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Innovative approach to mitigate aflatoxin and micronutrient problems in complementary foods (CFs) for health and economic wellbeing

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Abstract

Complementary foods (CFs) provided to infants and young children (IYC) in sub-Saharan Africa contain ingredients that are susceptible to aflatoxin contamination. Chronic dietary exposure to aflatoxins is associated with serious health consequences. CF for thin porridge was developed by combining seven ingredients namely, finger millet, soya beans, orange fleshed sweet potato, cassava, breadfruit, baobab and moringa aiming to produce a nutrient dense and aflatoxin safe CF. The developed formulation was evaluated for safety with a focus on total aflatoxin and aflatoxin B1 and microbiological parameters, fibre and micronutrients especially iron and zinc. After passing safety test, the formulations (normal and instant porridge types) were evaluated for acceptability, cookability preference and market performance. The formulated product passed safety and overall quality tests. Market response was very good suggesting that more work should be undertaken in the direction of scale up and dissemination for wider availability and use. Children and persons of other ages can now use a highly nutritional quality and guaranteed aflatoxin safe product.

Keywords: *Complementary food, nutrient dense, aflatoxin safe, porridge, micronutrients*

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Introduction

Sustainable development goal number 2 aims at ending hunger achieve food security and improve nutrition and promote sustainable agriculture. Apparently, investing in nutrition is critical to reach the goals in 7 of the SDGs. Recently, processed and pre-packaged complementary foods (CFs) commonly known as **Lishe** are popular in the shopping outlets including shelves in supermarkets. However, like in other parts of Sub Sahara Africa, several studies have demonstrated the occurrence of chronic dietary exposure and co-exposure of Infants and Young Children (IYC) in Tanzania to Aflatoxins (AFs) and other mycotoxins (Kimanya *et al*., 2014; Magoha *et al*., 2014; Kamala *et al*., 2018). Of late, therefore, there has been extensive attention on the risks of exposure and the effects of mycotoxins on consumers especially IYC as the most vulnerable group. Yet, there are limited attempts to strategically produce CFs that are safe to feed IYC. Aflatoxins are toxic secondary metabolites produced by molds mainly those of the species *Aspergillus flavus* and *Aspergillus parasiticus.* Their effects are evidenced by an increase of extreme health conditions such as malnutrition (as indicated by stunting in children), hepatitis B and tuberculosis which suppress immune system, economic issues, health outcomes, e.g. liver cancer and immune suppression. Available information on the incidence, public health importance, prevention and control of mycotoxins in many African countries is still lacking

Aflatoxin may enter the food chain directly or indirectly. Contamination may be found in cereals like maize, sorghum, pearl millet, rice and wheat, oilseeds such as groundnuts, soybean, sunflowers and cotton; spices like chilies, black pepper, coriander, turmeric and zinger; tree nuts such as almonds, pistachio, walnuts and coconuts; and milk and milk products. Cereals especially maize and groundnuts are probably the most contaminated food by fungi and are the most important ingredients used in preparation of complementary foods in Tanzania and East Africa (Kimanya *et al*., 2010). The population in these regions is exposed to aflatoxins (Figure 1). The daily human aflatoxin exposure varies between countries. It is estimated 4 184 ng/kg body weight in various African countries, 122 027 ng/kg body weight in Southern China and less than 3 ng/kg body weight in the USA. In 2004 acute toxicity of aflatoxin, a case aflatoxicosis which occurred in Kenya made 317 people ill 125 of them died after consuming aflatoxin contaminated cereals. In Tanzania, aflatoxin contamination and exposure in complementary foods has been reported to be very high. Studies have reported high exposure of infants and young children to aflatoxins through direct consumption of maize-based diet as well as through breast milk of mothers whose main diet is maize.

