UP's school feeding program in Kamuli district promotes nutrition and contributes to mitigating hunger among pupils in selected schools [8]. This SFP provides meals of maize grains and beans cooked together with various leafy vegetables and eggs and served at lunchtime to pupils in primary three to seven [9]. The program expanded its scope by feeding porridge to pupils in kindergarten as well as primary grades one and two given at break time. The initial formulation of the porridge used in the pilot phase was prepared from a composite flour with maize (44%), millet (26%), amaranth grain (18%), and sugar (12%). According to Keller *et al*. [9], ISU-UP's school feeding program has incentivized parents to keep sending their children to school.

Composite flours are blends of different flours used to make various food products including porridges [10]. Using composite flours allows better overall usage of domestic agricultural products, reducing in costs associated with imported foods while enhancing the nutritional quality of different foods. Porridges typically have low nutrient and energy density due to the low flour incorporation rate required for drinking viscosity [11]. Furthermore, several studies have reported fungi and mycotoxins as the primary food safety hazards in flours and grains [12]–[14]. Some studies have reported daily exposure of Ugandan rural children to high concentrations of aflatoxin B1 through consuming contaminated food [12].

This study, therefore, determined the microbiological safety and macronutrient content of the composite porridge flour planned for school feeding by the ISU-UP and assessed its contribution to the Recommended Dietary Allowance of the pupils. The results of this study can be used to guide the enhancement of school feeding programs to ensure meal safety and adequacy.

2. Materials and Methods

2.1. Sample Collection

Five samples of the composite porridge flour containing maize, millet, and amaranth grain were randomly taken from five consecutive production batches from the ISU-UP in Kamuli district over ten weeks (one sample was taken from each one of the consecutive batches every fortnight). Two porridge samples were randomly collected from Namasagali and Kasozi Primary Schools in Kamuli district, Uganda, during a trial school feeding program. The porridge samples were used to determine dry matter content and, thus, flour rates. The flour from each batch was collected in a clean, dry, opaque polythene bag. The porridge was put in a clean vacuum flask and transported to the laboratory for immediate analysis.

2.2. Analyses

2.2.1. Fungal Contamination

Fungal counts were determined according to the ISO [15] method. Briefly, samples were analyzed by spread plating selected serial dilutions onto sterile pre-poured Potato Dextrose Agar (Laboratorios CONDA, Madrid, Spain) containing 1% lactic acid, followed by incubating the plates at 25 °C for five days. The number of colonies forming units (cfu) was determined using a colony counter. The cfu/g of each sample was calculated as below:

$$CFU/G = \frac{\sum C}{V(N_1 + 0.1N_2)D_1} \quad (1)$$

where

$\sum C$–total number of counts in all plates in the countable range (30–300),

N_1–number of plates in the first dilution retained and counted,

N_2–number of plates in the second dilution retained and counted,

D_1–dilution factor of the first dilution retained and counted,

V–volume of the inoculum plated.

2.2.2. Aflatoxin Contamination

Aflatoxins were determined by Liquid Chromatography tandem Mass spectrometry (LC-MS/MS). In brief, 10 g of the flour were mixed with 100 mL of methanol-water solvent. The mixture was centrifuged, and the supernatant collected. A solid-phase extraction column was used for sample cleanup, followed by elution with methanol to obtain the aflatoxin-containing elute. Analysis was performed using a reversed-phase AflaStar™R-Immunoaffinity Columns (AF1306-1805). The results were compared with Biopure Mycotoxins Mix 1 (aflatoxin) reference standard. Aflatoxin peaks were monitored using a UV detector and mass spectrometry data were collected using electrospray ionization. Identification and quantification of aflatoxins were done based on retention times, mass spectra, and calibration curves.

2.2.3. Moisture Content

The moisture content was determined using the convection oven drying method described by AOAC [16] and Mauer and Bradley [17]. Briefly, 5 g of the flour, or porridge sample, was weighed into clean crucibles preconditioned at 100 °C for 4 h and cooled in a desiccator. The flour or porridge was spread evenly in the crucible and heated at 100 °C for 16 h in an oven (Gallenkamp, UK). The dried flour was cooled in a desiccator and weighed to determine the weight loss. The moisture content was determined using the formula below:

$$\%\ Moisture\ content = \frac{weight\ of\ wet\ sample\ (g) - weight\ of\ dried\ sample\ (g)}{weight\ of\ wet\ sample\ (g)} \times 100\% \quad (2)$$

