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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

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Table of Contents: Volume 9 Number 8 August, 2015

ARTICLES

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Full Length Research Paper

Improving the nutritional value of traditional finger millet porridges for children aged 7-24 months in Bujenje County of Western Uganda

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Child malnutrition is a major problem in Western Uganda where finger millet porridges are commonly used as complementary foods. This study aimed at improving the nutritional value and safety of finger millet porridges using *Moringa oleifera*, *Cucurbita maxima* and lactic acid fermentation. Proximate analysis, iron, zinc and vitamin A contents of composite flours was done according to AOAC methods while agar diffusion pouring technique and a seven point hedonic scale were used to analyse antimicrobial and organoleptic properties of the improved millet porridges, respectively. The porridges were developed with the aim of catering for at least 60% daily requirements for protein, vitamin A, iron and zinc for children aged 7-24 months. They were fermented using lactic acid starter cultures. Chi-square tests were used for comparing percentage acceptance of the porridges by mothers. Analysis of variance was carried out and differences among means were compared by Duncan's test at p<0.05. Fermented millet porridge with 7% *M. oleifera* leaves had the best nutrient composition and antimicrobial properties while fermented millet porridge with 17% *C. maxima* flesh was more acceptable by mothers. The porridges are thus a potential solution to inadequate nutrient intakes and diarrhoeal infections attributed to child malnutrition in Western Uganda and most developing countries.

Key words: Nutritional value, traditional millet porridges, western Uganda.

INTRODUCTION

Globally an estimated 165 million, 10 million and 52 million of children below five years of age are stunted, underweight and wasted, respectively (UNICEF, 2012). Micronutrient deficiencies of iron, vitamin A and zinc have also been reported as a major cause of death through diseases among these children, especially in developing countries (Black et al., 2008; West and Darnton-Hill, 2008; Bruno et al., 2005). In Uganda, 33% of preschool children are stunted, 14% underweight, 5% wasted and 49% anaemic (UBOS and ICF, 2012). Zinc deficiency is estimated to range between 20 to 69% while 20% are vitamin A deficient (Fanta, 2010). Most malnutrition in

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Uganda occurs during the period of complementary feeding (6-18 months) because of inappropriate complementary foods (UBOS and ICF, 2012). This is because of increased nutrient requirements for growth and development during this period but with limited gastric capacity (Compaore et al., 2011).

Critical stunting levels above 40% have been persistently reported in Western Uganda where millet porridge is a common complementary food (Harvey et al., 2010; UBOS and ICF, 2001, 2012). Our baseline survey in this area revealed that stunting, underweight and wasting levels among children aged 7-36 months were 31, 12 and 7%, respectively. Poor complementary foods and diarrhoea were found to be major factors attributed to this poor nutritional status among children. About 25-77% of the children aged 7-24 months were found to be consuming less than recommended daily intake of energy, protein, vitamin A, iron and zinc with finger millet porridges as the most common bulky complementary food (unpublished data).

Finger millet porridge is recommended as a weaning food since it is one of the least allergenic, non acid forming and easy to digest cereal foods (Paragya and Raghuvanshi, 2012). However, it has low energy and nutrient density (Paragya and Raghuvanshi, 2012), limited amino acids (Usha and Lakshmi, 2010; Bachar et al., 2013) and high content of anti-nutrients such as phytates and tannins. These contribute to poor digestibility of protein and starch and reduce the overall availability of vitamins and minerals in millet (Lei, 2006; Paragya and Raghuvanshi, 2012). These shortcomings in finger millet porridges suggest inadequate dietary intake for children who use it as a main complementary food. There is need to improve on the nutrient density and utilisation of nutrients in this common feeding medium in Western Uganda. This can help to avoid the effects of malnutrition at such early ages which can result in irreversible damage to intellectual and physical development (Fanta, 2010). *Moringa oleifera* and *Curcubita maxima* powders are locally available food resources with a potential to bridge the nutritional value gap in finger millet and lower morbidity due to their antimicrobial properties (Dhiman et al., 2009; Fuglie, 2005; Usha and Lakshmi, 2010). Lactic acid fermentation is a potential household food preparation technology underutilized in Bujenje County but can also help abate the nutritional functional problems related to finger millet such as poor bioavailability of iron and zinc and low digestibility of starch and protein (Paragya and Raghuvanshi, 2012; Ogbe and Affiku, 2012). In addition, the antimicrobial properties in moringa and lactic acid fermentation can promote the safety and keeping quality of millet porridges (Ogbe and Affiku, 2012; Onyango et al., 2005). This is an advantage especially in such farming communities where porridges are kept for long before feeding the children. In Bujenje County, *M. oleifera* powders are mainly used as herbal concoction especially for children with diarrhoea. Alternatively, pumpkins are a popular crop whose leaves and fruits can be relied upon in times of scarcity. Therefore, the aim of this study was to improve on the nutritional value of finger millet porridges using locally available resources such as moringa *(M. oleifera)*, pumpkin (*C. maxima*) and lactic acid fermentation in resource constrained and technologically under developed areas of Western Uganda.

MATERIALS AND METHODS

The experimental materials comprised of finger millet (*Eleusine coracana*), moringa (*M. oleifera*) and pumpkin (*C. maxima)* made into flour. A commonly used farmer's variety of finger millet known as 'Mugaali' was purchased from the local market, cleaned, dried and ground into flour. *M. oleifera* leaves and seeds were harvested, cleaned and dried indoors before being ground into flour by a local miller (mortar and pestle). A farmers' variety of *C. maxima*, identified as 'Okamanyaota' pumpkin was harvested when mature, washed, peeled and seeds removed. The flesh and seeds were sun dried for about 3-4 days before they were milled separately into flour.

Nutrient quality analysis of finger millet, *Moringa oleifera* and *Curcubita maxima* foods

Finger millet porridges consumed at baseline, finger millet flour and pumpkin (*C. maxima*) were analysed for proximate composition, zinc, iron and vitamin A contents in triplicates according to AOAC (2012). The Kjeldahl method (Jung et al., 2003) was used to determine the protein content (N × 6.25) while fat was determined by solvent extraction (Sukhija and Palmquist, 1988). Carbohydrates were determined by the difference of the sum of all the proximate composition from 100%. The calorific (energy) value was obtained by multiplying the value of carbohydrate, protein and crude fat by the Atwater factors of [energy content = (g carbohydrate x 4) + (g fat x 9) + (g protein x 4] (Papadopoulos et al., 1986). Iron and zinc were estimated using the atomic absorption spectrophotometer (AA 500 G., PG Instruments, Leicestershire, England) while vitamin A was determined by column chromatography (Parvin et al., 2014).

Formulation of the porridge flours

This was done basing on our baseline data. Study findings showed that in majority of children, only 40-50% of the daily requirements for kilo calorie, protein, vitamin A, iron and zinc were met by children's diets (unpublished data). Table 1 shows how these porridges were used to feed children in Bujenje County and the percentage of daily recommended nutrient intake (RNI) they covered in children aged 7-24 months. The average amount of porridge served to children varied from 150 to 300 ml per day. Children were given porridges approximately 2 to 3 times a day (servings) depending on their age. The % recommended nutrient intake (RNI) covered by these porridges was quite small and varied from zero to 55% depending on the nutrient and the age of the child.

The experimental porridge flours were developed using finger millet, *C. maxima* flesh and *M. oleifera* leaf flours, with the aim of providing at least 60% daily requirements for protein, iron, zinc and maximising vitamin A in 150 to 300 ml per serving of prepared porridges taken per day. Given that vitamin A content in millet flour was found to be almost zero, *C. maxima* (pumpkin) and *M. oleifera*

Table 1. Nutrient intake from traditional millet porridges at baseline per day.

Variable	7-8 months	9-11 months	12-23 months
Serving amount	150 ml	200 ml	300 ml
Amount of solids	10 g	15 g	20 g
Number of servings	1.75±0.18	2.65±0.06	2.95±0.04
Energy			
RNI	200 k cal	300 k cal	550 kcal
Total intake	69.5 k cal	157.8 k cal	234.1 k cal
% RNI covered	35	53	42
Protein			
RNI	9.1 g	9.6 g	10.9 g
Total intake	1.6 g	3.7	5.6
% RNI covered	18	39	51
Vitamin A (RE)			
RDA	250 µg	300 µg	400 g
Total intake	0 µg	0 µg	0 µg
% RNI covered	0	0	0
Iron			
RDA	11 mg	11 mg	11 mg
Total intake	1.8 mg	4.0 mg	6.0 mg
% RNI covered	16	36	55
Zinc			
RDA	2.8 mg	2.8 mg	2.8 mg
Total intake	0.4 mg	0.9 mg	1.3 mg
% RNI covered	14	32	46

Iron* Assuming medium iron bioavailability (10%). Zinc** Assuming moderate bioavailability (30%) References: World Health Organisations Recommendations (Ruel, Loechl, and Pelto 2004; Dewey and Brown 2002).

flours were formulated with finger millet flour at calculated rates of 300 µg, 400 µg and 500 µg based on the daily recommendation for vitamin A for children aged 7-24 months (Brown and Dewey, 2003). Six complementary porridge flours (M1, M2, M3, P1 P2 and P3) were formulated as follows:

Flour 1 (M1): *Finger millet*: *M. oleifera leaf powder* (95: 5).
Flour 2 (M2): *Finger millet: M. oleifera leaf powder* (93: 7)
Flour 3 (M3): *Finger millet: M. oleifera leaf powder* (91: 9)
Flour 4 (P1): *Finger millet: C. maxima flesh flour* (88: 12)
Flour 5 (P1): *Finger millet: C. maxima flesh flour* (83: 17)
Flour 6 (P1): *Finger millet: C. maxima flesh powder* (79: 21)

The formulated porridges consisted of two versions (fermented or non-fermented) and were compared with the control of traditional millet porridges (fermented and non-fermented) to ascertain the effect of fermentation and *M. oleifera* leaves and *C. maxima* flesh flours on the porridges formulated.

Fermentation of the formulated porridge

The porridges were fermented using lactic acid fermentation starter cultures prepared according to U.K patent, ARIPO Patent No. AP122 (Mbugua, 1992). Formulated flours were each slurred with water (50% solids), inoculated with 5% starter culture and incubated at room temperature for 24 h. The pH and titratable acidity in the porridges were measured to determine if fermentation had occurred.

The pH was measured using Metrohm 605 pH meter Swiss made, calibrated to 7.005. Ten millilitre samples of each of the porridges were put in a beaker. Five drops of 1% phenolphthalein solution were added and titration done to first persistent pink colour with 0.1 N NaOH. Titratable acidity was calculated on the basis of lactic acid equivalent according to the formula;

$$\% \text{ lactic acid} = \frac{\text{ml alkali} \times \text{normality alkali} \times \text{relative molecular mass of lactic acid}}{\text{Weight of sample in grams}}$$

Preparation of porridges

Fourteen types of porridges were prepared on the day of the test. They included the control of traditional millet porridge fermented and non-fermented and porridges with different amounts of *M. oleifera* and *C. maxima* (pumpkin) flours. 200 ml of smooth slurries made from 10-20% flour solids were mixed with 1 L of boiling water in cooking pots and the mixture stirred for at least 10 min to obtain smooth textured porridges. To ensure a comparable consistency in porridges, the total amount of water, flour and cooking time were adjusted for each of the porridge through visual examination by checking the fluidity of the porridge. The cooked porridges were kept in plastic jugs to maintain the serving temperature and sweetened before tasting.

Sensory evaluation

According to Gomiero et al. (2003) organoleptic parameters such as colour, aroma, taste, consistency and appearance are key measures of product quality (Compaore et al., 2011). Porridge samples from the different formulations were subjected to sensory evaluation using a seven point hedonic scale of 1-7, where 1 = dislike very much; 2 = dislike moderately; 3 = dislike slightly; 4= neither like, nor dislike; 5 = like slightly; 6 = like moderately and 7 = like very much (Carpenter et al., 2000). Twenty two (22) mother panellists from Bujenje County were used to evaluate the formulated porridges in terms of colour, consistency, flavour, taste and overall acceptability. Twenty two (22) mother panellists aged 22-35 years with children less than two years of age were used because they determine the choice for children's feeds and were familiar with millet porridges. Each judge was presented with 7 disposable cups containing different blinded porridge samples per day for two days. The porridges were provided at the interval of 5-10 minutes. On the first day non-fermented moringa and pumpkin fortified millet porridges, and ordinary millet porridge as control, were presented while on the second day, fermented millet based porridges were presented. In addition, spoons for evaluating each porridge sample and bottled water for rinsing of the mouth in between testing of the porridges were provided. Rinsing the mouth minimised the carry-over taste effects from previous sample. Each treatment was evaluated three times by each panellist. The most acceptable porridges were standardized by a Bostwick Consistometer (Mouquet et al., 2006) and their nutrient composition analysed.

Viscosity of the acceptable formulated porridges

For this study 100 g finger millet flour, 150 g finger millet with 7% *M. oleifera* fortificant and 120 g finger millet with 17% *C. maxima* flour were mixed separately with 1000 ml of clean tap water. They were brought to boil and simmered for at least 10 min while stirring to ensure a smooth consistency for porridges. The porridges were cooled to 40°C, the recommended temperature for young children. The flow distance of the porridges was measurement by a Bostwick consistometer in triplicate (Mouquet et al., 2006).