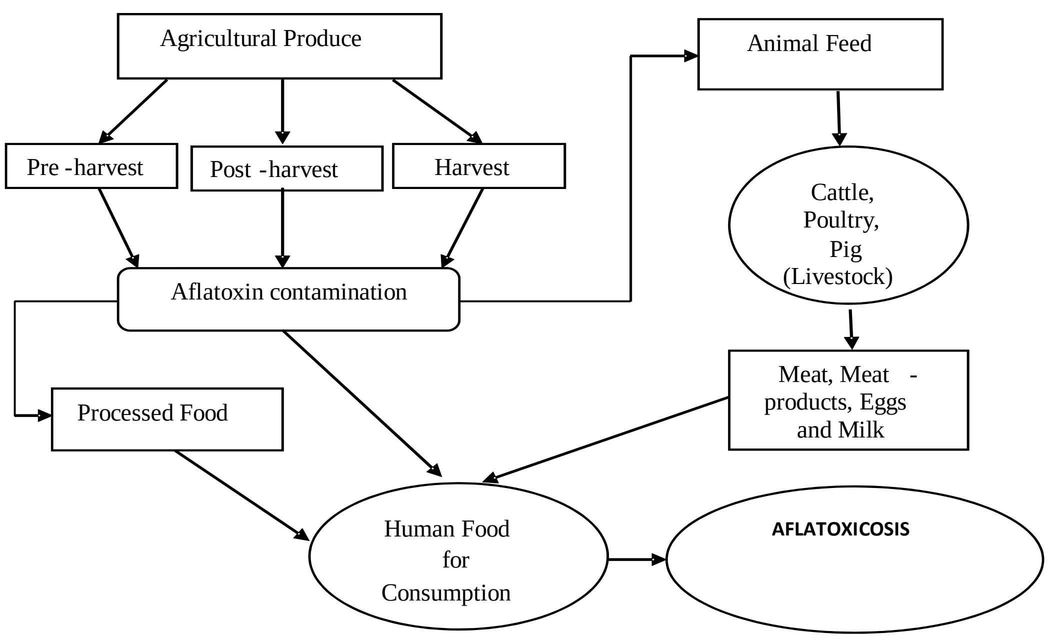

Figure 1: Entry of Aflatoxins into the Human Food Chain (Bhat and Vasanthi, 2003).

In order to reduce aflatoxins risks various strategies have been tried. They include use of insecticides and fungicides to reduce fungal and insects infestation and irrigation to avoid moisture stress; control of moisture through proper drying of crops prior to storage that will minimize fungal attack; dehulling and washing grains before processing to minimize contamination; use of chemicals and biological control of aflatoxins; mechanical sorting; use of organisms to reduce the occurrence of toxigenic *Aspergillus* in susceptible crops and in that way reduce aflatoxin contamination; use of enterosorbents that "trap"/bind aflatoxin in the gastrointestinal tract thus facilitating elimination; and consume less maize and groundnuts, in favour of other food crops that usually have lower aflatoxin levels such as sorghum and pearl millet (Ngoma *et al*., 2016a, 2016b; 2017; 2020). In Tanzania, it has been established that 82% of the parents/caregivers were not aware of aflatoxin contamination in complementary foods and their health effects (Ngoma *et al*., 2016; 2017).

While CFs consumption should improve health, deficiencies of micronutrients in many of the available formulations do not provide the levels of the required micronutrients (Chacha and Laswai 2020; Marcel *et al*., 2021; Mosha *et al*., 2020). Several underutilized plants (such as moringa, baobab and bread fruit) used in this innovation are available in communities, have great potential to be used and contribute to improved micronutrient nutrition. These ingredients including orange fleshed sweet potatoes (OFSP) have significant amount of pro-vitamin A, vitamin C which contribute to iron absorption and provide essential micronutrients iron, zinc and calcium.

