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Nutritional Quality, Mycotoxins and Antinutritional Factors in Quality Protein Maize-Based Supplementary Foods for Children in Tanzania

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Abstract
Most complementary foods used for children in Tanzania are low in energy and nutrient content. In addition, they may contain contaminants such as mycotoxins and also antinutritional factors. The aim of this study was to determine nutritional quality of quality protein maize-based supplementary foods and levels of mycotoxins (fumonisins, aflatoxins) and antinutritional factors (phytates, tannins). Three composite diets were prepared from quality protein maize namely; quality protein maize-soybeans; quality protein maize-soybeans-common beans and quality protein maize-soybeans-cowpeas. The fourth and fifth diets were prepared from plain quality protein maize and plain common maize. The formulations were made to meet the greatest amino acid scores and the desired amount of energy and protein according to the FAO/WHO (1985) recommendation for pre-school children. Concentrations of energy, protein, amino acid, aflatoxins, fumonisins, phytates and tannins were determined by standard methods. Quality protein maize-soybeans-common beans and quality protein maize-soybeans-cowpeas met RDA for both energy (360 kcal/100 g) and protein (16 g/100 g) for children aged 2-5 years. The amino acid scores for QPM-based diets were higher than the recommended scores (≥65%) for supporting optimal growth of children. Concentrations of fumonisin B1 and total fumonisin were 1687.82 and of 1717.16 µg/kg in quality protein maize and 1625.08 and 1745.22 µg/kg in plain common maize, respectively. These values were above the maximum tolerable limit of 1000 µg/kg recommended by the European commission. Efforts such as good agricultural practices and proper processing of food ingredients by sorting, dehulling and washing are recommended to reduce concentrations of fumonisins in maize grains.

Keywords: mycotoxins, soybeans, beans, cowpeas, tannin, phytate, extrusion-cooking, children, Tanzania

Introduction

Adequate nutrition and health care during the first several years of life is fundamental for child growth, development and survival. Although the causes of malnutrition are many and diverse, inadequate intake of foods and essential nutrients is a major contributory factor. At about six months of age, breast milk supply of energy and other nutrients such as proteins, vitamins and minerals is no longer adequate to meet child's body needs (WHO, 2009). Therefore, a high energy and nutrient dense complementary foods must be provided to the child. In many developing countries, Tanzania inclusive, cereals and legumes are used as a basis for these complementary foods (Kulwa et al., 2015). These foods are usually prepared as thin gruels. As a result, their energy and nutrient density are low. Furthermore, cereals and legumes are susceptible to fungal contamination. One of these contaminants is mycotoxins. Mycotoxins are fungal secondary metabolites produced by toxigenic strains of fungi that contaminate crops before or after harvest. The most common mycotoxin producing fungi belongs to the three genera of fungi: *Aspergillus*, *Penicillium* and *Fusarium* (Frisvad et al., 2006). Ingestion of mycotoxins contaminated grains by animals and human beings has enormous public health

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significance, because these toxins are capable of causing diseases (Gnonlonfin et al., 2013). These toxins are nephrotoxic, immunotoxic, teratogenic and mutagenic. They are also capable of causing acute and chronic effects in man and animals ranging from disorders of the intestinal tract, central nervous, cardiovascular and pulmonary systems and death (Lombard, 2014). These mycotoxins have recently been associated with cancers and stunting in young children (IARC, 2016). The most toxic mycotoxins are aflatoxins, ochratoxin A, fumonisins, trichothecenes and zearalenone (Pitt, 2000).