Determination of antimicrobial properties

Antimicrobial properties of the porridges were determined over a 24 period to gauge the extent to which microorganisms could be resisted in formulated porridges. Agar diffusion pouring technique was used to monitor growth of pathogens on selective media by measuring the zones of inhibition against diarrhoea causing bacteria of *E. coli, S. shiga, S. aureus* and *S. typhi* (Doughari et al., 2007). These bacterial cultures were sourced from the Department of Public Health, Bacteriology and Toxicology, University of Nairobi, Kenya. Their turbidity was standardised to 0.39 optical densities before being inoculated in the media.

Determination of growth of bacteria by plate count technique

The growth of pathogens was monitored during the 24 h using selective media at 35°C. Baird- Parker was used to monitor growth of *S. aureus*, Violet Red Bile Agar for *E. coli*, Brilliant green agar for *S. typhi*, and MacConkey agar for *S. shiga*. The media were each prepared aseptically in McCarthey bottles and put in a water bath at 45°C. About 15-20 ml media was poured on the PCA plate after being mixed with 0.2 millilitres of each of the standardised diluted bacteria pathogens and allowed to set. Three points were marked and holes 1 cm in diameter made using a cork borer in each of the plates equidistant from each other. Fermented porridges of finger millet, millet with 7% *M. oleifera* leaf powder and millet with 17% *C. maxima* flesh flour were each prepared and immediately cooled to 40°C. About 0.2 ml of each of the prepared porridges were put in each hole. The plates were left for about 15 min to allow for setting before being incubated at 35°C for 24 h. The diameter of a colony-free halo around the porridges was taken as a measure of the degree of antibacterial activity of the porridges.

Data analysis

Version 20 SPSS Inc., Chicago, IL was used for data analysis. Chi-square tests were used for comparing percentage acceptance of the porridges by mothers. Analysis of variance was carried out and differences among means were compared by Duncan's test at $p<0.05$.

RESULTS

Nutrient composition of finger millet, *Cucurbita maxima* and *Moringa oleifera*

Table 2 shows the results for proximate and micronutrient composition of finger millet, *C. maxima* (flesh and seeds) and *M. oleifera* (leaves and seeds) flours. Finger millet flour had the highest carbohydrate content while *C. maxima* had the highest amounts of moisture. The seeds of both *M. oleifera* and *C. maxima* contained higher amounts of protein, energy and zinc compared to the leaves and flesh. There was more vitamin A and ash (minerals) in *C. maxima* flesh and *M. oleifera* leaf powders than in their seeds.

The pH and titratable acidity in the porridges

Table 3 shows the pH and titratable acidity in the porridges. The pH and titratable acidity were significantly higher in fermented *C. maxima* fortified millet porridge.

Organoleptic properties of the nutritionally optimised millet porridges

Percentage acceptance of nutritionally optimised finger millet porridges by mother panelist compared with traditional finger millet porridge is shown in Table 4. Majority of the mothers preferred the flavour of fermented porridges to non-fermented porridges. Mothers also showed higher preferance in taste, colour and acceptability of fermented *M. oleifera* and *C. maxima* fortified finger millet porridges than the non-fermented. Addition of 9% *M. oleifera* leaf powder and 21% *C. maxima* flesh flours in finger millet porridges resulted in

Table 2. Proximate and micronutrient composition of millet, pumpkin and moringa.

Variable	Pumpkin flesh	Pumpkin seeds	Millet flour	Moringa seeds	Moringa leaves
Proximate composition					
Moisture %	10.99±0.01	5.4±0.20	10.44±0.02	3.65±0.03	7.56±0.02
Protein %	13.73±0.03	23.12±0.06	9.42±0.02	23.26±0.10	21.03±0.07
Fat/oil %	1.09±0.09	26.51±0.01	1.44±0.04	26.00±0.20	6.45±0.05
Fibre %	7.08±0.1	32.68±0.02	4.51±0.08	24.95±0.02	10.57±0.10
Ash %	7.57±0.10	3.96±0.02	2.58±0.04	4.33±0.03	11.10±0.05
Carbohydrates %	70.53±0.10	23.34±0.30	82.07±0.02	23.44±0.22	51.16±0.04
Energy(kcal)	346.88±0.10	424.23±0.4	375.99±0.99	420.77±0.77	332.15±0.15
Micro nutrient					
Vitamin A (µg/100 g)	2705.88±0.08	126.85±0.8	0.02±0.03	268.13±0.13	6076.37±0.70
Iron (mg/100 g)	8.34±0.10	8.71±0.03	10.18±0.18	7.51±0.51	12.44±0.44
Zinc (mg/ 100 g)	2.19±0.19	8.54±0.54	1.33±0.03	4.65±0.65	2.50±0.10

Results are mean ± standard deviation, n = 3.

Table 3. The pH and titratable acidity (%) in fermented porridges.

Variable	Millet -porridge	Millet -pumpkin	Millet- moringa	P-value
pH	3.57±0.006	3.46±0.012	3.64±0.12	0.000
Titratable acidity	0.095±0.002	0.091±0.006	0.100±0.006	0.002

Results are mean ± standard deviation, n = 3.

Table 4. Acceptance of experimental porridges compared with traditional millet porridges in percentages.

Porridge	Colour			Taste			Flavour			Acceptability		
	C5	C6	C7	T5	T6	T7	F5	F6	F7	A5	A6	A7
F1	18.2	27.3	54.5	18.2	31.8	50.0	22.7	45.5	31.8	22.7	27.9	49.4
FF1	13.6	39.8	46.6	18.9	33.7	47.4	22.0	45.2	32.8	28.2	31.2	40.6
F3	31.8	45.5	22.7	23.6	42.8	33.6	28.0	49.0	23.0	27.7	36.4	35.9
FF3	13.6	50	36.4	21.0	41.8	37.2	24.6	43.0	32.4	27.3	35.9	36.8
F6	18.2	31.2	50.6	18.0	37.8	44.2	13.7	45.5	40.8	22.2	37.3	40.5
FF6	16.7	29.7	53.6	15.0	37.7	47.3	18.4	39.8	41.8	18.2	34.3	47.5

7= Like very much; 6=like moderately and 5=Like slightly. F1= Traditional millet flour, FF1=Fermented traditional millet flour, F3 = millet with 7% moringa leaf, FF3= Fermented millet with 7% moringa leaf; F6= millet with 17% curcurbita flesh powder and FF6= Fermented millet with 17% curcurbita flesh powder.

less desired porridges in colour and overall acceptability.

Viscosity of acceptable porridges

The viscosities of the experimental porridges and traditional millet porridge are shown in Table 5 below. Finger millet porridges fortified with *M. oleifera leaves* had the highest amounts of flour solids and yet with the highest flow distance. The flow distance of traditional millet porridge was very low and yet it had the lowest amount of solid matter. The amount of flour solids in fermented and non-fermented porridges did not differ much. There was some difference in amounts of flour solids in fermented *C. maxima* fortified finger millet porridge compared with the non-fermented.

Nutrient composition of organoleptically acceptable millet based porridges

Table 6 shows the nutrient composition of organoleptically accepted ready to use finger millet based porridges compared with the non-fermented finger millet

Table 5. Viscosity of porridges at 40°C.

Porridge sample	Amount of flour solids / 100 ml porridge (g)	Flow distance mm/30 sec
Ordinary millet porridge	10	117±1.0
Fermented millet porridge	11	117±2.0
Non fermented millet pumpkin porridge	11	118±2.0
Fermented millet pumpkin porridge	13	119±1.5
Non-fermented millet moringa porridge	15	121±2.0
Fermented millet moringa porridge	15	122±2.0

Table 6. Nutrient composition of organoleptically acceptable millet based porridges.

Nutrient	F1	FF1	F3	FF3	F6	FF6	Recommended value (g)*
Carbohydrates (g/100 g DM)	81.61±0.61[a]	81.06±0.06[a]	79.67±0.20[a]	79.61±0.3[a]	79.91±0.01[a]	79.85±0.02[a]	64±4
Protein (mg/100 g DM)	9.24±0.10[a]	9.34±0.10[a]	10.25±0.27[b]	10.37±0.30[b]	10.01±0.17[ab]	10.15±0.15[b]	≥ 15
Energy (kcal/100 g DM)	396.86±0.85[a]	401.39±0.34[b]	402.02±0.20[b]	406.10±0.10[c]	396.28±0.10[a]	396.61±0.10[a]	400-426
Micronutrients							
Vitamin A (µg/100 g)	0.001±0[a]	0.003±0[a]	333.3±0.30[d]	329.20±0.18[e]	312.8±0.08[b]	319.5±0.05[c]	
Iron (mg/100 g)	10.13±0.10[a]	10.33±0.10[a]	17.92±0.20[d]	17.99±0.4[c]	9.80±0.01[a]	9.91±0.10[a]	16
Zinc (mg/100 g)	2.15±0.10[b]	2.21±0.10[b]	3.20± 0.20[a]	3.37±0.10[a]	2.01±0.01[b]	2.32±0.10[b]	3.2

Recommended value reference: Codex alimentarius reference (Ijarotimi and Keshinro 2012),Values are the means of triplicate determinations expressed on dry weight basis. F1= Traditional millet flour, FF1=Fermented traditional millet flour, F3 = millet with 7% moringa leaf, FF3= Fermented millet with 7% moringa leaf; F6=millet with 17% curcurbita flesh powder and FF6= Fermented millet with 17% curcurbita flesh powder.

based porridges. *M. oleifera* fortified porridges were superior in energy, protein, zinc, vitamin A and iron content when compared with all porridges. *C. maxima* fortified finger millet porridges were better than traditional millet porridge in protein, vitamin A and zinc contents. Only moringa fortified porridges could meet the iron, zinc and energy recommendations of the CODEX Alimentarus Guidelines on formulated supplementary foods. There were some slight increases in energy, protein, vitamin A, iron and zinc contents after fermentation. However the carbohydrate content slightly reduced.

Expected nutrient intake from improved millet porridges basing on baseline data and WHO guidelines

Table 7 shows the number of servings, amount per serving, amount of solids in the serving, cost of the porridges per day and the % RNI expected to be met by the porridges. After adjusting on the viscosity and the expected number of porridge servings per day basing on WHO guidelines for infant feeding, traditional millet porridges showed the potential of catering for ≥ 60% energy requirements in children aged 7-24 months. However, the iron and zinc gaps still remained especially for children aged 7-11 months. Traditional millet porridges still have a big challenge of catering for vitamin A requirements. Finger millet porridges fortified with *M. oleifera* and *C. maxima* have the potential of meeting ≥ 60% RNI for energy, protein, vitamin A, iron and zinc in Bujenje County of Western Uganda. The porridges all costed less than $ 1 per day and there were no significant difference in their prices.

Antibacterial activity of the porridges

The diameters of inhibition halos against test pathogens of *E. coli*, *S. aureus, S. typhi and S. shiga* are shown in Table 8. All porridges offered some resistance against tested diarrhoea pathogens. Fermented *M. oleifera* fortified finger millet porridges offered the best inhibition against test pathogens of *E. coli, S. shiga* and *S. typi* while fermented *C. maxima* fortified finger millet porridge and fermented millet porridges mostly resisted *S. aureus.*

Diameter inhibition halos showed *S. aureus* as the most inhibited by all the porridges and *S. typhi* as the

Table 7. Nutrient intake from improved millet porridges compared basing on baseline data and WHO guidelines.

Variable	7-8 months	9-11 months	12-23 months
Serving			
Number of servings	4	3	3
Amount of serving	150 mls	200 mls	300 mls
Amount of solids			
Traditional millet	15 g	20 g	30 g
Cucurbita fortified	18 g	25 g	35 g
Moringa fortified	20 g	30 g	40 g
Cost per day			
Traditional millet	$ 0.02	$ 0.02	$ 0.04
Cucurbita fortified	$ 0.03	$ 0.03	$ 0.04
Moringa fortified	$ 0.03	$ 0.03	$ 0.05
Energy (RNI)	200 k cal	300 k cal	550 k cal
Traditional millet	238.1 kcal (119)	238.1 k cal (79)	357.1 k cal (64)
Cucurbita fortified	285.6 kcal (143)	297.5 k cal (99)	416.4 k cal (76)
Moringa fortified	324.9 kcal (162)	365.5 k cal (122)	487.3 k cal (89)
Protein (RNI)	9.1g	9.6g	10.9g
Traditional millet	5.5g (61)	5.5 g (58)	8.3 g (76)
Cucurbita fortified	7.2 g (80)	7.6 g (79)	10.7 (98)
Moringa fortified	8.3 g (91)	9.3 g (97)	12.4 g (114)
Vitamin A (RNI)	250 µg	300 µg	400 µg
Traditional millet	0.002 µg (0)	0.002 µg (0)	0.003 µg (0)
Cucurbita fortified	230.0 µg (92)	234.4 µg (78)	328.1 (82)
Moringa fortified	263.4 µg (105)	296.3 µg (98)	395.16 (99)
Iron*	11 mg	11 mg	11mg
Traditional millet	6.0 mg (55)	6.0 mg (55)	9.1 mg (83)
Cucurbita fortified	7.1 mg (65)	8.9 mg (96)	11.9 mg (108)
Moringa fortified	14.4 mg (131)	16.2 mg (147)	21.6 mg (196)
Zinc**	2.8 mg	2.8 mg	2.8 mg
Traditional millet	1.3 mg (46)	1.3 mg (46)	1.9 mg (69)
Cucurbita fortified	1.7 mg (60)	2.0 mg (62)	2.7 mg (99)
Moringa fortified	2.0 mg (96)	3.0 mg (108)	4.0 mg (144)

Iron* Assuming medium iron bioavailability (10%). Zinc** Assuming moderate bioavailability (30%) References: World Health Organisations Recommendations (Dewey and Brown 2002; Ruel, Loechl, and Pelto 2004).Numbers in brackets represent % RNI met. Curcurbita fortified = 17% curcurbita maxima; Moringa fortified =7% moringa oleifera

most resistant tested pathogen in all porridges.