Tanzania government has invested a lot to deal with the aflatoxin problem. Many studies have been conducted worldwide to try to improve the micronutrient composition of complementary foods. Most of these works have concentrated on use of OFSP, nuts and varieties of grains. Reports on the use of combinations of vegetables and fruits are very scanty. We innovated by utilizing locally available non-conventional vegetable and nutrient sources to enrich CFs and produce micronutrient dense formulation that is acceptable to consumers. In the case of safety concern we used a Dilution Techniques to reduce aflatoxins in CFs to within acceptable standards. The objective of the study was to develop nutrient dense complementary food flour for thin porridge that meets aflatoxin safety standards.

Materials and Methods

We innovated by using a dilution technique to attain acceptable levels of total and aflatoxin B1 in the final product and food based formulation to obtain levels of nutrients that meet recommended daily intake. Through literature search ingredients naturally prone to aflatoxin contamination and those less sensitive were identified. More sensitive ingredients mainly maize and groundnuts were avoided in the formulation. Less sensitive ingredients prepared onsite or from trusted suppliers were combined and analysed for total aflatoxin and aflatoxin B1 content. Microbial parameters of *Salmonella spp*., *E coli*, total plate count and yeast and molds were analysed. *Salmonella spp* in 25 g was determined by TZS 122 (2007), Total Plate Count by TZS 118 (2007), *E. coli* by ISO 16649 (2018) and Yeast and Molds by TZS 131 (2020).

In order to ensure a high level of food safety is achieved all persons engaged in production were trained in Good Manufacturing Practices focusing on identification of food hazards and allergens, points of occurrence, source and mitigation or eradication measures. Other areas included personal hygiene, illness and food contamination, pests safeguarding and eradication, premises layout, cleaning and sanitizing, waste management (generation, sorting, storage and disposal, equipment care and maintenance, production raw materials, quality control, storage and dispatch. Other topics included labeling, reporting, traceability and recall and emerging issues such as fraud and food defense.

In order to attain the desired levels of focus micronutrients (iron, zinc, beta carotene, fibre and vitamin C, combinations of seven ingredients namely finger millet, soya beans, orange fleshed sweet potatoes, cassava, bread fruit flours, baobab and moringa powders were optimized. The three tested formulations are shown in Table 1

Table 1

Ingredient proportions of test formulations

Ingredient	Proportion (%)		
	Formula 1	Formula 2	Formula 3
Millet	50	50	50
Soya bean	30	30	30
OFSP	5	8	8
Cassava	5	5	0
Bread fruit	6	0	9
Baobab	2	3	2
Moringa	2	4	1

Apart from nutrient content, consumer acceptability, cooking time, particle size distribution and reconstitutability when mixed with water were also optimized. The formulations were made to produce two types of composite flour one requiring cooking (normal) and another instant for preparing thin porridge. The instant type was prepared from the normal cooking flour by extrusion. Orange fleshed sweet potatoes, cassava and bread fruit were washed, peeled, sliced, dried in a dehydrator (Model ST-00, China) at temperatures between 60 and 65°C until 12% moisture content. Moringa leaves were blanched in boiling water for 2 minutes, cooled immediately immersing in cold water followed by drying in the dehydrator at 45C until 12% moisture content. Baobab powder was purchased from a certified supplier. To produce extruded flour for instant porridge (Uji Automatic) all seven ingredients in the test formulations were mixed together and passed through a Twin-Screw Extruding machine (Model Js-60D; KY Chemical Machinery, China). The extruded mixture was then milled into flour using an electric blender (Blendtec FIT Model; Blendtec Inc., USA). Similarly, soya bean grains were washed, boiled for about 45 min to inactivate trypsin and soften the cotyledons for easy dehulling. They were then cooled, dehulled, dried in a hot air oven and milled to flour.