Maize and beans are important food crops in Tanzania (Barreiro-Hurle, 2013 and ProFound, 2012). They are commonly used as ingredients in making complementary foods for children. Other cereals such as sorghum, rice, wheat and finger millet are rarely used. Apart from nuts, maize is more susceptible to mycotoxin contamination than any other crops. This is because corn production frequently encounters a period of drought and heat stress during flowering and kernel development. These weather conditions have been reported to increase mycotoxin contamination in maize than in other crops (Kebede et al., 2012). Maize contains about 45% carbohydrate, 5% protein and 2% fat in general (Enyisi et al., 2014). The protein content of beans ranges between 20- 30% and 5% fat. Legumes are also rich in folate (50%), and minerals such as iron (4%), zinc (1%), and calcium (24%) (Messina, 1999). In addition to nutritional values of cereals and legumes, their use is limited due to presence of antinutritional factors. Phytic acid for example, reduces the availability of many minerals such as iron, zinc, calcium and magnesium (Asuquo & Etim, 2011). Ability of phytate to form complexes with these minerals makes the minerals not bioavailable. Tannins are known to bind proteins, including digestive enzymes leading to poor protein digestibility (McSweeney et al., 2001). Formation of protein complexes lowers protein digestibility, hence reducing food utilization and growth in children. It is therefore recommended that, when preparing complementary foods for children various methods should be used in inactivating and/or reducing these antinutritional factors. In Tanzania, some efforts have been made to determine nutrient content, levels of mycotoxins and antinutritional factors in cereal-based complimentary foods for children in plain or composite maize with legumes and/or nuts (Kimanya et al., 2014: Magoha et al., 2014). There is however, limited information documented about mycotoxin and antinutritional factors in quality protein maize (QPM)-based formulations for children in Tanzania although QPM use has been growing steadily in the past decade. This study was carried out to determine the nutritional quality, mycotoxins (aflatoxin and fumonisin) and antinutritional factors (phytate and tannins concentrations) in QPM-based composite formulations used for supplementing children in Tanzania.

Materials and Methods

Materials

Quality protein maize was purchased from Seliani Research Station in Arusha, Tanzania. Common maize (*Zea mays*), soybeans (*Glycine max*), common beans (*Phaseolus vulgaris*), cowpeas *(Vigna unguiculata*), edible vegetable oil and sugar were purchased from Morogoro Municipal central market.

Product Formulation and Processing

Blend formulation

Formulations of high-protein-energy supplementary foods were made to meet the greatest amino acid score and the desired amount of energy and fat according to the FAO/WHO (1985) Codex Alimentarius guidelines for supplementary foods for infants and young children. The blend ratios of QPM, soybean, common beans, cowpeas and normal maize are shown in Table 1.

Table 1: Composition of the maize-based supplementary food formulations

	Formulations[1](g/100 g)				
Ingredient	QS	QSC	QSB	QQ	CM
QPM	68	46	45	82	-
Soybean	14	6	7	-	-
Cowpeas	-	30	-	-	-
Common beans	-	-	30	-	-
Common maize	-	-	-	-	82
Cooking oil	10	10	10	10	10
Multi-mix[2]	3	3	3	3	3
Sugar	5	5	5	5	5
Total	100	100	100	100	100

[1] QS=Quality protein maize-Soybean; QCS=Quality protein maize-Cowpeas-Soybean: QBC=Quality protein maize-common Beans -Soybean QQ=Quality protein maize; CM= Common Maize

Food processing

Separately, QPM, common maize (CM), soybeans, common beans and cowpeas were sorted to remove extraneous materials and pebbles and washed twice in distilled water. Then, QPM and CM were separately dehulled. Thereafter, each of these ingredients was separately milled into fine flour (mesh size 0.4 mm) using a commercial hammer mill (Intermek, Tanzania) to fine flour. Three formulations were developed; QPM-Soybeans (QS), QPM-soybeans-common beans (QSB) and QPM-soybeans-cowpeas (QSC). The fourth product was made from QPM alone (QQ) and fifth products from common maize alone (CM). Each food product was conditioned to 22% moisture content and 5% vegetable oil and allowed to equilibrate for 30 minutes. The food mixtures were separately extruded using a commercial twin-screw extruder (Model JS 60 D, Qitong Chemical Industry Equipment Co. Ltd, Yantai, China) with two electrically heated zones. The following extrusion conditions were adopted: Temperatures 130ºC (Zone 1) and 122ºC (Zone 2), main motor speed was set at 10.48 rpm and feeder speed at 10.26 rpm. Desired barrel temperature was maintained by circulating cold water. Temperature was controlled by inbuilt thermostat and a temperature control unit. The extruder was cleaned after each run with moist maize flour. After extrusion, the extrudates were allowed to cool and dry at room temperature, thereafter milled, fortified and packaged in polyethylene packets ready for laboratory analysis.