DISCUSSION

Nutrient composition of the raw materials used in the formulation

Studies have reported carbohydrate content of 79.5%, iron content of 9.9 mg/100g flour and crude fibre content of 4.8% in finger millet which are very close to the present study findings (Bachar et al., 2013; Obilana, 2003; Paragya and Raghuvanshi, 2012). The protein content in this study is within the reported of 5.6-12.7 mg by Paragya and Raghuvanshi (2012) while the fat content is within 1.3-1.8 g reported by Bachar et al. (2013).

Present study findings on ash and zinc content of finger millet are also within 1.7- 4.13 mg and 0.92-2.55 mg per 100 g finger millet flour, respectively reported (Amadou et al., 2013; Bachar et al., 2013). Gopalan et al. (1999) have

Table 8. Diameters of inhibition halos (mm) for microorganisms in fermented porridges.

Variable	FF1	FF3	FF6
E. coli halo size	0.93±0.03	1.03±0.09	1.33±0.12
S. aureus halo size	1.00±0.06	1.33±0.09	1.27±0.19
S. typhi halo size	0.37±0.09	0.43±0.08	0.53±0.18
S. shiga halo size	0.53±0.18	0.53±0.18	1.100±0.15

Results are mean ± standard deviation, n = 3; FF1= Fermented millet porridge; FF3 = Fermented millet-moringa porridge (7.12 g moringa fortificant/100g flour); FF6 = Fermented millet-pumpkin porridge (16.61 g moringa fortificant/100g flour).

reported 45 µg carotene per 100 g of finger millet while nap.edu reported 6 retinol equivalent (Paragya and Raghuvanshi, 2012). Present study findings concur with Bhaskaracharya (2001) reports of finger millet being a very poor source of β-carotene with values ranging from 0 to 1 µg/100 g (Paragya and Raghuvanshi, 2012).

Results for *Moringa oleifera* leaves compare closely with findings by Ogbe and Affiku (2012) but contrast for zinc and energy content in moringa leaves. Ogbe and Affiku (2012) reported 6.0 mg of zinc and 1440 kcal energy against 2.5 mg zinc and 332.15 kcal energy observed in the present study. Thierry et al. (2013) also reported protein content of 31.62-35.59 g and iron content of 20.34-33.68 mg which are quite higher than the present study findings.

M. oleifera seeds have been reported to vary in protein content from 29.63-31.36g, fat from 30.36-40.39, ash from 6-8 and 9% carbohydrates (Compaore et al., 2011). However, the reported carbohydrate content is low compared to the present research findings of 23.44% while for protein, fat and ash contents, they are slightly higher. These differences in nutrient content may be attributed to differences in soils and stage of maturity of *M. oleifera* plants.

High moisture, carbohydrates and vitamin A levels and lower protein, fat/oil and zinc levels in the *C. maxima* flesh compared to the seed is comparable to findings by Mohammad (2004). Low moisture indicates good storage properties since growth of micro-organisms can be hindered. Findings in this study show lower levels of protein in *C. maxima* (23.12 g) compared to 36.2g and 39.3g reported by Mohammad (2004) and Fedha et al. (2010), respectively. Cucurbita flesh protein of 13.73 g was also different from the 4.0 g proteins reported by Fedha et al. (2010). However present findings concur with Usha and Lakshmi (2010) results for protein, fat and ash contents of 15.69g, 1.62g and 5.7g respectively. According to Compaore et al. (2011), pumpkin seeds contain 42% protein, 13.4% carbohydrate, 42.9-57.3% lipids and 4.33-7.25% ash (minerals). These values are higher than the present study findings. The differences in nutrient composition can be attributed to the variety of pumpkin, nature of soils and maturity stage (Fedha et al., 2010).

High amounts of protein, energy and zinc in *C. maxima* and *M. oleifera* seeds compared to the leaf and flesh justify their suitability as good raw materials for promotion of high nutrient density diets in infant food formulation (Compaore et al., 2011). Fibre prevents constipation but high amounts of fibre in the seeds could be a challenge in infants diet since it causes irritation of the gut mucosa (Elinge et al., 2012; Mohammad, 2004). Though moringa and pumpkin seeds have more protein, fat/oil and energy than the leaf and flesh powders, they were not suitable for fortifying finger millet porridges. This was because of their comparatively low levels of vitamin A when compared to the leaves of moringa and pumpkin flesh and the fact that finger millet had almost no vitamin A. *M. oleifera* leaves and *C. maxima* (pumpkin) flour have very high amounts of vitamin A which could be utilised in improving vitamin A content in millet porridges. This would promote vitamin A intake in children, build body immunity and perhaps reduce on infections in children that are common in Bujenje County (Ring and Develo, 2009). The high iron content in moringa leaves could help address the iron deficiencies that are common among preschool children in Western Uganda.

Effect of lactic fermentation, moringa and pumpkin fortificants on the organoleptic properties of the formulated porridges

The reduction in acceptability of 21% *Cucurbita maxima* fortified finger millet porridges can be attributed to the yellow colour imparted by the carotenoids pigment naturally present in *Cucurbita maxima* (Usha and Lakshmi 2010). The dark green colour of the *M. oleifera* leaves was also responsible for the reduced acceptability in finger millet porridges with 9% *M. oleifera* leaves powder. Pumpkin (Cucurbita) fortified millet porridges were more acceptable than moringa fortified because of being familiar foods. Higher preference for fermented products in terms of flavour can be attributed to the production of lactic acid, alcohols and carboxylic acids in fermented products that promote production of a variety of flavour of the existing food (Blandino et al., 2003; Lei, 2006). The oven temperatures slightly altered the colour in the process of drying. This could explain why majority of mothers preferred the colour of fermented *M. oleifera* and *C. maxima* fortified porridges to the non-fermented versions.

Effect of lactic fermentation, moringa and pumpkin flours on the nutrient content of the formulated porridges

M. oleifera leaf powder and *C. maxima* flesh powder were able to improve the nutrient content of finger millet porridges. Through optimisation of nutrients and costs, the nutrient content of the porridges was improved and the prices kept to the minimum. *M. oleifera* and *C. maxima* flesh flours had a negative impact on gelatisation of porridges compared to millet flours. This was also observed by Usha and Lakshmi (2010) when formulating weaning mix and was attributed to high pectin levels in Cucurbita. This property of a weaning mix is ideal because diets that form gel at higher concentrations allow dilution in attempts to increase the digestibility of the weaning mix without losing the density of nutrients as compared to weaning mixes with least concentration (Usha and Lakshmi, 2010). Such a property is especially vital for developing countries where low calorie and protein intake in children have been attributed to dilution of complementary foods (Hossain et al., 2005). Finger millet porridges fortified with *M. oleifera* powders resulted in more nutritious porridges within the recommended viscosity for children (Mouquet et al., 2006). Complementary porridge consistency has been reported to have considerable variation depending on character, size, proportion of suspended particles (Bruyeron et al., 2010). There was some decrease in carbohydrate content after fermentation since lactic acid bacteria use sugars during fermentation (Thierry et al., 2013). This decrease in carbon ration causes the nitrogen in fermented slurry to be concentrated and must have affected the total mass resulting in slight increases in energy, protein, vitamin A, iron and zinc in the fermented porridges (Onyango et al., 2005; Thierry et al., 2013).

Moringa fortified porridges had the best nutrient composition and were able to meet the energy, iron and zinc recommendations of the CODEX requirements. Scholars have shown the potential of moringa in improving child nutrition (Odinakachukwu et al., 2014; Thierry et al., 2013). Moringa has also demonstrated the potential of improving vitamin A in serum depleted rats (Thurber and Fahey, 2009). *C. maxima* fortified millet porridges had better protein, vitamin A and zinc compared to traditional millet porridges. Consumption of pumpkins (Cucurbita) has been promoted as a means of promoting vitamin A intake among resource constrained populations (Dhiman et al., 2009). *C. maxima* has also demonstrated the potential of providing economic, nutritious and organoleptically acceptable weaning mix (Compaore et al., 2011; Usha and Lakshmi, 2010). Adequate processing and judicious blending of the locally available foods has been encouraged as a measure of improving intake of nutrients among children in areas with limited resources (Lombor et al., 2009).

Antimicrobial properties of formulated millet porridges

All lactic fermented porridges had some inhibition properties against bacterial pathogens. Lactic acid bacteria are reported to produce antimicrobial substances which inhibit certain diarrhoea causing microorganisms such as E. *coli*, salmonella t*yphi* and shigella (Gabriel-Ajobiewe et al., 2014; Guslandi, 2005). Efficient lactic acid fermentation is reported to produce a pH of 4 or less at which growth of pathogens is inhibited (Lei, 2006). This explains the better inhibition against S. *aureus* observed in pumpkin (Cucurbita) fortified finger millet porridges with high titratable acidity. Other factors responsible for safety of fermented foods include production of bacteriocins, hydrogen peroxide, carbon dioxide, ethanol and antibiotic like substances (Gabriel-Ajobiewe et al., 2014; Lei, 2006). Presence of *M. oleifera* leaf powder in the finger millet porridge must have contributed to the larger halo size against E. *coli*. Moringa also had better inhibitory characteristics against *Shigella shiga* and salmonella *typhi*. This is because of the complex chemical compounds such as kaempferol and rutin contained in *M. oleifera* leaves that have antioxidant and antibiotic properties (Patel et al., 2011; Fuglie, 2005). Moringa leaf extract is also reported to have exhibited broad spectrum activity against test organisms of E. *coli* and S. *typhi* (Bukar et al., 2010). Bacteria inhibition in curcubita fortified porridges could also be attributed to photochemicals found in pumpkin (Dhiman et al., 2009). Inhibition of growth of bacteria in these porridges was therefore due to combined effects of lactic acid fermentation and presence of antioxidants in moringa and pumpkin powders (Dhiman et al., 2009; Patel et al., 2011). These antimicrobial properties of the formulated porridges justify them for use in communities like Bujenje County where high infection rate has been reported (Ring and Develo, 2009). Perhaps this would reduce on the high levels of diarrhoea infections that are common during the weaning period not only in Uganda but also in developing countries.

Nutritional potential of improved millet based porridges

Foods for infants should be energy dense because of the limited gastric capacity in young children coupled with the need for increased nutrient intake. The present recipe for traditional millet porridges if intergrated in the feeding practices of this local community and combined with nutritional education could address children's energy needs. However the iron and zinc gaps would still remain. These are very essential nutrients in young children whose effects cannot be neglected especially at this critical stage of growth and development. These results confirm the superiority of finger millet to other cereals in catering for energey needs of children (Obilana, 2003; Paragya and Raghuvanshi, 2012). They also reflect the need for enriching the content of vitamin A, iron and zinc in millet porridges to avoid long term effects of these micronutrients.

Experimental porridges have the potential of meeting the target point of ≥60% RNI for children. The porridges were designed to be intergrated in the feeding practices of children aged 7-24 months and therefore could reduce malnutrition in Western Uganda. The formulated porridges are thus better complementary foods compared to traditional millet porridges. This is qualified not only by their increased nutrient content but also the antimicrobial properties they possess. Increased digestibility of starch and protein and bioavailability of iron and zinc are some of the benefits of lactic fermented millet porridges that have been reported by various scholars (Onyango et al., 2005; Lei, 2006). Lactic acid fermentation has also been reported to increase iron availability, gastric digestibility of protein and to reduce on the level of phytates in moringa powder (Thierry et al., 2013).