Extrusion was accomplished using a twin screw extruder zone 1 temperature (189C), zone 2 temperature (107C), with feed speed of 14.5kg per hour. 50Kg of flour was mixed with 5 litres of water to condition it in order to obtain extruded flour of desired particle size. NutriSurvey (2007) software was utilized to determine and select complementary flour formulation that would meet at least 50% of the recommended daily allowance (RDA) of nutrients of interest/targeted nutrients (energy: 900 Kcal; protein: 13 g; vitamin A: 300 µg; iron: 7 mg; zinc: 3 mg) for young children of age 6–12 months and East Africa Standard for Processed cereal based foods for infants and young children specification EAS 72 (2013). Acceptable candidate composite flour formulation based on safety and nutrient content was accomplished using mother child pair assessors.

Total and aflatoxin B1 were determined by FCL/SOP-JM/13-02 method (ISO16050, 2003), fibre by FT-NIR, iron and zinc by MP AES method. Moisture content was determined by volumetric method, crude protein by micro-kjeldahl, total fat by Soxhlet methods while total carbohydrates were determined by difference. A sample for nutrient and microbiological analysis was submitted to the Tanzania Bureau of Standards for analysis.

Acceptability was determined by using mother – child pair and through test marketing. Test marketing was done through exhibitions at trade fairs, radio advertisement and promotion among shop owners. Particle size distribution was determined by using shaking machine (FRITSCH, RoHS, Germany using sieves with retention size ranging from 1mm to 63 µm. Cooking time was established by using a stop watch to find the time required to produce smooth porridge during cooking or using hot water for instant porridge flour. Reconstitutability was also assessed by measuring the time and effort required to produce smooth porridge and absence of clogs.

Results

Flow chart for production of CF formulation

Figure 2 shows the flow chart for production of composite Complementary Food (CF) flour that combines seven ingredients in its formulation. It is apparent that the different ingredients pass through a series of steps before combining them. In order to ensure final product quality that guarantees better nutrient retention and safe food, GMP and GHP practices are strictly observed. As part of the food safety management system Food Business Operators (FBOs) were trained in GMP and GHP and to observe sanitation standard operation procedures (SSOPs). Following training, in order to close all loopholes that may result in unsafe food all FBOs commit themselves to different levels. First, individually, each FBO commits to define and take up individual role in delivering safe food; second, he commits to take initiative to help his colleagues play their part in delivering safe food; third, he commits himself to proactively intervene when his colleagues do anything that compromises the safety of the food in their factory.

Quality and safety of the candidate CF formulation

The test CF flour that was produced following the flow chart presented in Figure 1 was evaluated for targeted safety (Table 2) and nutritional quality parameters (Table 3. The results were compared with East Africa Standards requirements. The evaluation considered test formula 1 (Table 1). The results of the analysis were very encouraging. The sample passed the tested safety parameters indicating that the test CF was very safe to consumers. Of great interest is the non-detection of both total aflatoxins and aflatoxin $B_{1.}$ Numerous literature show varied levels of aflatoxin combination with significant proportions exceeding the allowed limits in Tanzania of 10 μ/kg for total aflatoxin and 5 μg/kg for afalatoxin B_1 (Guchi, 2015; James and Zikankuba, 2018; Kaale *et al*., 2021; Kamala *et al*., 2017,2018a; 2018b; Kimanya *et al*., 2012; 2015; Mollay *et al*., 2022; Magoha *et al*., 2014). The results in Table 3 show that the test flour exhibited high levels of zinc, iron and fiber. The levels of nutrients such as protein (24.89%), fat (19.84%), iron (18.2mg/kg), zinc (21) and fibre (2.56%) were exceedingly higher than those targeted (protein 13 g; iron: 7 mg; zinc: 3 mg) based on RDA as per NutriSurvey (2007).