Chemical Assays

Crude protein, energy and amino acid determination

Crude protein was determined according to AOAC (1995). Carbohydrate content was calculated by difference. Energy values (kcal) were calculated by using Atwater conversion factors 4, 9 and 4 for each gram of protein, fat and carbohydrate, respectively (AOAC, 1995). Amino acid concentrations (except tryptophan) were determined by a high performance liquid chromatography using the Waters Pico-Tag method (Cohen et al., 1989). For all amino acids except methionine, cysteine and tryptophan, food samples were hydrolysed in 6 N HCl. The methionine and cysteine in foods were oxidised by performic acid to methionine sulfone and cysteic acid prior to hydrolysis by 6 N HCl. All amino acids (except tryptophan) were derivatised by phenyl isothiocayanate and detected at 254 nm. Tryptophan was analysed by ion exchange chromatographic method as described in the AOAC (1995) method 988.15. The protein in the food was hydrolysed under vacuum with 4.2 N NaOH. After pH adjustment and clarification, tryptophan was separated by ion exchange chromatography (DC5A cation exchange resin) with measurement of the ninhydrinchromophore.

Amino acid score

The amino acid score was calculated using the ratio of a gram of the limiting amino acid of test diet to the same amount of the corresponding amino acid in the reference protein multiplied by 100. The reference proteins suggested by FAO/WHO/UNU (1985) for children aged 2-5 years was used for calculating amino acid score.

Aflatoxins and fumonisins determination

Aflatoxin concentration was determined according to method described by Kimanya et al. (2008). Determination of fumonisins B1 and fumonisins B2 was carried out using HPLC method described by Samapundo et al. (2006) and Kimanya et al. (2008). Phytic acid concentration was determined by the method described by Wheeler and Ferrel (1971). Tannin was determined following the method described by Porter et al. (1986).

Statistical analysis

All data (except for amino acids), were subjected to analysis of variance (ANOVA) using Genstat (1998, version 4). For amino acids, mean values were computed. The concentrations of mycotoxin and antinutritional factors in the composite products were compared by using the Turkeys Least Significant Difference (LSD) test when difference existed. Differences were considered significant at $p \leq 0.05$.

Results and Discussion

Energy, protein content and amino acid profile of QPM-based composite foods

Chemical assays showed an increase in protein content of the composite diets blended with soybeans. The energy and protein density of the diets were 386 kcal and 17 g per 100 g of QSB, 389 kcal and 16 g per 100 g of QSC, 381 kcal and 16 g per 100 g of QS,387 kcal and 6 g per 100 g of CM. Comparing with FAO/WHO reference, diets QSB and QSC met both energy (360 kcal) and protein content (16 g) per 100 g of edible food required for children aged 2-5 years. This could partly be attributed to the higher contents of protein supplemented to QPM blended flour. These findings were in line with those reported by other investigators (Madukwe et al., 2013: Kadam et al., 2012). Consumption of cereal based supplementary food with other protein rich diet is of great nutritional benefit since nutrient complementarity can be achieved. Mohsen *et al. (*2009) reported contrasting findings indicating that soybean supplementation did not improve the protein composition of wheat cookies. From this study, carbohydrate content decreased from

90.5 and 88.7% in plain CM and plain QPM products, respectively, to 79, 79 and 81% in QS, QSB and QSC diets, respectively. This decrease could be explained by the low carbohydrate content of legumes, soybean, common beans and cowpeas (Nwosu 2013: Khan, 2009). Soybean stores energy at approximately 21% fat and 4% crude fibre, whereas maize stores 73% energy as carbohydrate (starch) (Rajendar et al., 2013). Therefore, blending maize with soybean diluted the carbohydrate concentrations of the composite products.