Conclusion

Formulation and fortification of millet porridges using locally available food resources and technologies can provide nutritious, cost effective and safe foods to prevent malnutrition. This is believed to be a practical food based approach aimed at combating malnutrition among infants and children in Western Uganda and other developing countries. However certain aspects like digestibility of macronutrients and bio-availability of vitamins and minerals in these formulated porridges need further investigation. The porridges can therefore be evaluated for their effectiveness among malnourished children in Bujenje County, Western Uganda.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Toxic trace elements in different brands of milk infant formulae in Nairobi market, Kenya

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World Health Organization (WHO) among other health agencies recommends exclusive breast feeding during the first six months of infancy. However, Infant formula milk (IFM) has been increasingly used as a breast milk substitute due to maternal occupation, death and illness. The product has been associated with infant health complications and even deaths due to its contamination with aluminum (Al), cadmium (Cd), lead (Pb) and nickel (Ni). Both the Kenya Bureau of Standards (KEBS) and WHO have set maximum levels of concentration of these metals in IFM and the latter has set levels of provisional tolerable weekly intake (PTWI). Seven brands of IFM (for age 0-6 months) imported into the Kenyan market and shelved in stores in Nairobi County, were analysed for levels of Cd, Al, Pb and Ni. Except for Cd, all brands of IFM (0-6 months) contained the rest of the trace elements ranging as follows: Al (1.054±0.085 to 2.156±0.423 µg/g); Pb (0.018±0.001 to 0.059±0.002 µg/g) and Ni (0.022±0.001 to 0.032±0.001 µg/g). The estimated weekly intakes were below the PTWI thus indicating safety of the brands of IFM. Other than affirm presence of the elements Al, Pb and Ni in IFM though below the WHO set limits, the findings are a pointer to caution on consumption of IFM although this would be inevitable under unavoidable circumstances. The caution is justified since the consumption of IFM would pose health risk to infants due to exposure and bioaccumulation of the elements. Lead particularly was higher than the limits set by KEBS in most brands supporting this caution.

Key words: Infant formula milk, 0-6 moth infants, heavy metals, WHO, Kenya market.

INTRODUCTION

The World Health Organization (WHO), international and national health agencies recommend exclusive breastfeeding for the first six months of infancy (CDC, 2006; WHO, 2015). In Kenya, about 32% of lactating mothers exclusively breastfeed their babies during the first six months of infancy (Kenya National Bureau of Statistics, 2010). Under unavoidable circumstances, safe and nutritious alternatives such as infant formula milk (IFM), dairy milk and sweetened liquids should be employed. Circumstances that divert breastfeeding include, but are not limited to death of the mother, risk of mother to child transmission of diseases, absence of the mother from the infant for an extended period, personal preferences and beliefs and societal pressure (Lawrence, 2004; WHO, 2004; Mamiro et al., 2005; CDC, 2006). Infant formula milk is the only product which is

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Table 1. The AAS operating conditions.

Operating parameters	Al	Cd	Pb	Ni
Wavelength (nm)	309.3	228.8	217	232.0
Slit width (nm)	0.5		1.0	0.2
Flame type	N_2O-acetylene	Air-acetylene		N_2O-acetylene
Oxidant flow rate (l/min)	4.5	1.5		
Sensitivity (ppm)	8.4	0.011	0.055	0.066
Detection limit (ppm)	0.02	0.005		0.008
Lamp current (mA)	6	5	8	5
Optimum working range (ppm)	1.0-50.0	0.5-2.0	2.0-8.0	3-12

considered nutritionally acceptable for infants under the age of one year (WHA, 2001; Gian et al., 2009). According to Natural Resources Defence Council (NRDC), the infant formula industry is an $8 billion per year business (NRDC, 2005). Across the globe, huge advertising budgets are spent to convince women that it is better and more convenient to bottle-feed their babies. Infant formula milk (IFM) is categorized by age brackets: 0-6 months, 7-12 months and beyond 1 year. In Kenya, more than seven brands of IFM are imported into the market. Kenya is a signatory to all global conventions with a commitment to promote, protect and support infant and young children feeding practices (KEBS, 2014; Komen, 2009).

Metal pollution as a result of increasing industrialization has penetrated into all sectors of the food industry and as such pose fears for IFM (Gian et al., 2009). Labelling on the package, however, does not indicate the minimal levels of the elements present. As a matter of fact, elements and ions may find route in foods as a result of processing, packaging, farming activities and industrial emission (Khalifa and Ahmad, 2010; Ljung et al., 2011). Food and Agricultural Organization (FAO) of WHO has set provisional tolerable weekly intake limits for metal ions by infants but their poisoning effect even at low levels of exposure cannot be overemphasized and especially that they can bio-accumulate in vital body organs to persist in adulthood (Nevin, 2000; Needleman et al., 2002; Lanphear et al., 2008; Ljung et al., 2011; Salah, 2012). The Kenya Bureau of Standards (KEBs) in Kenya has set standards for the levels of elements in not only IFM but all food products among other commodities (KEBS, 2014). Studies have reported contamination of IFM by various substances such as nitrates, nitrites, aluminium (Al), cadmium (Cd), mercury (Hg), nickel (Ni), lead (Pb) and melamine (Gian et al., 2009; Khalifa and Ahmad, 2010; Burrell and Exley, 2010 and Ljung et al., 2011). In 2008, melamine contamination of IFM in China led to deaths and illness of several infants (Nakashima et al., 2009). China reported an estimated 300,000 victims with approximately 54,000 babies being hospitalized and six infants dying from kidney stones and other kidney damage (Branigan, 2008). A study to determine the concentration of selected trace and toxic elements in breast milk and IFM reported the concentrations to be tenfold higher than in breast milk; thus confirming IFM as an exposure route of toxic elements to infants (NDRC, 2005).

The aims of the study were to quantify the content of aluminium, lead, cadmium and nickel found in individual cow's milk-based infant formula and to evaluate the weekly intake in comparison with the Provisional Tolerable Weekly Intake established by Joint FAO/WHO Expert Committee on Food Additives.

MATERIALS AND METHODS

Materials

Seven brands of IFM powders for the age bracket 0-6 months were purposively sampled from stores in Nairobi, Kenya and coded T, U, V, W, X, Y and Z.

Chemicals and reagents

All chemicals and reagents were of analytical grade. Concentrated nitric acid, hydrogen peroxide and aluminum sulphate, were sourced from Thomas Backers Chemicals Ltd Mumbai India. Standards for nickel, cadmium and lead were purchased from Fluka Chemie GmbH Aldrich chemical company, INC, USA.

Laboratory procedures

Digestion of the powders followed the procedure by Picciano (2001). Briefly, 2.5 g of powder was accurately weighed into a Kjeldahl flask, 15 ml of concentrated nitric acid and 5 ml of 10% hydrogen peroxide were added and the resulting solution heated until there were no more brown fumes. The resulting mixture was filtered through Whatman paper No.1 into 50 ml volumetric flask and its volume topped up with deionized water to the mark. The measurements for Al, Cd, Pb and Ni were done in triplicates using computerized Varian Atomic absorption Spectrometer (Model: AA-10, Varian, USA). The instrumental parameters are presented in Table 1.

Method validation procedures

Freshly prepared standard stock solutions were serially diluted and

Table 2. Concentration range of standards for calibration curves.

Instrument	Element	Concentration range (ppm)	Correlation coefficient (r^2)
AAS	Aluminium	0.5-15.0	0.9998
	Cadmium	0.5-2.0	0.9998
	Lead	0.1-5.0	0.9993
	Nickel	0.5-4.0	0.9988

Table 3. Mean concentrations of selected trace metals in infant formula milk brands in Nairobi market, Kenya.

Brand code	Concentrations (µg/g) ($\overline{X}$ ±SE); n=9*			
	Al	Cd	Pb	Ni
T	1.054±0.085^{a}	ND	0.018±0.002^{a}	0.032±0.001^{b}
U	2.069±0.447^{b}	ND	0.022±0.001^{a}	0.029±0.002^{b}
V	1.545±0.089^{ab}	ND	0.018±0.001^{a}	0.032±0.002^{b}
W	1.099±0.068^{a}	ND	0.059±0.002^{c}	0.031±0.003^{b}
X	2.156±0.423^{b}	ND	0.032±0.007^{b}	0.022±0.001^{a}
Y	1.543±0.245^{ab}	ND	0.022±0.002^{a}	0.030±0.003^{b}
Z	1.405±0.031^{ab}	ND	0.040±0.002^{b}	0.028±0.001^{b}

*Values are means ± standard deviation of nine replicate analyses. Means within a column (for each variable) marked with different letters are significantly different at (p< 0.01); ND- Not detected; (One way ANOVA, α =0.05 SNK test).

used to obtain calibration curves with linearity values as presented in Table 2. The correlation coefficient values were a good indicator of the linearity for AAS instrument for precision and accuracy of results (Duan et al., 2003). Further, using recovery tests where samples were repeatedly spiked with known amounts of standards prior to measurements were performed to confirm the accuracy of the instruments (Duan et al., 2003). The percentage recoveries obtained were good, falling between 98.1-99.9%.

Statistical analysis

Data were analyzed with SPSS 17.0 for windows. The mean and standard deviation of means were calculated. The Estimated Weekly Intake (EWI) per body weight of the infant for the metal ions in each brand of formula milk were then calculated based on the feeding table provided in each brand of formula milk (0-6 months). The data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range tests was used to separate means (P < 0.05).

RESULTS AND DISCUSSION

The levels of Al, Cd, Pb and Ni in seven brands of IFM for infants aged 0-6 months sold in Nairobi County, Kenya are presented in Table 3 and the EWI are presented in Table 4.

Except for Cd, all brands of IFM (0-6 months) contained Al, Pb and Ni. These ranged as follows: Al (1.054±0.085 µg/g in brand T to 2.156±0.423 µg/g in brand X); Pb (0.018±0.001 µg/g in brand V to 0.059±0.002 µg/g in brand W), Ni (0.022±0.001 µg/g in brand X to 0.032±0.001 µg/g in brand T). Except for brands T and V, all other brands had levels of Pb above the KEBS maximum limit of 0.02 ppm. Infants are at a critical point of their brain development and exposure to elements pose severe health risks since the effect is compounded by the fact that even at low levels of exposure, metals bio-accumulate in vital organs and persists in adulthood (Nevin, 2000; Needleman et al., 2002; Lanphear et al., 2008). The findings therefore raise a health concern to

Table 4. Estimated weekly intake of trace elements by infants compared with PTWI values.

Brand code	Estimated weekly intake (µg/kg)		
	Al	Pb	Ni
T	56.02	2.90	1.72
U	109.98	1.15	1.74
V	82.11	0.98	1.70
W	39.95	2.15	1.11
X	322.07	4.84	3.26
Y	60.58	1.89	2.01
Z	59.32	2.52	2.61
PTWI value*	**1000**	**0.5**	**35**

*(µg/kg of infant body weight).

infants who consume the brands. A further concern is the labelling of the packages. In this study, Pb was identified in the samples, but not reported on the labels of the containers. These study findings indicate an increased health risk with the consumption of IFM resulting in not only the exposure, but the bioaccumulation of the elements Al, Pb and Ni.

There were statistically significant differences of the elements between brands (p<0.001). This would be expected and these differences can be attributed to the different manufacturing practices, quality of raw materials, and packaging containers used (Khalifa and Ahmad, 2010). Comparing the metal ion levels and the maximum possible weekly intake (Table 4), the EWI of Al and Pb were below the Provisional Tolerable Weekly Intake (PTWI) set by the Joint Committee on Food Additives of the FAO and the EU Scientific Committee for infant feeding, an indication that the IFM are safe for consumption. These findings may generate a debate on whether the campaigns on consumption of IFM should be encouraged although it is understandable that under some unavoidable circumstances exclusive breast feeding is an impossible undertaking. The labels on the IFM packages should include even the minimal safe amounts of all elements as this not only informs the consumers, but have implications on their choice in promoting their consumption.

Conclusion

In this study, we affirm the presence of trace elements (Al, Cd, Pb and Ni) in IFM brands in the Kenyan market. Although below the WHO set limits, the findings are an indication of caution regarding consumption of IFM unless under unavoidable circumstances as the risks of their bioaccumulation are inevitable.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Assessment of essential trace elements in selected food grains, herbal spices and seeds commonly used in Kenya

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Trace elements are essential in preserving good health and body immunity to diseases. The study was undertaken to determine the levels of chromium (Cr), vanadium (V), selenium (Se) and zinc (Zn) in herbal spices, food grains and edible seeds commonly used in Kenya. The levels of elements were determined using atomic absorption spectroscopy (AAS). The herbal spices, food grains and seeds considered in this study included coriander (*Coriandrum sativum*), ginger (Z*ingiber officinalis*), garlic (*Allium sativum*), cloves (*Syzygium aromaticum*), lemon grass (*cymbogon citratus*) rosemary (*Rosmarinus officinalis*), wheat (*Triticum cestivum L.),* brown rice (*Oryza sativa*), finger millet (*Elusine coracana*), bulrush millet ((*Pennisetem glaucum*), sorghum (*Sorghum bicolor*), sunflower seeds (*Helianthus anuus)*, watermelon seeds *(Citrullus lanatus)* and pumpkin *(Cucurbita maxima*) seeds. The results indicated that lemon grass had the highest V levels (14.40±1.20 mg/kg) followed by ginger at (14.38±0.31 mg/kg) while coriander seeds had the highest Cr levels (13.00±0.42 mg/kg), followed by lemon grass (12.80±1.47 mg/kg). Bulrush millet had the highest Se levels (198.38±3.75 µg/k) followed by sorghum (151.20±12.8 µg/kg). Pumpkin seeds had the highest level of Zn (53.54±1.44 mg/kg) followed by watermelon seeds at 41.00±5.79 mg/kg. The food grains, seeds and herbal spices could provide the body with the required daily intake. Consumption of mixed diet could therefore provide the body with essential trace elements that could boost the body immunity especially to those people with compromised health.