2.2.4. Total Ash

Total ash was determined by dry ashing, as described by Harris and Marshall [18]. About five grams of the porridge flour was weighed into a preconditioned crucible and ignited in a muffle furnace (Mustek Limited, UK) at 550 °C for 6 h. The ashed sample was cooled in a desiccator and weighed. The ash content was determined using the expression below:

$$\%\ Ash = \frac{(Weight\ after\ ashing\ (g) - Tared\ weight\ of\ crucible\ (g))}{Original\ sample\ weight\ (g)} \times 100\% \quad (3)$$

2.2.5. Crude Protein

The Kjeldahl method, as described by AOAC [16], was used. Half a gram of the flour was weighed into a digesting Kjeldahl tube. Ten milliliters of concentrated H_2SO_4 and one spatula of mixed complex Kjeldahl catalyst were added to the tube, and the mixture digested at 360 °C in the digesting block to a clear solution which converted the nitrogen in the protein to ammonium sulphate. The digested sample was cooled and diluted to 50 mL with distilled water. Five milliliters of the diluted sample were taken in the Markham distillation apparatus to distill off the ammonia. Five milliliters of 40% NaOH were added to neutralize the acid and facilitate further liberation of ammonia gas trapped in a calibrated 2% boric acid solution containing three drops of 1% phenolphthalein. The trapped ammonia was titrated against 0.1 M HCl, and the protein content was calculated, as shown in the equation below:

$$\%\ Crude\ protein = \left(\left(Net\ titre\text{-}blank\right) \times total\ volume\ of\ diluted\ sample \times 6.25 \times 14 \times M\ HCl\right) / \left(Weight\ of\ sample\ (g) \times volume\ of\ sample\ pipetted\ for\ distillation \times 1000\right) \times 100 \quad (4)$$

where

$Net\ titre$ = mL of HCl required for the test sample

M_{HCl} = Molarity of HCl.

2.2.6. Crude Fat

Crude fat was determined by the Soxhlet extraction method as described by AOAC [16]. Five grams of the flour were weighed into a thimble and placed in the Soxhlet apparatus (Soxhlet system 1043 Tecator, Sweden). The fat was extracted with petroleum ether by boiling at 100 °C for 30 min and rinsing the extracted fat into pre-weighed Soxhlet beakers for 1 h. Fat content was calculated as:

$$\%\ Crude\ fat = \frac{[Final\ weight\ of\ flask\ (g) - initial\ weight\ of\ flask\ (g)]}{Sample\ weight\ (g)} \times 100 \quad (5)$$

2.2.7. Crude Fiber

Crude fiber was determined by acid digestion followed by alkaline digestion as described by AOAC [16]. Half a gram of the flour was weighed into a 600 ml round-bottomed flask, followed by 100 mL of 0.1 M H_2SO_4 and refluxing for 30 min in a fiber analyzer (Labconco, UK). The digest was filtered through a cheese-cloth to collect the residues, which were digested again with 100 mL of 0.3 M NaOH for 30 min and filtered again. The final residue was oven-dried in a pre-weighed crucible at 100 °C for 45 min. The dried residue was weighed and ashed. The % crude fiber was determined as shown below:

$$\%\ Crude\ fiber = \frac{\left(Weight\ of\ Crucible\ + dry\ residue - of\ crucible\ + ash\right)}{weight\ of\ sample} \times 100\% \quad (6)$$

2.2.8. Total carbohydrates

The carbohydrate content was determined by difference as:

$$\%\ Carbohydrate = 100\% - (\%crude\ protein + \%crude\ fat + \%crude\ fiber + \%ash + \%moisture) \quad (7)$$

2.2.9. Gross Energy

The gross energy of the composite porridge was determined from the proximate components using conversion factors (4 kcal/g of carbohydrates, 4 kcal/g protein and 9 kcal/g of fat) [19].