Amino acid profiles of the experimental and control diets are presented in Table 2. Amino acid composition of the composite diets ranged between 15 mg/100g for tryptophan (QQ diet) and 109 g/100g for leucine (CM diet). Lysine and tryptophan contents of the composite diets increased with soybean supplementation. Lysine content in QPM-based diets ranged from 67 to 74 mg/100 g protein in QS and QSB, respectively. Tryptophan concentration ranged from 16 to 18 mg/100 g protein in QS and QSB, respectively. Lysine and tryptophan contents of QSB and QSC were higher than the levels in reference protein for children aged 2-5 years (58 mg/g Lys and 11 mg/g Trp) (FAO/WHO/UNU, 1985). Plain common maize diet (CM) contained lower concentration of lysine and tryptophan than the recommended levels of Lys (58 mg/g) and Trp (11 mg/g) protein, for children 2-5 years. This study revealed that, histidine, threonine, valine, leucine, lysine, tryptophan, sulphur containing amino acids (methionine and cysteine) and aromatic amino acids (phenylalanine and tyrosine) were present in adequate amounts in the QPM-based diets when compared with the recommended levels for children 2-5 years by (FAO/WHO/UNU 2002). Amino acid that is in short supply is referred to as the most limiting amino acid (Häffner et al., 2003). Results of amino acid score in the test diets indicated that lysine was the most limiting amino acids in all the diets. This observation was in agreement with (Caire-Juvera et al., 2013) (Nuss & Tanumihardjo, 2011) and (Häffner et al., 2003) who reported lysine as the most limiting amino acid in cereals. Amino acid scores for the QPM-based diets were higher than the minimum value (≥65%) recommended by the FAO/WHO/UNU (1985) Codex Alimentarius for children aged 2-5 years. This could be attributed to the inclusion of soybeans which improved their amino acid profile. Common maize was the only diet with limiting amino acid less than the recommended value of ≥65%. Several studies have shown that, common maize is limiting in lysine and tryptophan (Nuss & Tanumihardjo, 2011; Sofi et al., 2009).

Table 2: Amino acid profile and scores of the various foods designed for children aged 2-5 years

	CM	AAS	QS	AAS	QQ	AAS	QSB	AAS	QSC	AAS
Amino acid (mg/g)										
Histidine	31	162	36	189	41	216	34	179	37	195
Threonine	29	85	43	126	41	121	44	129	43	126
Valine	45	128	59	169	60	171	58	166	59	169
Leucine	121	184	103	156	106	161	96	145	98	148
Lysine	26	45	67	116	50	86	74	128	73	126
Tryptophan	7	67	16	145	15	136	18	164	17	155
SAA	41	165	37	148	46	184	44	176	42	168
AAA	76	120	91	144	73	116	91	144	88	140
Limiting AA		Lys		Lys		Lys		Lys		Lys

[a] CM= Common maize: QS= Quality protein maize- Soy bean: QQ= Quality protein maize: QSB= Quality protein maize-Soy bean-common Bean: QSC= Quality protein maize-Soy bean-Cow peas: SAA= sulphur containing amino acid (methionine and cysteine): AAA= aromatic amino acids (phenylalanine and tyrosine)
AAS= Amino acid Score

Mycotoxin contamination of the composite diets

Aflatoxins and fumonisins concentrations of the various food samples are summarized in Table 3. Concentrations of aflatoxin B1 and total aflatoxins in all the food samples were less than one microgram per kilogram. These concentrations were far below the Tanzania recommended concentrations of 5 µg/kg for aflatoxin B1 and 10 µg/kg for total aflatoxin in supplementary foods for children (Tanzania Bureau of Standards, 2010). Likewise, the European Commission (2006) recommends the upper limits of 2 µg/kg aflatoxin B1 and 4 µg/kg (total aflatoxin) for foodstuffs. High aflatoxin contaminations in maize has been reported in Tanzania recently (Gheysens, 2015).