Key words: Trace elements, nutrition, herbal spices, food grains, fruit seeds, AAS.

INTRODUCTION

Good nutrition is essential for achieving and preserving health while helping the body to protect itself from infections (WHO and FAO, 2002). Vitamins and minerals are essential for good health for the body against opportunistic infections by ensuring that the lining of the skin, lungs and gut remain healthy and that the immune system functions properly (WHO and FAO, 2002). Trace elements such as selenium, zinc, chromium and vanadium play a major role in improving the body immunity against diseases (Al Durtsch, 1999; American

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Cancer Society, 2004). Available and reliable sources of these minerals in terms of absorption are plant sources. Plants absorb much of the essential elements from the soil in which they grow. Herbal spices and food grains contain essential trace elements in amounts that are helpful to the body. In spite of advances in diagnosis and treatment, cancer continues to be a major health burden (American Cancer Society, 2004). With the fear associated with diagnosis of cancer, it is not surprising that the public may have considerable interest in easily implementable measures such as use of vitamins and trace element supplements for cancer prevention. Selenium is effective in cancer reduction because it prevents development and progression by causing cancer cells to die before they can have a chance to grow and spread in a process called apoptosis (American Cancer Society, 2004). Selenium is also said to preserve elasticity in body tissue, slow aging process, improve the flow of oxygen to the heart, prevent abnormal blood clotting and stimulate the formation of antibodies in response to vaccines (American Cancer Society, 2004). Selenium is an excellent anti-oxidant. Various studies have shown that people with epilepsy have significant low levels of selenium and supplementation with selenium results in fewer seizures (Mahyar, et al., 2010). Zinc in the diet can greatly reduce cases of malaria, diarrhoea, pneumonia and other infections (Fox, 1998). These diseases are the major killers of children all over the world. Zinc is essential for human body growth and development of normal brain function. It is a component of many enzymes and thus involved in numerous metabolic processes (Rink and Gabriel, 2000). It is an important co-factor of important enzymes such as DNA, RNA and protein synthesis and so on, is important for cell division (Hambidge et al, 1986). It plays an important role in the protection of cell membrane integrity and may be protective against free radical injury (Prasad, 1983).

Replacing complex carbohydrates with refined simple carbohydrates (white flour and sugar) will eventually wear out chromium supply and possibly cause diabetes (Al Durtsh, 1999). Chromium is found in tiny amounts in the body and has the main function of glucose metabolism. Insulin, a hormone that controls blood sugar cannot function without glucose tolerance factor (GTF) and the molecule contains chromium. Chromium is also required by pregnant mothers to build the babies stock to last them up to 10 years of age when they start ingesting Cr (Al Durtsh, 1999). Vanadium is essential in humans, as it helps to promote healthy glucose levels in people with lack of insulin sensitivity. It also plays a role in building healthy bones and teeth as well as promoting healthy cellular replication in the body (Cohen et al., 1995).

There is great interest in healthy benefits of whole grain foods like brown rice, millets sorghum, whole wheat and spices in countries around the world. Hence, this study was done to: (1) analyse some essential trace elements in selected food grains and spices, and (2) compare with recommended daily in intake (RDI).

MATERIALS AND METHODS

Sampling

The herbal spices, grains and edible seeds were obtained from supermarkets, open air markets and agrochemical shops in the Central Business District (CBD), Nairobi city, Kenya. The pumpkin and watermelon seeds were freshly scooped from the fruits, washed and dried. All the samples were dried in the oven at 80°C to a constant weight and ground using a laboratory mini miller (Glen Crescent Company, England) and homogenized to make a representative sample. The samples were finally put in clean polythene bags, labeled and stored safely awaiting analysis in the laboratory. For zinc, analysis was done at Government Chemist laboratory using AAS model AA-668 (Shimadzu, Japan) while analysis of vanadium, selenium and chromium was done at Mines and Geology laboratory using AAS VGA-77 Model (Varian, Australia).

Chemicals and reagents

All chemicals and reagents used in this research were of analytical grade. Nitric acid, sulphuric acid and perchloric acid were sourced from Thomas Baker Chemicals Ltd. Mumbai India whereas hydrochloric acid and sodium hydroxide were from Sd. Line Chem. Lab. Mumbai India. Sodium hydroxide was from Sigma Aldrich Chemi Kallen GmbH, United States. The standards (Zn, Se, Cr and V) were prepared by serial dilution of commercially pre-prepared stock solutions purchased from Fluka Chemie GmbH Aldrich Chemical Company, Inc. USA. The stock solutions were in 1% nitric acid with concentration of 1000 µg/ml.

Cleaning of glassware and plastic containers

All glassware were cleaned with detergent and hot water, rinsed several times with tap water and then soaked for several hours in 10% nitric acid solution. Finally they were rinsed thoroughly with distilled deionized water, dried in the oven at 105°C and then stored in clean and dry drawers. The plastic bottles were cleaned with detergent and tap water, soaked with 1:1 nitric acid overnight and rinsed with distilled deionized water and then dried on open racks.

Sample preparation for AAS

A 2.0 g sample was weighed and placed in a 250 ml Kjeldhal flask and digested with 27 ml mixture of HNO_3, H_2SO_4 and $HClO_4$ in the ratio of 6:3:1 (Horwitz, 2001; Khan et al., 2011). The digest was cooled and filtered using Whatman No. 42 filter paper into 100 ml volumetric flask and then diluted to the mark with distilled deionized water. The sample was then transferred into a plastic bottle. A blank was prepared in the same way as the samples. Standards containing known concentrations of the elements were prepared from pre-prepared stock solutions by serial dilutions. Using values for standards, a calibration curve was obtained and used to quantify the trace element levels in the digested samples and blank. The samples were analysed in triplicates. Samples, standards and blank were analysed against calibration curve for each element (Figure 1) under same conditions using AAS (Horwitz, 2001; Khan et al., 2011). The operating conditions of the AAS (Shimadzu model AA-668) are given in Table 1. Selenium was analysed using a hydride generator (Varian Model VGA-77) connected to AAS (Model Spectra AA-10, Varian, Australia).

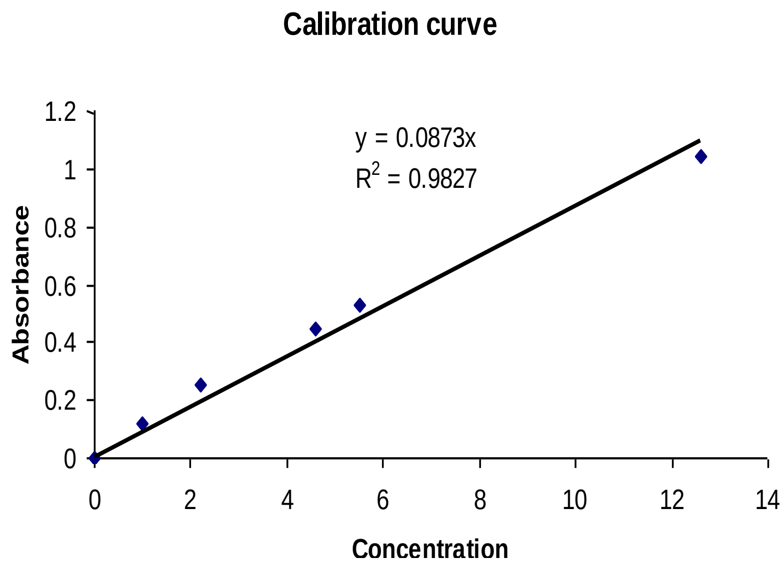

Figure 1. Zn Calibration curve

Table 1. Atomic absorption spectroscopy (AAS) operating conditions.

Parameter	Cr	Zn	Se	V
Wavelength (nm)	357.9	213.9	197	318.5
Slit width (nm)	0.2	1.0	1.0	0.2
Flame	Air/Acetylene	Air/Acetylene	Air/Acetylene	No_2/Acetylene
Oxidant flow rate (Litres/min)	1.5	1.5	1.5	4.5
Detection limit	0.02 mg/kg	0.01 mg/kg	0.03 µg/kg	0.02 mg/kg
Sensitivity	0.015	0.01	0.02	0.4

Calibration curves

Calibration curves (Figures 1-4) from the standards (Zn, Se, Cr and V) were used to determine the concentrations of the trace elements in the samples. The Zn, V and Cr, concentration was indicated in mg/kg while that of Se was given in µg/kg. To eliminate some of the errors associated with a single measurement, the working curve method was used. A working curve was prepared by plotting absorbance of solutions of standards of the trace elements as a function of their concentration. The standard solutions that were used to prepare the working curve had a broad range of concentrations to encompass the measured absorbance of all sample solutions.

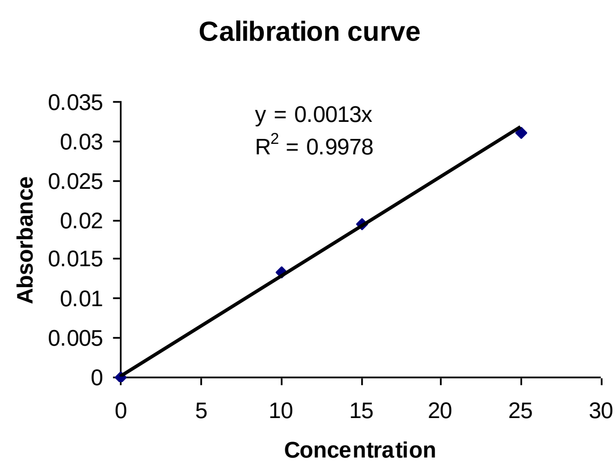

Figure 2. Se calibration curve

RESULTS AND DISCUSSION

The results of all food items analyzed for Zn, Se, Cr and V are shown in Table 2. The results indicate that the samples considered in this study have the trace elements V, Se, Cr and Zn though the amounts varied from sample to sample.

Zinc levels varied from 10.9±0.39 in Rosemary to 53.54±1.44 in pumpkin seeds. Zinc levels were found to be the highest in pumpkin seeds (53.54±1.44 mg/kg), followed by watermelon and sunflower seeds respectively. There is significant difference ($p<0.05$) in the Zn levels in pumpkin and sunflower seeds but no

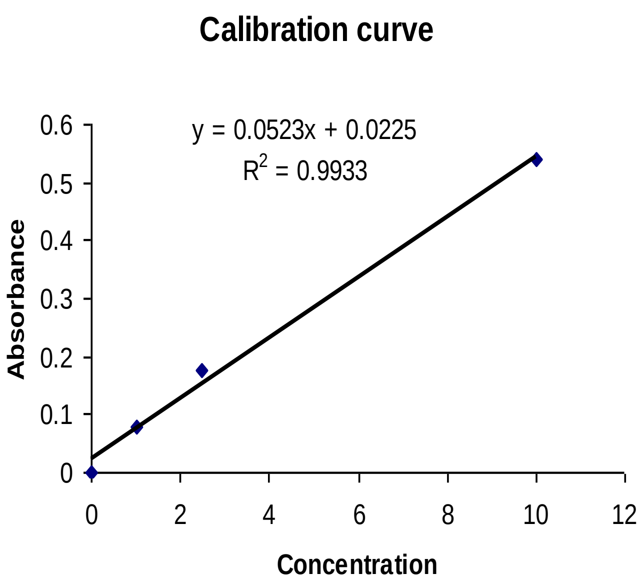

Figure 3. Cr calibration curve.

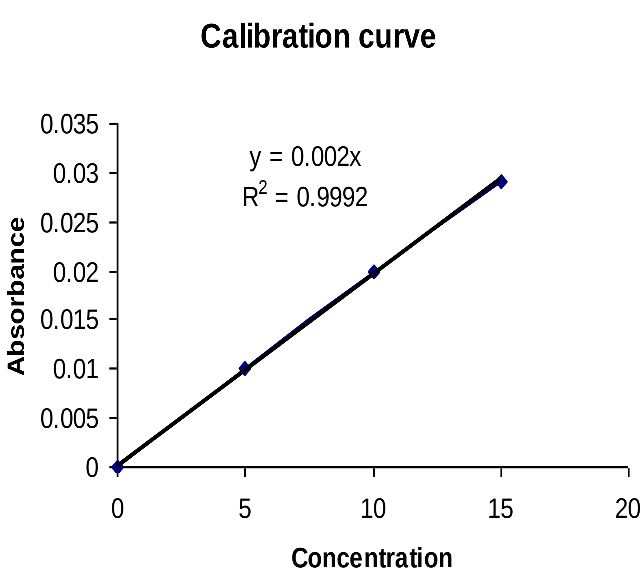

Figure 4. V calibration curve.

significant difference in Zn levels found in pumpkin and watermelon seeds. Among the grains, bulrush millet had the highest level of Zn (28.78 ± 0.65) while sorghum had the lowest level. Among the spices, coriander seeds had the highest level of Zn at 34.5 mg/kg. Zinc is the component of more than 270 enzymes and its deficiency in the organism is accompanied by multisystem dysfunction (Zinpro, 2000). It is responsible for sperm manufacture, fetus development and proper function of the immune response (Serfor et al., 2002). Zinc plays a role in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids (Hambidge et al., 1986). Severe Zn deficiency effects include impaired reproduction, immune disorders, dermatitis and impaired wound healing, night blindness, poor appetite, liver cirrhosis, enhanced sensitivity to drugs, most of which are treatable with adequate amounts of Zn (Walsh et al., 1994). This indicates that a mixed diet of the fruit seeds, food grains and spices are a good source of zinc and their consumption could be encouraged to individuals with low levels of zinc. The RDI levels of zinc are 2.5-9.4 mg/kg (Giridhar et al., 2013).