Table 2

Safety parameters of test CF formulation

Safety parameter	Result	Requirement (EAS 72:2013)
Salmonella spp. (/25g)	Not detected	Absent
E. coli (cfu/g)	<1 x 10^1	< 1
Total bacterial count (cfu/g)	8.5 x 10^2	-
Yeast and Mold (cfu/g)	< 1.0 x 10^1	Max 10^4
Aflatoxin B_1(mcg/g)	Not detected	5
Total aflatoxins (μ/g)	Not detected	10

Table 3

Nutrient content of test CF formulation

Chemical/nutrient content	Result	Requirement (EAS 72:2013)
Moisture (%)	8.2	8.0
Carbohydrates (%)	41.0	60.0
Crude protein (%)	24.9	14.0
Crude fat (%)	19.8	8.5
Fibre (%)	2.5	5.0
Iron (mg/kg)	18.2	10.0
Zinc (mg/kg)	21.0	-

Candidate CF formulation

After passing the initial nutritional quality and safety test evaluations candidate formulation (Table 4) was developed further for subsequent evaluation including acceptability, consumer preference and market response. The candidate formulation had soya bean and moringa proportions reduced. More moringa, though nutritious was not preferred by consumers due to unappealing green colour. Soya bean was adjusted down to a level that was cost effective and able to meet the required protein level as per EAS. Findings from mother-child pairs assessmet indicated overall acceptability based on impressive composition and flavor. However, some mothers noted some undesirable after taste bitterness. Overall, they were more impressed and looking forward to finalization of the instant flour development. All mothers indicated willingness to pay Tanzania shillings 4 000 per kg which was higher that other CF on the market.

Table 4

Ingredient proportion of candidate CF formulation

Ingredient	Proportion (%)
Millet	55
Soya bean	25
OFSP	5
Cassava	5
Bread fruit	5
Baobab	3
Moringa	1

Particle size of instant flour suitable for smooth porridge

The results of sieve analysis and ease of preparing smooth thin instant porridge are presented in Table 5. Initial tests using extruded instant flour that produced fine flour presented challenges to reconstitute when mixing with boiling water by forming lumps and requiring more than 5 minutes of continuous stirring in order to break lumps and obtain smooth porridge. This was deemed unacceptable to users. Testing of flour passing through various sieve sizes ranging from 1mm to 63 µm exhibited different reconstitution behavior. Flour passing through 250µm produced very smooth porridge with some lumps. Therefore, if flour is milled to pass between 400µm and 250µm it would produce satisfactory porridge and hence be recommended.

For larger size fractions there was a tendency of particles settling for failing to hydrate quickly. As a way forward it is recommended that a sieve of about 300µm should be used when milling the extruded materials

Table 5

Particle distribution and time for reconstituting instant porridge flour

Particle size	Percent	Time to smooth porridge (seconds)	Remarks
1mm	30.1	15	Poor particle dehydration; porridge not smooth, settling particles
800 µm	8.3	15	Poor particle dehydration; porridge not smooth, settling particles
630 µm	11.7	15	Porridge not smooth, layer separation
560 µm	7.8	30	Porridge not smooth, layer separation

400 µm	13.0	30	Porridge not smooth, layer separation
250 µm	25.8	90	Smooth porridge with few lumps remain at bottom
140 µm	3.2	30	Smooth porridge
90 µm	0	No fraction	-
63 µm	0	No fraction	-
Pan	0	No fraction	-

Table 7

Distribution of demand for normal flour type as of 25th April 2023

District in Tanzania	Number of enquiries
Morogoro Municipal	50
Ifakara	6
Gairo	6
Kilosa	4
Handeni	3
Bagamoyo	3
Tanga	1
Arusha	2
Mwanza	2
Dar es Salaam	15
Kisarawe	3

Consumer acceptability and market test response

The results of consumer acceptability in terms of sales and repeated demand for normal and instant flour types are presented in Table 6. while the geographical distribution of demand for flour is shown in Table 7. Normal (cooking type) **Kongano** flour that has been on the market testing for 11 months indicates a clear increase in demand. The trend is determined by a number of factors including coincidence with times of exhibitions like Farmers Day in Morogoro popularly known as 8.8, International Trade fare in Dar es Salaam and Innovation Week in Dodoma, advertisement on Radio which tends to increase sales volume. The instant porridge flour was market tested for a short period and requires more time to determine the trend and response. Enquiries for our flour nutrient dense aflatoxin safe flour branded **Kongano** are presented in Table 7. Customers who call us inform us that they have heard about the flour and that they are very interested in the product and would want to know where they can get the product. They advise that we arrange to have agents in each district of Morogoro region.