According to Gheysens (2015), aflatoxin B1 concentration in samples of maize collected in rural Tanzania was 94.23 µg/kg while total aflatoxin concentration was 219.45 µg/kg. A study by Kumi (2014) in Ghana reported mean total aflatoxin contamination of 145.2 µg/kg in weanmix. In a recent study in Tanzania (Kamala et al., 2016), 45% of the maize samples evaluated were contaminated with aflatoxins with levels ranging from 0.1 to 269 µg/kg. Low levels of total and aflatoxin B1 observed in the current study might be due to the processes that were made on the maize before extrusion. Maize and legumes that were used in this study were washed and dehulled. These processes have been reported to reduce mycotoxin levels. Fandohan et al.*(*2005) reported that, processing methods such as sorting, winnowing, washing and dehulling of maize grains removed up to 93% of aflatoxin and 48% of fumonisins.

This study showed that plain maize diets (QQ and CM) had higher levels of fumonisin contamination than blended diets. Diet QQ had fumonisins B1 concentrations of 1687.82 µg/kg and total fumonisins concentrations of 1717.16 µg/kg, whereas diet CM had fumonisin B1 concentrations of 1625.08 µg/kg and total fumonisins concentrations of 1745.22 µg/kg. These two diets, QQ and CM were the only samples with fumonisins concentrations above the upper limit of 1000 µg/kg recommended by the European commission (EC, 2006). Fumonisin B1 and total fumonisins levels reported in this study were lower than levels reported from other studies conducted in Tanzania. A recent study by Gheysens (2015) in rural Tanzania reported values of fumonisin B1 and total fumonisin higher than those observed in this study.

According to Gheysens (2015), concentrations of mycotoxins in maize grains were fumonsin B1 (0.00-5461.00 µg/kg), fumonisin B2 (0.00-1756.80 µg/kg) and total fumonisins (0.00-6761.00 µg/kg). The maize used for their study were just crushed prior to analysis of mycotoxins unlike the maize grains used in our study which were sorted, winnowed, washed, dehulled, milled and extruded before analysis. These processes were missing in the Gheysens (2015) study. Magoha et al. (2014) reported that 68% of the flour samples collected from Northern Tanzania were contaminated with total fumonisin concentration above the maximum tolerable limit (MTL). A study by (Kumi et al., 2014) in weanmix observed that, mean fumonisin contamination was 4.6 µg/kg in the composite flour. Furthermore, 58.3% of the weanmix samples were contaminated with fumonisin concentration above the U.S. FDA limit of 4 µg/kg. Chronic mycotoxin exposure has major effects on nutritional status in human. It suppresses body's immunity and nutritional status. Aflatoxin B1 is known to be acutely toxic and a cause of liver cancer in humans (Shephard, 2008). Aflatoxin exposure has also been associated with an increased risk for liver cirrhosis (Kuniholm *et al.*, 2008). Fumonisin and aflatoxin exposure early in life has been associated with impaired growth, particularly stunting in Tanzania (Kimanya 2010) and West Africa (Gong et al., 2003).