Selenium was highest in bulrush millet (198.38 µg/kg) followed by watermelon seeds, ginger, garlic and sorghum respectively. The lowest level was in brown rice with 27.73 µg/kg. There is no significant difference ($p>0.05$) in the levels of selenium in bulrush millet and watermelon seeds. However, bulrush millet is commonly consumed by a larger population of Kenyans as part of diet but unfortunately, the watermelon seeds are thrown away as people consume the fresh juicy part. The spices are consumed in small amounts but they are believed to have a therapeutic effect. Selenium improves sperm motility and hence human reproduction (Hawkes and Turek, 2001). It is also an essential co-factor of antioxidant enzyme such as glutathione peroxidase (Zhang et al., 1999) and its deficiency has been associated with cardiovascular diseases, diabetes, arthritis (Stranges et al., 2006; Coppinger and Diamond, 2001). It has also been shown to have protective role against cancers such as prostrate (Bjelakovic et al., 2004; Yu et al., 1997) and to improve immune function (Broome et al., 2004). Deficiency of selenium causes hair loss, skin and fingernail discoloration, low immunity and fatigue. Increasing selenium level intake improves immune function when body stores are not at an optimal level (Broome et al., 2004). The bulrush millet, sorghum, watermelon seeds and spices such as garlic and ginger have selenium levels, which could provide the recommended daily intake. The required daily intake of Se is 55 µg for health adults with 40 µg being the minimum requirement while a daily dose of 100-200 µg inhibit genetic damage and cancer development in humans because a methylated form of Se is necessary for cancer reduction (Whanger, 2003).

Vanadium was found to be highest in lemon grass (14.4 mg/kg) followed by ginger 14.38 mg/kg, cloves 13.60 mg/kg and coriander seeds (12.4 mg/kg) respectively. There was no significant difference between levels of vanadium in lemon grass and ginger at P= 0.05. Vanadium levels were found to be higher among the spices studied (11.8-14.4 mg/kg) and therefore recommended for use in food preparation. Vanadium is used for treating diabetes, high cholesterol, heart disease, tuberculosis, syphilis, anemia, water retention (edema) and for improving athletic performance in weight training; and for preventing cancer (Cusi et al., 2001; Guo et al., 2010; Samantha, 2008). It is believed to prevent

Table 2. Amount of trace elements present in the food grains, herbal spices and seeds

Food item	Zn (mg/kg)	Se (µg/kg)	V (mg/kg)	Cr (mg/kg)
Brown rice	19.2 ± 1.16	27.73 ± 1.68	5.76 ± 0.51	2.24 ± 0.28
Wheat	27.29 ± 2.61	140.69 ± 5.7	11.48 ± 1.32	5.69 ± 0.34
Finger millet	19.0 ± 0.82	91.38 ± 5.68	9.58 ± 0.56	2.32 ± 0.54
Bulrush millet	28.78 ± 0.65	198.38 ± 3.75	6.28 ± 0.6	2.70 ± 0.38
Sorghum	17.89 ± 2.33	151.2 ± 12.8	7.23 ± 1.18	5.86 ± 0.27
Garlic	18.9 ± 0.77	151.9 ± 11.90	8.1 ± 0.58	1.76 ± 0.25
Ginger	20.5 ± 2.37	153.9 ± 0.90	14.38 ± 0.31	10 ± 1.01
Rosemary	10.9 ± 0.39	96.2 ± 3.9	11.80 ± 1.50	4.25 ± 0.75
Cloves	27.07 ± 4.9	30.1 ± 6.30	13.60 ± 1.20	4.6 ± 0.80
Lemon grass	31.30 ± 0.81	110.7 ± 15.20	14.4 ± 1.2	12.80 ± 1.47
Coriander seeds	34.50 ± 0.71	82.7 ± 16.3	12.4 ± 2.5	13.0 ± 0.42
Pumpkin seeds	53.54 ± 1.44	16.54 ± 1.02	1.54 ± 0.17	5.26 ± 0.57
Watermelon seeds	41.00 ± 5.79	182.70 ±13.2	8.50 ±1.15	6.20 ± 1.11
Sunflower seeds	36.8 ± 0.74	36.50 ± 3.26	10.53 ± 1.31	5.0 ± 1.29

Table 3. Comparison of t-critical for Zn, Se, Cr and V.

Food item	Mean levels of Zn (mg/kg) (n=10)	Zn t-value at P=0.05	Mean levels of Se (µg /kg) (n=10)	Se t-value at P=0.05	Mean levels of Cr (mg/kg) (n=10)	Cr t-value at P=0.05	V mean levels in mg/kg (n=10)	V t-value at P=0.05
Brown	21.01 ±1.21	15.70	27.73 ± 1.68	-51.32	2.24 ± 0.28	23.04	6.61 ± 0.97	21.52
Wheat	30.95 ± 1.23	25.34	140.69 ± 5.7	47.54	5.68 ± 0.34	50.96	11.47 ± 1.32	27.45
Finger millet	21.80 ± 0.82	17.48	91.38 ± 5.68	20.25	3.00 ± 0.26	34.06	9.58 ± 0.56	54.04
Bulrush millet	32.44 ± 0.70	78.78	198.38 ± 3.75	23.77	2.79 ± 0.44	18.61	6.28 ± 0.60	33.05
Sorghum	21.01 ± 2.3	8.26	151.20 ± 12.80	120.90	5.86 ± 0.27	66.24	8.25 ± 0.74	35.21
Garlic	53.54 ± 1.44	84.63	152.01 ± 11.90	161.46	1.76 ± 0.25	19.73	8.1 ± 0.58	44.11
Ginger	41.00 ± 5.79	14.20	153.81 ± 0.89	351.08	10.00 ± 1.01	30.68	14.38 ± 0.31	146.59
Rosemary	36.80 ± 0.74	93.16	30.07 ± 0.63	-125.14	4.60 ± 0.80	17.39	13.63 ± 1.20	35.92
Cloves	24.68 ± 1.20	25.51	96.22 ± 3.90	33.42	3.78 ± 0.96	11.79	11.80 ± 1.50	24.86
Lemon grass	23.32 ± 2.26	11.64	110.69 ± 15.20	11.86	12.80 ± 1.47	27.10	14.38 ± 1.20	37.87
Coriander	30.00 ± 0.44	10.78	82.71 ± 16.30	5.38	13.00 ± 0.42	96.37	12.38 ± 2.50	15.65
Pumpkin	12.82 ± 0.84	-8.20	16.54 ± 1.02	-120.84	5.26 ± 0.57	28.07	1.54 ± 0.17	28.46
Watermelon	33.30 ± 2.77	20.89	182.70 ± 13.20	30.59	6.20 ± 1.11	17.09	8.50 ± 1.15	23.34
Sunflower	36.84 ± 0.58	119.08	36.50 ± 3.26	-17.94	5.00 ± 1.29	11.76	10.53 ± 1.37	25.39

cholesterol formation in blood vessels and nervous system as well as improving insulin sensitivity and reducing blood sugar in people with type 1 and type 2 diabetes mellitus (Cusi et al., 2001). Vanadium may also be needed for iodine metabolism and/or thyroid function. However, vanadium deficiency causes reduced growth, poor bone development, impaired reproductive capacity and poor teeth development (Kreider, 1999). The spices (lemon grass, ginger, cloves) and coriander seeds as well as wheat are good sources of vanadium, which could provide the body with sufficient levels required to improve and maintain body immunity. The RDI requirement for vanadium is 10 µg/kg (NAS, 1998).

The chromium levels ranged from 13.0-1.76 mg/kg. Chromium was the highest in coriander seeds (13.0 ± 0.42), followed by lemon grass, and ginger respectively. There is no significant difference between Cr levels in coriander seeds and lemon grass. However, both plants are rarely consumed. Chromium is found in tiny amounts in the body and has the main function of glucose metabolism (Table 3). The presence of Cr in plants may be correlated with therapeutic properties against diabetic and cardiovascular diseases (Perry, 1972). It has been shown to increase humoral and cell mediated immunity (Khangarot et al., 2002). Large amounts of Cr are found in adrenal glands, indicating its importance in the production of stress coping hormones. White blood cells contain Cr and this explains why one is susceptible to illness when under stress, and not able to consume enough of the mineral. Chromium activates vitamin C, and is therefore needed to reap the full benefit from it (Al Durtsch, 1999). Deficiency of Cr causes body fatigue, decrease in energy levels, muscle weakness, slow growth rate in children (Al Durtsh, 1999). Coriander seeds, lemon grass and ginger are good sources of chromium and their consumption could be encouraged to people with the symptoms of its deficiency. The RDI levels of chromium are 50-200 µg/kg (Kumpulainen et al., 1979). A diet of mixed food components would provide the body with the required daily intake (RDI) as indicated in Table 3. Some of the foods analysed in this study are readily available in the market and could be fed on people with compromised immunity.

Conclusion

All the samples analyzed contained the four elements (Zn, Se, V and Cr). The amount varied from sample to sample. Seeds had the highest levels of zinc with pumpkin seeds having the highest level followed by watermelon seeds and sunflower seeds. Herbal spices had the highest level of vanadium with lemon grass and ginger leading. Bulrush millet was found to have the highest levels of Se followed by watermelon seeds, ginger, garlic and sorghum, respectively. Coriander seeds were found to have the highest levels of Cr followed by lemon grass and ginger respectively among others.

Consumption of whole food grains, herbal spices and fruit seeds should be encouraged as good sources of trace elements which boost the body immunity. Recently there have been increases in lifestyle diseases in Kenya such as diabetes, high blood pressure and cancers and considering the cost of treatment is prohibitive, good nutrition is wealth. It is therefore important to encourage people to consume whole grain foods (cereals, pasta, porridge) containing finger millet, bulrush millet, sorghum, brown rice, wheat as part of daily diet as they contain the essential elements and high in fiber. From the results obtained it can be concluded that a mixed diet, would provide the body with the required daily intake of the trace elements as indicated in (Table 3).

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Effects of incorporation of cassava flour on characteristics of corn grit-rice grit-chickpea flour blend expanded extrudates

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A rice grit-corn grit-chickpea flour blend was used as a model feed system for extrusion. Response surface methodology was used to study the effects of level of incorporation of cassava flour in feed composition (5-25%), feed moisture (12-16%), screw speed (1000-1400 rpm) and barrel temperature (80-120°C) by using a single screw extruder. Among the independent variables, feed moisture was found to be the most influential ($p<0.05$) to bulk density (BD), water solubility index (WSI) and water absorption index (WAI). Cassava flour level affected lateral expansion (LE) most significantly, however, temperature affected all the dependent variables equally at $p<0.05$, while screw speed altered significantly BD, WSI and WAI only.

Key words: Single screw extrusion, response surface methodology, rice and corn grit, chickpea flour, cassava flour.

INTRODUCTION

Snack foods (SF) are popular worldwide as they provide taste, convenience, are manageable portions for enjoyment and fulfill short-term hunger. Among several technologies for production of SF, extrusion is highly efficient and versatile in terms of differentiation in texture and size of SF. The physical properties and sensory attributes of extruded products are generally influenced by large number of process variables, of which the amount and structure of starch and protein, and their interaction in native form and during processing are of primary importance (Liu et al., 2000). Parameters such as degree and extent of gelatinization (leading to partial or complete destruction of the crystalline structure), shear induced molecular fragmentation of starch polymers, denaturation of protein are known to affect the quality of extruded SF (Harper, 1981).

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Table 1. Proximate composition of raw materials.

Raw materials	Moisture content (%)	Crude Fat (%)	Crude Protein (%)	Crude Fiber (%)	Ash Content (%)	Starch Content (g/100g)	Bulk Density (g/100ml)	Particle size (%)		
								> 600 µm	150-600 µm	>150 µm
Rice grit	12.15±1.1	0.58±0.1	6.51±0.3	0.21±0.3	0.67±0.7	75±1.4	74.52±1	42.4	56.8	0.8
Corn grit	12±1.1	3.21±0.3	8.88±0.2	3.72±0.2	1.54±0.2	60±2	45.23±2.1	77.7	21.2	1.1
Chickpea flour	11.6±0.8	5.1±0.23	20.76±0.1	1.2±0.5	2.82±0.5	45±2	62.1±0.9	0	31	69
Cassava flour	9.2±1.2	1.09±0.2	5.5±0.6	1.59±0.4	3.23±0.4	80±1.6	62.45±3.7	0	30	70

* Values are means ± standard deviation of triplicate analyses.