2.3. Nutritional Adequacy of the Composite Porridge Flours

The amount of nutrient intake from the composite porridge per pupil per day was determined by calculating the total amount of composite flour used to prepare one typical cup of porridge based on the recipe used in the schools. The nutrient composition was determined based on the proximate analysis components corrected for their respective digestibility factors. The proportion of nutrients contributed by the composite porridge was

TABLE I: Fungal and Aflatoxin Concentrations of Composite Flour Used by the ISU-UP for School Feeding

Parameter	Result	Specification (US EAS 1024:2019)
Fungi (log cfu/g)	4.4 ± 0.1	4 (Maximum)
Aflatoxins B_1, G_1, B_2, G_2 (µg/kg)	Not detected	5 (Maximum)
Total aflatoxin (µg/kg)	Not detected	10 (Maximum)

Note: Value for fungi is a mean ± standard deviation.

determined using the formula below [20] and The RDA from FAO/WHO [21] was followed:

$$\%\ Recommended\ Dietary\ Allowance = \frac{Nutrient\ intake\ from\ one\ cup\ of\ porridge}{Recommended\ Dietary\ Allowance\ from\ the\ nutrient} \times 100\% \quad (8)$$

2.4. Data Analysis

All laboratory analyses were performed in triplicates. Descriptive statistics analyzed the data. A one-sample T-test was performed to compare the fungal levels with the maximum acceptable limit.

3. Results and Discussion

3.1. Fungal and Aflatoxin Contamination

Table I summarizes the fungal and aflatoxin concentrations of the composite flours. The mean fungal count was 4.4 ± 0.1 log cfu/g whereas the maximum acceptable limit was 4 log cfu/g. Aflatoxins were undetectable. *Aspergillus* spp. and *Penicillium* were the common mold species identified (Fig. 1).

Aflatoxins are toxic secondary metabolites produced by certain molds, primarily *Aspergillus flavus* and *Aspergillus parasiticus* [22]. Although they are a group of several metabolites, those common in food are B1, B2, G1, and G2 [22]. They are a global concern because of their high prevalence and severe health and economic implications [23], [24]. Apart from their association with liver cancer and death, they are also implicated in stunting and immune suppression, which are crucial, particularly in children [25], [26]. Aflatoxigenic molds thrive in tropical and subtropical regions. Hence, many populations worldwide are routinely exposed to aflatoxins through diet, particularly cereals and legumes, which are staples [27]. Indeed, studies have reported aflatoxins in cereal products [28], [29].

The presence of aflatoxigenic molds does not always guarantee aflatoxin production. Certain environmental factors, such as temperature, humidity, and substrate composition, impact aflatoxin generation [30]–[32]. Aflatoxin production may be restricted if these conditions are not favorable. According to Kaushal and Bhatnagar [33], *A. flavus* and *A. parasiticus* are semi-thermophilic and semi-xerophytic, thriving favorably between 12 °C and 48 °C and at lower water potentials. The optimum growth of these fungi occurs between 25 °C and 42 °C and low water whereas the optimum temperature for aflatoxin biosynthesis is between 28 °C and 35 °C and above this range biosynthesis is inhibited due to the attack of transcription genes *aflr* and *afls* [34].

The results for fungi and aflatoxin analyses of the composite flour agreed with those by Larissa *et al*. [35], who also reported the presence of fungi but no aflatoxin in complementary porridge flour. The presence of fungi in food, even though mycotoxic, does not necessarily signify mycotoxin contamination of food. Even though the aflatoxins in the composite flours in this study were below the detectable limit (Table I), the presence of aflatoxigenic fungi species (Fig. 1) still poses a food health hazard once the conditions for aflatoxin production are made available.

Harvest and postharvest practices influence the contamination of grains and subsequently flours with fungi. Delayed harvesting, drying, storage, and milling of grains using mold-contaminated surfaces such as bare ground all increase the chances of mold infection of produce. Additionally, inadequate drying of grain, that is, moisture content greater than 14%, also encourages mold growth and lowers the shelf life of composite flours [36]. Moisture contents above 17.5% favors mold growth and aflatoxin biosynthesis, especially by *A. flavus* [37]. Despite the presence of fungi in the flours in this study (Table I), the levels