Antinutritional factors of the formulated diets

Concentrations of phytate and tannin in the composite foods are presented in Table 3. Concentrations of phytic acid among the food products were significantly different (p<0.05), increasing with the level of legume substitution (16.6 to 24.6mg/100g). The concentrations of phytates in the supplementary foods studied were lower than 25 mg/100g, the amount considered lethal to health (Nagel, 2010). Similarly, tannin levels increased with legume supplementation. Tannins concentrations were very low (0.12-3.5 mg/100g) while the reported lethal dose is 90 mg/100g (Ifie & Emeruwa, 2011). This indicated that, the concentrations of phytates and tannins in the composite diets were of acceptable safe levels and the foods were therefore safe for human consumption. According to Kumar*et al.* (2010), high levels of phytates in human foods limits the bioavailability and hence utilization of minerals, specifically calcium, magnesium, iron and manganese by forming insoluble compounds that are indigestible. These minerals play important roles in children bodies by supporting growth, bone and tissue development. Observed low phytate in extruded diets might be associated with heat treatment which is known to reduce concentrations of antinutritional factors. Processes such as extrusion have been reported to destroy antinutritional factors and inactivated lipoxygenase enzyme responsible for the beany flavour development. Beany flavor has been reported to adversely affect utilization of legumes in foods. Several authors have reported on the effects of extrusion on inactivating antinutritional factors; phytic acid in soybean (Ariet et al., 2012), cowpeas (Olapade & Umeonuorah, 2013: Olapade & Aworh, 2012), trypsin inhibitor activity in pinto beans (Balandran-Quintana et al.,*1998)* and phytic acid in pigeon pea (Anuonye et al.,2012). A study by (Anton et al. *(*2009) reported that, extrusion cooking reduced phytic acid inhibitor levels to nearly 50% in maize-common bean blend. Concentrations of phytates and tannins observed in this study posed no health risk to the consumers.

Table 3: Concentrations of aflatoxins, fumonisins and antinutritional factors in the various food formulations

Diet	Mycotoxins (µg/kg)								Antinutrients (mg/100 g)	
	Aflatoxins					Fumonisins				
	B1	B2	G_1	G_2	Total	B1	B2	Total	Phytates	Tannin
CM	0.15^{d}	0.02^{a}	0.07^{d}	0.07^{a}	0.66^{a}	1625.08^{b}	120.22^{a}	1745.22^{b}	16.61^{d}	0.12^{a}
QS	0.23^{c}	ND	0.01^{d}	0.02^{a}	0.26^{a}	112.60^{e}	86.29^{b}	198.89^{e}	22.63^{c}	0.56^{ab}
QQ	0.33b	ND	0.02^{d}	0.05^{a}	0.40^{a}	1687.82^{a}	29.34^{c}	1717.16^{a}	17.54^{d}	0.23^{c}
QSB	0.50^{a}	ND	0.05^{ab}	ND	0.55^{a}	ND	ND	ND	24.62^{c}	3.45^{d}
QSC	0.50^{a}	0.11^{a}	ND	0.05^{a}	0.56^{a}	330.72^{d}	ND	330.72^{d}	22.93^{b}	2.74^{e}
LSD	**0.04**	**0.00**	**0.03**	**0.06**	**0.07**	**4.631**	**0.115**	**4.634**	**6.612**	**0.7278**

*Mean values in a column with different super script are significantly different at p<0.05: ND= Not Detected

Conclusion and Recommendations

Supplementation of plain maize flour with soybeans, common beans and cowpeas increased the energy density and protein content. Lysine and tryptophan contents of QSB and QSC diets were higher than the FAO/WHO/UNU (1985) recommended levels for children aged 2-5 years. The amino acid scores for QPM-based diets were higher than the recommended scores of ≥65% needed for supporting optimal growth of children aged 2-5 years. Diets QSB and QSC had both aflatoxin and fumonisin concentrations below the maximum tolerable limits set by Tanzania Bureau of Standards and European Commission. Conversely, both CM and QQ diets had higher concentrations of fumonisin above the maximum tolerable limits set by Tanzania Bureau of Standards and European Commission. Based on these results, it is recommended that efforts must be made to reduce the levels of fumonisins in maize grain. This can be achieved through good agricultural practices and proper processing practices of food ingredients such as sorting, dehulling and washing when making supplementary foods for children.

Conflict of interests

The authors declare that they have no conflict of interest.

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