Though cereals are the main raw material for extruded snack foods, the use of tubers in product formulation of extrudates is becoming popular (Chiu et al., 2013; Hashimoto and Grossmann, 2003; Jisha et al., 2010; Seth et al., 2013). Among tubers, cassava flour can be used as a good alternative due to its relatively high starch content, and low protein and lipid content compared to cereals. In addition, cassava starch is odorless and has a high paste clarity with low stickiness (Adejumo et al., 2011) making it a suitable ingredient for extrusion (Santillán-Moreno et al., 2011). Furthermore, cassava starch does not block the extrusion barrel even at low moisture conditions (Rampersad et al., 2003).

Chickpea flour has also been widely used in extrudates (Meng et al., 2010; Shirani and Ganesharanee, 2009). However, contradictory effects have been reported regarding the properties of extrudates with chickpea flour. Shirani and Ganesharanee (2009) found that the addition of chickpea flour on extrudates significantly reduced their lateral expansion of extrudate. In contrast, Meng et al. (2010) found positive correlation between the amount of chickpea flour and lateral expansion in a work related to multiple blend of chickpea flour, potato starch, protein concentrate, and other additives. The interactive effect of cassava on major commercial extrusion ingredients such as corn grits, rice grits and chickpea flour has not been fully assessed yet so this report is focused on evaluating effects of cassava levels in properties of extrudates based on rice grits, corn grits and chickpea flour.

MATERIALS AND METHODS

Raw materials

Corn grits, rice grits, and chickpea flour were supplied by a local company (CG Foods Pvt. Ltd., CUG, Nawalparasi, Nepal). The mature cassava tubers (grown locally) were harvested, trimmed using stainless steel knife, sorted and peeled manually before dipping in a 2% NaCl solution at room temperature (28°C) for 30 min. This process prevents the initial browning. The tubers were sliced to 4 mm thickness and immersed in solution containing (0.5%) potassium meta-bisulphate and (0.5%) citric acid for 30 min. Then slices were dried at 60°C to 9% moisture content (wet basis). The dried slices were milled and fractions between 600 and 150 µm sieve were stored in an air tight container until further use. The particle size distributions of each raw material are presented in Table 1.

Methods

Triplicate samples of each raw material were taken to measure moisture (AOAC 935.29), fat (AOAC 922.06), protein content (AOAC 992.23), ash content (AOAC 923.03), and crude fiber (AOAC 962.09) using standard AOAC (2005) methods. Protein content was calculated using conversion factors 5.8, 5.95, 5.3, and 6.25 for corn grits, rice grits, chickpea flour and cassava flour, respectively. Starch content was analyzed using a modified iodine binding method (Dhital et al., 2010).

Experimental design

Response surface methodology (RSM) was used for the experimental design using a five-level, four-factor Central Composite Rotatable Design (Mayers et al., 1976). RSM can be used for the modeling and analysis of the problem in which a response of interest is influenced by several variables. Design Expert software (STAT-EASE Inc., USA, version 6.0) was used to apply RSM.

Extrusion of composite feed blends

In the blend, corn flour was substituted by cassava flour at levels of 5, 10, 15, 20, and 25% by weight while keeping the proportion of rice flour (50%) and chickpea flour (8%) constant. Detailed formulations for extrusion are given in Table 2. Extrusion experiments were performed using a single-screw extruder (main motor capacity 30kw; model DLG100, Jinan Shengrun Machinery Co., Ltd). The moisture was adjusted by sprinkling calculated amount of water into dry ingredients and the composite mixture was homogenously mixed in a small scale planetary mixture (Jiangmen Cheongfai Electronic Manufactory Ltd, China, 250W) for 20 min followed by sieving through a 2 mm sieve to break up the lumps formed due to the addition of moisture. The blends were kept at 30°C for 12 h in an air tight container for moisture equilibrium.

The single screw extruder was kept running for 30 min to stabilize the set temperatures and samples were then fed into the hopper at a rate of 75 kg/h. The extrudates exiting from 3 mm diameter were dried at 60°C to 6% moisture (wet basis) and packed in an air tight container for future analysis.

Determination of properties of extrudates

Lateral expansion and bulk density: Lateral expansion (LE, %) and Bulk density (BD, g/cm^3) were calculated by equation 1 and 2 respectively (Stojceska et al., 2008).

Table 2. Experimental combinations in both Coded and Uncoded levels for cassava flour with rice grit, corn grit and chickpea flour.

Coded variables				Uncoded variables			
A	B	C	D	Feed Proportion (%)	Feed moisture (%)	Temperature (^{0}C)	Screw speed (rpm)
-1	-1	-1	-1	58:32:10	13	90	1100
1	-1	-1	-1	58:22:20	13	90	1100
-1	1	-1	-1	58:32:10	15	90	1100
1	1	-1	-1	58:22:20	15	90	1100
-1	-1	1	-1	58:32:10	13	110	1100
1	-1	1	-1	58:22:20	13	110	1100
-1	1	1	-1	58:32:10	15	110	1100
1	1	1	-1	58:22:20	15	110	1100
-1	-1	-1	1	58:32:10	13	90	1300
1	-1	-1	1	58:22:20	13	90	1300
-1	1	-1	1	58:32:10	15	90	1300
1	1	-1	1	58:22:20	15	90	1300
-1	-1	1	1	58:32:10	13	110	1300
1	-1	1	1	58:22:20	13	110	1300
-1	1	1	1	58:32:10	15	110	1300
1	1	1	1	58:22:20	15	110	1300
-2	0	0	0	58:37:05	14	100	1200
2	0	0	0	58:17:25	14	100	1200
0	-2	0	0	58:27:15	12	100	1200
0	2	0	0	58:27:15	16	100	1200
0	0	-2	0	58:27:15	14	80	1200
0	0	2	0	58:27:15	14	120	1200
0	0	0	-2	58:27:15	14	100	1000
0	0	0	2	58:27:15	14	100	1400
0	0	0	0	58:27:15	14	100	1200
0	0	0	0	58:27:15	14	100	1200
0	0	0	0	58:27:15	14	100	1200
0	0	0	0	58:27:15	14	100	1200
0	0	0	0	58:27:15	14	100	1200
0	0	0	0	58:27:15	14	100	1200

$$LE = \frac{(diameter\ of\ product - diameter\ of\ die\ exit)}{diameter\ of\ die\ exit}\ X\ 100 \quad (1)$$

$$BD = \frac{4\,m}{\pi\, d^2\, L}\ (\text{g/cm}^3) \quad (2)$$

Where, m is weight (g), L is length (cm), and d is diameter (cm) of extrudate.

Since the extrudates were not uniform in diameter, 10 extrudates were picked randomly and the diameter was measured at various positions. The maximum value was selected from each and the diameter was expressed as mean. For bulk density, a section of extrudate having maximum diameter was selected and then corresponding length and weight was measured. Bulk density of 10 individual sections was measured and final value was expressed as mean of 10 readings.

Water absorption and solubility index: Water absorption index (WAI) and water solubility index (WSI), of the extrudates were determined using the methodology described by Anderson et al. (1969). In brief, 2.5 g (dry basis) ground extrudate (< 60 mesh) was suspended in 30 mL of water at 30°C for 30 min followed by centrifugation at 3000 g for 10 min. The supernatant was separated from the gel and dried at 105°C till constant weight. WAI and WSI were calculated using equations 3 and 4 respectively.

$$\text{WAI (g/g)} = \frac{\text{Weight gain by gel}}{\text{Dry weight of extrudate}} \quad (3)$$

$$\text{WSI (\%)} = \frac{\text{Weight of dry solid in supernatent}}{\text{Dry weight of extrudate}}\ \text{X}\ 100 \quad (4)$$

Data analysis

The responses (bulk density, lateral expansion, WAI and WSI) as affected by independent variables namely cassava flour, moisture content, screw speed and barrel temperature, were modelled by

Table 3. Regression coefficients of second order polynomial and their significance for dependent variables

Factors	Lateral Expansion			Bulk density			Water absorption index			Water solubility index		
	Coefficient	*F* value	*P* value	Coefficient	*F* value	*P* value	Coefficient	*F* value	*P* value	Coefficient	*F* value	*P* value
Intercepts	127.17	9.1937	<0.0001**	0.294	9.50	< 0.0001**	5.47	15.28	< 0.0001**	8.45	15.0200	<0.0001
A	7.94	32.0125	< 0.0001**	-0.015	7.15	0.0173*	-0.09	6.34	0.0236*	-0.39	14.9665	0.0015
B	-3.78	7.249	0.0167*	0.019	11.75	0.0037*	0.12	12.60	0.0029*	-0.78	58.9524	<0.0001
C	4.11	8.5763	0.0104*	-0.016	8.09	0.0123*	-0.08	4.88	0.0432*	-0.44	19.0647	0.0006
D	0.81	0.3320	0.5730	0.014	6.03	0.0268*	-0.11	9.64	0.0072*	0.57	31.2313	< 0.0001
AB	1.80	1.0933	0.3123	0.021	9.25	0.0082*	-0.09	4.95	0.0419*	-0.23	3.3977	0.0851
AC	-2.89	2.8246	0.1135	-0.001	0.04	0.8484	-0.01	0.06	0.8137	0.31	6.2956	0.0241
AD	-5.05	8.6360	0.0102*	0.014	4.04	0.0626	0.01	0.04	0.8398	0.26	4.5286	0.0503
BC	-7.51	19.0681	0.0006*	0.003	0.14	0.7093	0.10	6.27	0.0243*	0.89	51.4957	< 0.0001
BD	2.43	2.0008	0.1776	0.016	5.46	0.0337*	0.08	3.47	0.0823	-0.04	0.0888	0.7698
CD	7.63	19.7259	0.0005*	-0.021	8.93	0.0092*	-0.17	15.86	0.0012*	0.28	5.1599	0.0383
A^2	1.79	1.8589	0.1929	-0.029	29.66	< 0.0001**	0.18	30.12	< 0.0001**	-0.29	9.5114	0.0076
B^2	-0.23	0.0309	0.8628	-0.036	46.72	< 0.0001**	0.21	43.12	< 0.0001**	-0.17	3.2057	0.0936
C^2	-6.26	22.7273	0.0002*	-0.018	12.20	0.0033*	0.19	35.90	< 0.0001**	-0.19	3.9016	0.0669
D^2	-0.05	0.0016	0.9686	-0.014	7.60	0.0147*	0.31	97.06	< 0.0001**	-0.20	4.2751	0.0564
R^2		0.89			0.89			0.93			0.93	
Adj R^2		0.79			0.80			0.87			0.87	
Adeq precision		11.87			9.70			12.43			15.13	
Lack of fit		0.28	0.9602		1.85	0.2575		2.26	0.1904		1.70	0.2898

*Significant at $P < 0.05$, **Significant at $P < 0.001$.

multiple regression analysis and the statistical significance of the terms was examined by analysis of variance (ANOVA) for each response. Second degree polynomial equation considered for modeling was as follows:

$Y=\beta_o+\beta_1 A+\beta_2 B+\beta_3 C+\beta_4 D+\beta_{11} A^2+\beta_{22} B^2+\beta_{33} C^2+\beta_{44} D^2+\beta_{12} AB+\beta_{13} AC+\beta_{14} AD+\beta_{23} BC+\beta_{24} BD+\beta_{34} CD+\varepsilon$ (5)

Where A, B, C and D are the coded values of independent variables namely feed composition (% cassava), feed moisture content (%), extrusion temperature (°C) and screw speed (rpm), respectively. The coefficients of the polynomial were represented by β_0 (intercept), β_1, β_2, β_3, β_4 (coefficient of linear effects); β_{12}, β_{13}, β_{14} β_{23}, β_{24}, β_{34} (coefficient of interaction effects); β_{11}, β_{22}, β_{33}, β_{44} (coefficient of quadratic effects); and ε (random error). Design Expert software (STAT-EASE Inc., USA, version 6.0) was used to analyze data.

RESULTS AND DISCUSSION

Effects of process variables on lateral expansion

The expansion of the extrudate varied between 95.1 and 148.7%. The expansion in lower extreme level (5%) (S No.17, Table 2), upper extreme level (25%) (S No.18, Table 2) and central level (15%) of cassava flour were found to be 118, 148.7 and 127.2% (average of six centerpoint combinations, S No. 25-30, Table 2) respectively. Table 3 shows the coefficients of the model and other statistical attributes of LE.