Table 6

Consumer acceptability through market testing sales (kg) in Morogoro Municipal

Month	Normal cooking type of flour	Extruded flour for instant Uji
Year 2022		
May	78	-
June	96	-
July	120	-
August	168	-
September	135	-
October	136	-
November	151	-

December	155	-
Year 2023		
January	140	5
February	170	6
March	185	10

Discussion

The flow chart for the entire process helps to conduct hazard analysis for the process. The analysis helps to identify steps where health hazards can occur, which type of hazard, physical, chemical, biological or allergens is likely to be encountered. Based on the knowledge of the nature and characteristics of the hazard it is possible to determine the risks that the hazard possesses. This is followed by identifying the source of the hazard and determination of preventive or control measures that can be applied. With the knowledge of the process and likely risk it is then possible to determine the critical control points to ensure total elimination or reduction of the hazard to an acceptable level. In this case FBO will have accomplished one of the critical behaviour in the processing of complementary food being produced. The FBO will commit himself first, to identify different hazards that can get into food at various points in the process; second, to implement programmes that reduce and eliminate significant hazards throughout the entire food process; third, he commits himself to actively intervene when he is concerned about risk levels for possible hazards.

The findings on absence of aflatoxin are also interesting in that the quality the flour fits for use beyond East Africa such as in the European Union and the United States of America. The findings confirm that the innovative dilution technique used to achieve aflatoxin safe product has been successful. In the absence of affordable aflatoxin test methods for ingredients used in CF preparation and given the fact that ingredients commonly used are obtained from unknown sources, makes the dilution technique a viable method to achieve aflatoxin safe food. This technique can also work for other combination of ingredients if known to be less susceptible to aflatoxin contamination. Mollay *et al.,* (2022) found about 82.14% of analysed CF ingredients to be contaminated with Afaltoxin B_1 in the range of 317µg/kg with aflatoxin B_1 exposure ranging from 0.33 to 1168ng/kgbw/day. Susceptible ingredients such as groundnuts and maize should be avoided unless their safety status is known. The absence of *Salmonella* and *E.coli* demonstrated that implementation of GMP (e.g. use of all stainless steel mill) and GHP (e.g. use of PPE) works towards ensuring food safety.

Recently, micronutrient nutrition has been given a lot of weight in promoting nutrition (Chacha and Laswai, 2020; Ekesa *et al.,* 2019; Khamis *et al.,* 2019; Marcel *et al.,* 2021; Mosha *et al.,* 2000; Shija *et al.,* 2019). In the test sample analysed, except for crude fibre and total carbohydrates, all other parameters were favourably above the requirement of EAS. There was an impressively higher level of iron and zinc that are currently through consumption of micronutrient dense foods as a nutrition strategy to combat anaemia and promote body immunity. Several producers of CF in Tanzania are struggling to incorporate pumpkin seed flour in their formulation to provide zinc and used as a product marketing strategy. However, based on cost consideration and processing losses of soya beans the candidate formula was modified to lower soybean proportion from 30 to 25% (Table 4). The lowering of soya bean content as a major contributor to protein and fat resulted in reduced content of these contents but still met the East African Standards requirements. The incorporation of breadfruit flour that is gluten free and baobab flour provided the needed folic acid and vitamin C that enhances iron absorption.