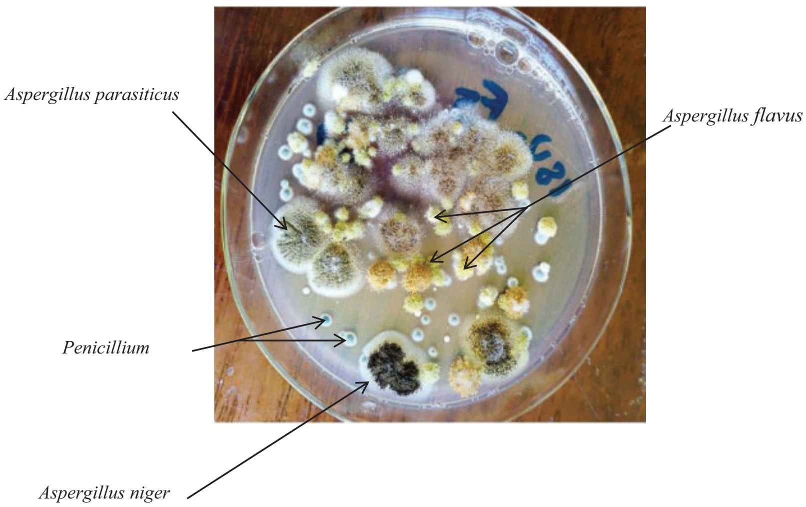

Fig 1. Common mold species from the composite flour.

were not alarming. More so, there were even no aflatoxins detected (Table I). This could also be attributed to the fact that the study was conducted in a school feeding program with a well-established postharvest handling facility where proper drying (mean moisture content = 11.7% ± 0.1), sorting, and hermetic storage of the grains used for the processing of the composite flour lowered the risk of aflatoxin contamination. Regular monitoring and testing for aflatoxins is recommended since it is crucial in ensuring food safety.

It is noteworthy that this being a pilot study, the significance of the results of the current study in estimating the actual aflatoxins level of the composite flours was, however, limited by the small sample size (n = 5) and the short period during which the sampling was conducted (2 months).

3.2. Nutritional Composition of the Composite Flour

The average nutritional composition of the composite flour on a dry matter basis is shown in Table II. The flours contained a high percentage of carbohydrates (81%) and hence a low composition of other constituents. The flour also had a high-energy content.

The high carbohydrate content (81.0% ± 0.7) of the composite flour (Table II) was due to the high inclusion of carbohydrate-rich ingredients in the formulation, that is, 44% maize, 26% millet, 18% amaranth grains and 12% sugar. Maize, millet and amaranth grains contain about 73% [38], 65%–75% [39] and 65% [40] carbohydrates, respectively. Similarly, a high carbohydrate content (88.8%) was reported for cowpea-maize composite flour [41]. The high carbohydrate crops, particularly, maize and millet used in the school feeding program, are easily affordable and widely grown in Kamuli district and throughout the country. Based on its high carbohydrate content, the composite flour is a good source of dietary energy for the pupils which is important for activity, growth and development [42]. The high carbohydrate content also suggests the usefulness of the composite flour in combating protein-energy malnutrition (PEM), as there is carbohydrate to provide energy to the body to spare protein, which can be used for its primary functions of building and repairing worn-out tissues, instead of being used as source of energy [42]. Primary school children are very physically active and engage in different physically demanding activities both at school and home so a high-energy porridge is important.

The low crude protein content (8.2% ± 0.1) of the composite flour (Table II) was due to the low protein content of the ingredients in the formulation that is; 18% amaranth grain, 44% maize and 26% millet with approximately 14% [40], 9% [38] and 7%–12% [39] protein, respectively. The protein content of the composite flour in this study was lower than what Gemede [43] reported for flour blends of maize, peas and anchote, which were 15%–21%. Similarly, the protein content was lower than reported by Ijarotim and Keshinro [44] and Bojňanská *et al*. [45] who reported 23.9%–28.8% for complementary food from fermented and germinated popcorn and 12.6%–16.3% for lentil-chick pea-wheat flour blend diets, respectively. These differences could be attributed to the ingredients' variations and proportions in the composite flours. It should be noted that adequate protein intake is necessary among young pupils to support fast growth rates and development for the repair of body tissues and boosting immunity [46]. These are crucial mainly for primary-age children since they are still actively growing and prone to stunting and various illnesses.

The crude fiber content of the composite flour was below the acceptable maximum limit (5%) according to the standard for composite flours [47]. Low fiber content could be attributed to the use of highly polished flours. The crude fiber content reported in the current study was very low compared to the 11.7%–15.3% reported by Ajifolokun *et al*. [48] for maize-wheat composite flour. Contrary to the current, the previous study reported an increase in the crude fiber content with an increase in the maize added to the composite flour [48]. Crude fibers are indigestible to the human digestive system and delay the release of gastric juice, modulate inflammations, and improve the bulk of food [49]. Adequate fiber intake is important for lowering the risk of colon cancer, constipation, and other digestive disorders [50].