Among the linear terms, all independent variables except feed moisture had a positive effect on LE, indicating that extrudates are more expanded when the proportion of cassava flour in feed rate; extrusion temperature and screw speed is increased. Similarly, increases in LE could be the result of a decrease in fiber content in feed because cassava flour contains less fiber than

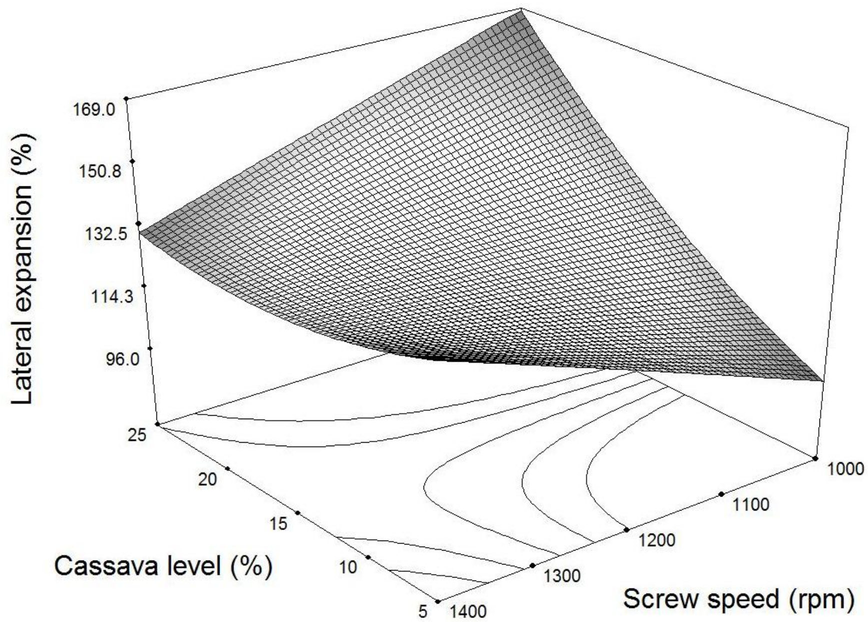

Figure 1. Response surface plot for lateral expansion ratio as a function of screw speed and feed composition at center value of feed moisture and barrel temperature.

corn grits (Table 1). Generally fiber associated with lowered LE is due to a reduction in cell size, which is caused by premature rupturing of gas cells, which reduces overall expansion and results in a less crispy product (Liu et al., 2000). However, increasing moisture significantly decreases the LE, which is consistent with previous findings (Bartholomew and Osualo, 1986; Hashimoto and Grossmann, 2003). Positive coefficient of barrel temperature (Table 3) shows that lateral expansion increases with increase in temperature which is in accordance to previous report of Ding et al. (2006). The increase in LE may be due to a higher degree of water heating in the extruder which induces the bubble formation. Although screw speed did not significantly ($p>0.05$) affect LE, a positive coefficient (Table 3) indicated that LE increased, with an increase in screw speed. This may be the result of a high degree of mechanical shear; resulting in higher rates of expansion as reported by Ding et al. (2006). However, with lower cassava content, lateral expansion was found to be decreased with increasing screw speed (Figure 1). The reason might again be effect of fiber from corn grits, since fiber breaks the bubbles in molten extrudate mass causing decrease in expansion.

Effects of process variables on product bulk density

Bulk density is a major physical property of the extrudate products. The BD, which considers expansion in all direction, ranged from 0.14 to 0.37 g/cm^3. BD in lower extreme level, upper extreme level and central level of cassava flour were found to be 0.23, 0.14 and 0.29 g/cm^3 (average of six centerpoint combinations; Table 2) respectively, with Table 3 showing the model coefficients and statistical attributes of BD.

Moisture content showed a highly significant positive effect on the BD of extrudates, which is attributed to the reduction in melt elasticity due to change in molecular structure of amylopectin at higher moisture level as described in several reports (Fletcher et al., 1985). As expected, the coefficient of feed composition was negative and also both LE and BD share negative correlation with each other (Table 3). At low cassava substation, the high fiber content from corn grits reduced the bulk density BD was found to be negatively correlated with die temperature (Table 3) which is in agreement with previous reports (Ding et al., 2006; Fletcher et al., 1985; Meng et al., 2010) With an increase in the die temperature, there was an increase in degree of superheating of water vapor in the extruder increasing expansion and decreasing BD (Fletcher et al., 1985). Positive coefficient of the screw speed shows that BD increases with increasing screw speed indicating molecular breakdown of starch in large extent at higher speed. It was observed from (Figure 2) that at a lower screw speeds, BD decreased with an increase in cassava level. However, at higher cassava level, BD increased with increase in screw speed.

Effects of process variables on product WSI

The WSI values ranged from 5.22 to 10.21%. The

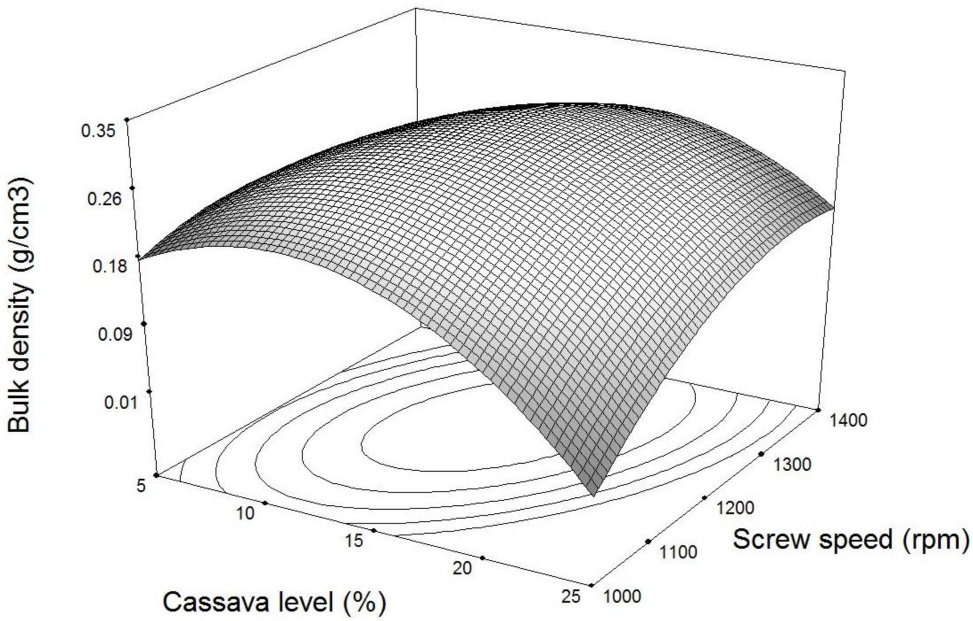

Figure 2. Response surface plots for the variation of bulk density as function of cassava level and screw speed at central level of feed moisture and barrel temperature.

coefficients of the model and other statistical attributes of WSI are shown in Table 3.

It was apparent from Table 3 that feed moisture content was the main determinant of WSI among linear terms. However, negative effect of moisture content on WSI indicated that the value will decrease significantly with increasing feed moisture content. WSI is an indicator of degree of degradation of starch and reflects the amount of free polysaccharide or polysaccharide released from the granule after addition of excess water (Sriburi and Hill, 2000). Variation in WSI as affected by feed moisture was consistent with results of study on wheat -based extruded products (Ding et al., 2005) and wheat semolina /pea hull extrusion (Sabota and Rzedzicki, 2009). It was hypothesized that feed moisture, when increased, acted as plasticizer allowing less friction between feed material and extruder wall. Figure 3 shows the interactive effect of temperature and moisture indicating a decrease in both parameters increases WSI. Although linear effect of temperature on WSI is positive, which is consistent with (Sabota and Rzedzicki, 2009); however, simultaneous increase in temperature and moisture decreased WSI which may be due to fairly higher influence of moisture on WSI than temperature. This can be apparent by comparing F- value of moisture and temperature which are 58.95 and 19.06 (Table 3) respectively.

Negative coefficient of feed composition shows that WSI increases as cassava level in feed decreases. Sabota and Rzedzicki (2009) also reported increase in WSI by extrusion in their extrusion studies of fiber enriched product. The result may be due to degradation of fiber present in corn grits. Screw speed showed significant positive effect of product WSI which is in accordance with results reported for corn meal and corn and wheat extrudates (Jin et al., 1995). The increase of screw speed induced a sharp increase in SME, which in turn degraded starches and fibers possibly through chain splitting increasing product WSI (Colonna and Mercier, 1983).

Effects of process variables on product WAI

WAI, measures of amount of water absorbed by extrudate, ranged from 5.31 to 6.91 g/g. Table 3 shows the coefficients of the model and other statistical attributes of WAI.

Negative coefficient of feed composition indicates that decrease in cassava flour proportion in feed will increase the WAI of the product. Increasing the fiber content resulted in an increase in WAI, which can be explained by fiber having a higher water absorption capacity, when starch content was reduced. Similar findings were reported by Artz et al. (1990) in extrusion of corn fiber and corn starch blend. The model coefficient suggests that increase in feed moisture caused increase in WAI. The other reason might be difference in water absorption capacity of corn and cassava starch. The negative coefficients of temperature indicate that increasing in temperature will decrease the WAI value of the product. This decrease in WAI values with an increase in temperature might be due to an increase in starch degradation (Pelembe et al., 2002). Ding et al. (2005) also stated that the WAI decreased with increasing

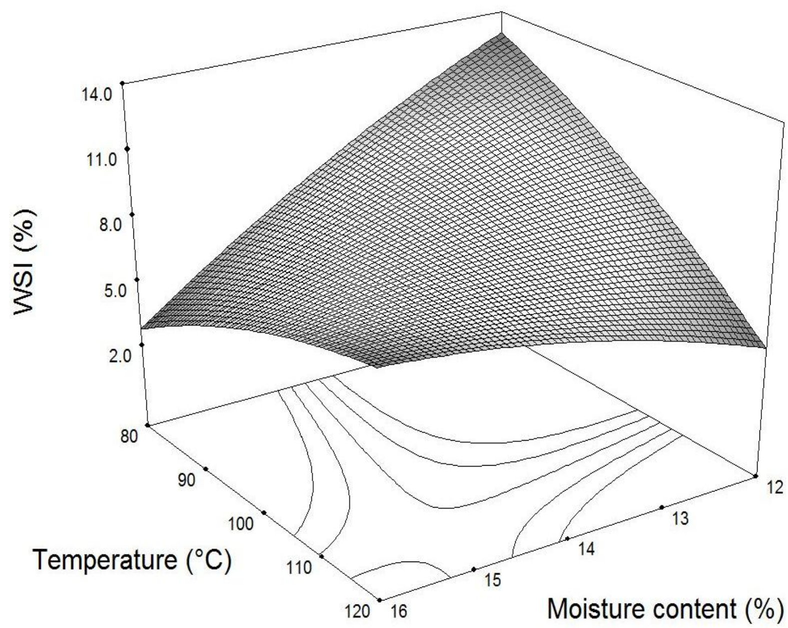

Figure 3. Response surface plot for water solubility index as a function of feed moisture and barrel temperature at center value of feed composition and screw speed.

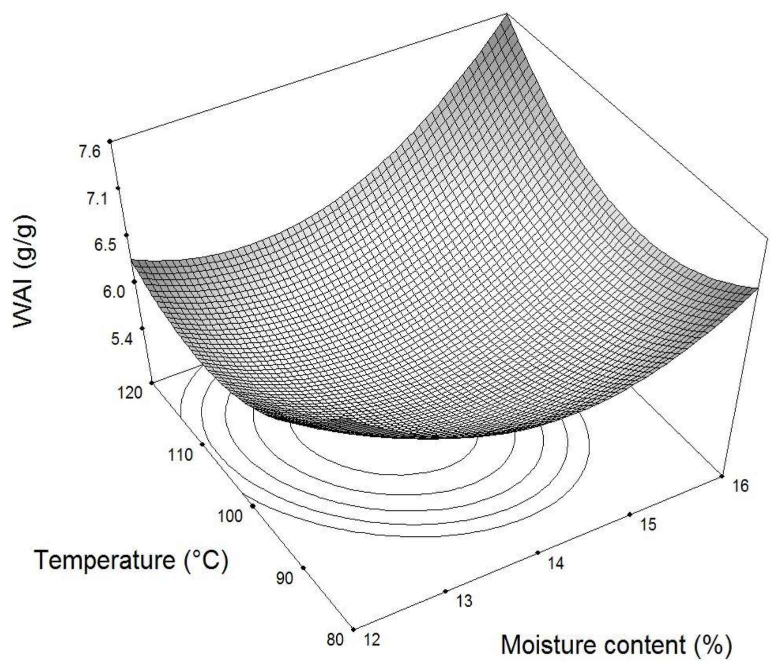

Figure 4. Response surface plot for water absorption index as a function of barrel temperature and feed moisture at center value of feed composition and screw speed.

temperature if starch dextrinization or starch melting prevails over the gelatinization phenomenon. At higher temperature, increase in feed moisture caused increase in WAI when keeping screw speed and feed composition constant at centre value respectively (Figure 4). This might be due to higher moisture content, acting as a plasticizer during extrusion which reduced the degradation of starch granules and simultaneously favors gelatinization resulting in an increased capacity for water absorption (Bartholomew and Osualo, 1986; Colonna and Mercier, 1983). Screw speed had significant negative effect on WAI which showed that increase in screw speed will decrease WAI. The reduction of WAI suggested that there might have been some starch degradation and the structural modification of fiber at higher shear conditions. At a low shear rate (low screw speed) and/or low temperature, there will be more undamaged carbohydrate polymer chains and a greater availability of hydrophilic groups which can bind more water resulting in higher values of WAI (Gomez and Aguilera, 1983).

Conclusions

The results showed that the product response variables were almost equally affected by changes in cassava flour level, feed moisture, extrusion temperature and by screw speed. Increasing barrel temperature resulted in an increase in expansion with a decrease in BD, WAI and WSI. A higher proportion of cassava flour in feed resulted in maximum expansion with a minimum BD, WAI and WSI. It was apparent that cassava starch could play a key role to enhance its functional attributes of expanded extrudate.

Conflict of interests

The authors did not declare any conflict of interest.

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