Several underutilized plants (such as moringa, baobab and bread fruit) used in this innovation are available in communities, have great potential to be used and contribute to improved micronutrient nutrition. These ingredients including OFSP have significant amount of pro-vitamin A, vitamin C which contribute to iron absorption and provide essential micronutrients iron, zinc and calcium to children over six months of age and individuals who wish to improve their immunity, lower blood sugar and pressure. Known as a miracle tree nutritionally, the dry *Moringa oleifera* leaves are rich in multiple micronutrients including calcium (2,003 mg), iron (28.2 mg), β-carotene (16.3 mg), Vitamin E (113 mg) (Marcel, 2021; Shija *et al*. 2019). Moringa leaves have 7 times more vitamin C than oranges, 4 times more vitamin A than carrots, 4 times more than calcium, 2 times more than milk, 3 times more than bananas, 3 times more vitamin E than spinach and 3 times more than almonds. A study conducted in Kisarawe district Tanzania (Shija *et al*., 2019) showed that *Moringa oleifera* supplementation reduced anaemia in children below 2 years. Feeding resulted in increased weight (79.3%), increased Hb (62.1%), reduced illness (55.2%), improved health (44.8%), increased appetite (24.1%), and retained Hb after illness (17.2%) and active children (13.4%). When needed, as for example to correct a given ailment such as high blood sugar more moringa powder can be added in the formula to realize more benefits from its medicinal effects.

Conclusions

Selection and combining safe nutrient dense ingredients as a Hazard Dilution Technique innovation provide a solution to aflatoxin problem in the infant and young children complementary foods available on the market. By employing Good Manufacturing Practices and Good Hygienic Practices other chemical and physical and biological hazards are effectively controlled. Careful selection and combination of less utilized ingredients such as moringa, baobab, OFSP and moringa which complement one another resulted in CF **Kongano** with high density of micronutrients that enhance availability consequently having potential to improve health of consumers. The instant type (**Uji Automatic**) is convenient and time saving for mothers and care givers. This innovation apart from health benefits it creates an opportunity for increased incomes of Actors in the supply chain.

Recommendation

Given the success in producing a safe and nutrient dense complementary food, it is desirable to promote the approach and the product in order to deal effectively with health problems associated with consumption of aflatoxin contaminated complementary foods used mainly to feed babies and children and even needy adults. We seek to explore avenues for scaling up and dissemination for benefit of a wider population within and outside Tanzania.

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Appendix

Ingredient						
Millet	Soybean	OFSP	Breadfruit	Cassava	Baobab	Moringa
RECEIVING AND STORING ↓ DRY CLEANING (winnowing, hand picking) ↓ WASHING ↓ DRAINING ↓ DRYING IN THE SUN ↓ STORAGE ↓ MILLING ↓ STORE	RECEIVING AND STORING ↓ DRY CLEANING (winnowing, hand picking) ↓ WASHING ↓ BOILING ↓ COOLING ↓ DEHULLING ↓ WASHING ↓ DRYING IN THE SUN ↓ STORAGE ↓ MILLING ↓ STORE	RECEIVING AND STORING ↓ WASHING ↓ PEELLING ↓ SLICING ↓ DRYING IN THE SUN, SOLAR OR ELECTRIC ↓ STORAGE ↓ MILLING ↓ STORE	RECEIVING AND STORING ↓ WASHING ↓ PEELLING ↓ SLICING ↓ DRYING IN THE SUN, SOLAR OR ELECTRIC ↓ STORAGE ↓ MILLING ↓ STORE	RECEIVING AND STORING ↓ WASHING ↓ PEELLING ↓ SLICING ↓ DRYING IN THE SUN, SOLAR OR ELECTRIC ↓ STORAGE ↓ MILLING ↓ STORE	RECEIVING AND STORING ↓ POUNDING ↓ SEIVING ↓ STORAGE ↓ MILLING ↓ STORE	RECEIVING AND STORING ↓ SORTING ↓ WASHING ↓ DRAINING ↓ DRYING IN SOLAR OR ELECTRIC ↓ STORAGE ↓ MILLING ↓ STORE

MIXING AND PACKAGING

WEIGHING
↓
MIXING
↓
LABELLING
↓
FILLING
↓
CLOSING
↓
STORAGE

Figure 2: Flow chart for production of CF flour for thin porridge