The composite flour had a high energy density, which could be attributed to the high carbohydrate content (Table II). The caloric contribution was 76% (324 kcal) from carbohydrates, 16% (68.4 kcal) from fat, and 8% (32.8 kcal) from protein. This deviated from the dietary reference intake of 20%–35% of calories from fat, 45%–65% from carbohydrates, and 10%–35% calories from protein [46]. It is, therefore, important to consider reformulating the composite flour to ensure that it meets the recommendations.

TABLE II: Proximate Composition of the Composite Porridge Flour Used by the ISU-UP for School Feeding (On a Dry Matter Basis)

Parameter	Result
Carbohydrates (g/100, g)	81.0 ± 0.7
Crude protein (g/100, g)	8.2 ± 0.1
Crude fat (g/100, g)	7.6 ± 0.5
Total ash (g/100, g)	1.8 ± 0.1
Crude fiber (g/100, g)	1.7 ± 0.6
Gross energy (kcal/100, g)	424.3 ± 2.9

3.3. Nutritional Contribution of Composite Porridge

Table III summarizes the percentage contribution of the porridge to the RDA of the different age groups of the pupils. The average intake (g/day) of carbohydrates, protein, fat, fiber, and per child was 25.6 ± 3.7, 2.6 ± 0.4, 2.4 ± 0.4, and 0.6 ± 0.1, respectively. The gross energy was 126.8 ± 25.9 kcal/day per child.

Due to the low dry matter intake from the porridge per pupil, the general contribution of the porridge to the RDA of all the different age groups and sexes was meager (Table III). Of all the nutrients, the contribution to carbohydrates was highest, though still below the recommendations. These results agreed with the findings of Horiuch *et al*. [51] and Le Nguyen *et al*. [52]. However, the results of the current study were contrary to the findings of Wasswa *et al*. [53], who reported a 100% contribution of the cowpea-maize and cowpea-millet composite flour

TABLE III: Contribution of the Composite Porridge to the Recommended Daily Intake of the Pupils

Parameter	RDA (g/day)			% Contribution of porridge to RDA		
Age (years)	(4–8)	(9–13)		(4–8)	(9–13)	
Sex	Males & females	Males	Females	Males & females	Males	Females
Carbohydrate	130	130	130	19.7 ± 2.8	19.7 ± 2.8	19.7 ± 2.8
Protein	19	34	34	13.7 ± 0.4	7.6 ± 1.2	7.6 ± 1.2
Crude fiber	25	31	26	2.4 ± 0.4	1.9 ± 0.3	2.3 ± 0.4
Energy	1742	2279	2071	7.3 ± 1.5	5.6 ± 1.1	6.1 ± 1.3

Note: Values are means ± standard deviations. RDA: Recommended Dietary Allowance. The average dry matter intake of porridge per child was 10 g ± 1.3.

porridge to the RDA of the children and infants. It is important to note that unlike in the current study, where the average intake was about 10 g/day taken once a day at breakfast, the study by Wasswa *et al*. [53] was based on an estimated intake level of 200 g/day.

4. Conclusions

This study assessed the microbiological safety and nutritional adequacy of a composite flour used in a School Feeding Program in Kamuli district, Uganda. The results of the study highlighted the need for a critical balance between microbial safety and nutritional adequacy. The flours had an acceptable microbial and aflatoxin safety profile, which was underscored by minimal fungal counts and the absence of aflatoxins. The flours, however, had deficits in protein, fat, and fiber content, thus making them inadequate for child feeding. These insights reinforce the contribution of rigorous post-harvest handling practices but also highlight the need for nutritional enhancement of the composite flours. Before scaling out the breakfast porridge-feeding program, it is important to optimize the nutritional content of the composite porridge by increasing the proportions of ingredients such as amaranth or incorporating additional nutrient-rich ingredients such as beans to increase protein content. It is also important to explore the use of extruded flours to improve the bulk and nutrient density of the porridge. This way, even when served once a day at breakfast, each pupil would consume more nutrients than currently provided.

Acknowledgment

The authors thank the Iowa State University-Uganda Programme, Kamuli district, Uganda, for funding this research. Special appreciation is given to the Department of Food Technology and Nutrition, Makerere University, for the technical support in conducting this study.

Conflict of Interest

Authors have no conflict of interest to declare.

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