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Sensory evaluation and consumer acceptability of orange-fleshed sweet potato by lactating women and their children (<2 years old) in Kaffrine, Central Groundnut Basin, Senegal

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This study evaluated the sensory properties and consumer acceptance of boiled, fried, and mashed orange-fleshed sweet potato (OFSP), by rural lactating women (n=80) and their children <2 years old (n=77). Sensory evaluation and acceptability were performed using a 7-point hedonic scale. Intake parameters (food intake and eating rate), and vitamin A content in the OFSP were measured. The relationships between the sensory descriptors and the type of OFSP preparations was analysed using principal component analysis (PCA) plot. Frying and boiling the OFSP decreased the vitamin A content by 50%, but an acceptable level of retinol was maintained. For all sensory attributes, mothers preferred fried OFSP over boiled, while their children gave high acceptability scores to both boiled and mashed OFSP. The success of the OFSP cultivar, which is newly introduced in Kaffrine, and its high acceptability are promising for the prevention of maternal and child vitamin A deficiency in the rural areas of Senegal.

Key words: Orange-fleshed sweet potato, sensory evaluation, consumer acceptance, Senegal.

INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.), a hardy crop that produces storage roots, has the potential to combat increasing food shortages as it provides a high yield of

edible energy per unit area and per unit time, while also supplying substantial amounts of vitamins and minerals (van Oirschot et al., 2003). In the Senegal River Valley,

sweet potato is produced annually and is mainly grown in the northern regions following two cropping systems, namely, conventional and flood recession cropping (ISRA/ITA/SAED, 2009). Annual production of sweet potato is approximately 35000 tons (FAOSTAT, 2016) and mostly consists of white- and yellow-fleshed varieties. In many Senegalese households, sweet potato has replaced potatoes and provides variety in the diet (ISRA, 1986). However, the production of orange-fleshed sweet potato (OFSP) is recent and rare, and generally, the crop is intended for export. In the Central Groundnut Basin of Senegal, especially the Kaffrine region, OFSP has not been introduced in the region until recently. In 2014, OFSP cultivars were introduced in northern Senegal through a development project for combating undernutrition (USAID, 2017) because of its high content of β -carotene (Hotz et al., 2012a, b). This project improved the diet quality and the nutritional status of children under five and women, but its impact on vitamin A status was not evaluated. Several studies have shown the beneficial effects of OFSP consumption in improving vitamin A reserves and status (van Jaarsveld et al., 2005; Low et al., 2007; Hotz et al., 2012a). Undernutrition and micronutrient deficiencies (MNDs) are prevalent in Senegal. The first national MNDs survey carried out in 2011 showed iron, zinc, folate, and vitamin A (VA) deficiencies were common among children and women, and particularly in the rural central region of Senegal (Agne-Djigo et al., 2012; Ndiaye et al., 2015, 2017). The main strategies implemented in Senegal to prevent vitamin A deficiency are vitamin A supplementation every six months for children aged 6 to 59 months, large-scale fortification of refined vegetable oil with vitamin A, and promotion of breastfeeding (DAN, 2016a). Despite these preventive interventions, undernutrition and MNDs remain (Fiorentino et al., 2013; DAN, 2016b; ANSD, 2017). Thus, the prevention of these problems requires a holistic and sustainable approach that integrates food availability and accessibility, women's empowerment, advocacy and the promotion of micronutrient-rich foods (Masset et al., 2012; Ruel and Alderman, 2013). To address the promotion and consumption of carotenoid-rich foods such as OFSP, this study implemented a community farm of 5 ha through a Development Research Project (PRD) in Sagna, a village with 1478 inhabitants located in the Kaffrine region of central Senegal, and introduced an OFSP cultivar to this region for the first time. In Senegal, sweet potato is usually boiled and consumed as vegetables, but the fried form is also used as taste, snack or accompaniment. As the sensory properties and degree of acceptance of OFSP have not been established in this area, the aim of this

study was to conduct sensory evaluation and acceptability tests of boiled, fried, and mashed OFSP preparations by mother-child (<2 years old) pairs in Kaffrine.

MATERIALS AND METHODS

OFSP samples, preparations, and carotenoids contents

The OFSP was from healthy cuttings of the Kande variety (Kapinga et al., 2010) provided by the Senegalese Institute of Agricultural Research (ISRA). Healthy cuttings were planted in the farm in Sagna and grown using a drip irrigation system. After two cycles of culture (dry and wet seasons), the introduction of Kande was considered successful (Sylla et al., 2017). Mature OFSP roots were harvested 5 months after planting as recommended for this variety (Kapinga et al., 2010). For at least 2 months after harvest, the OFSP roots remained available for food consumption by the populations of Sagna and its surrounding area. The storage time before sensory evaluation and consumer acceptability tests was approximately 5 days.

The OFSP roots were sorted to remove diseased and insect-damaged roots, and the acceptable samples were peeled and chopped into roughly equal sized pieces of 30 to 50 g each. A portion of the roots was immersed in tap water and boiled (approximately 20 min) until the texture, as assessed by a knife, was considered tender and suitable for eating. Boiled OFSP were cut into smaller pieces and used for sensory testing by the mothers and older children (≥ 1 year). For children <1 year of age, the boiled OFSP were mashed. The remaining portion of the raw OFSP roots was fried (10 min) in refined vegetable oil until the texture, as assessed by a knife, was considered correct for eating. Fried OFSP was used for sensory evaluation by the mothers (Figure 1).

Raw, boiled and fried OFSP samples were assessed for total carotenoid contents by colorimetric assays using an i-Check device (iCheck™ carotene BioAnalyt GmbH, Germany) (Schweigert et al., 2010). All samples were analysed as ready-to-eat samples instead of based on their dry matter content. The β -carotene content in each preparation was estimated to account for 90% of the total carotenoid content (Rodriguez-Amaya and Kimura, 2004; Bengtsson et al., 2008). The retinol activity equivalent (RAE) was estimated using a bioconversion factor of 12 mg of β -carotene to 1 mg of retinol activity (FAO/WHO, 2004).

Participants, design, and ethics approval

A sample size of at least 70 subjects per targeted group is required by the ISO method (ISO, 2017) to determine the degree of liking (DOL) from a 7-point hedonic scale with a probability of error of 0.05 and power ≥ 0.80 . Overall, 160 subjects (80 mothers and 80 children <2 years old) living in Sagna and the surrounding area were selected to participate in the test. Ethical approval was obtained from the Ethical Committee of the University Cheikh Anta Diop of Dakar. An information session was conducted in the local language with the mothers to obtain their verbal consent. On the same day, Child's hearty appetite pre-test was performed using boiled OFSP. In addition, to be eligible for the study, the children had to appear healthy and have a weight-for-length z-score > -2 .

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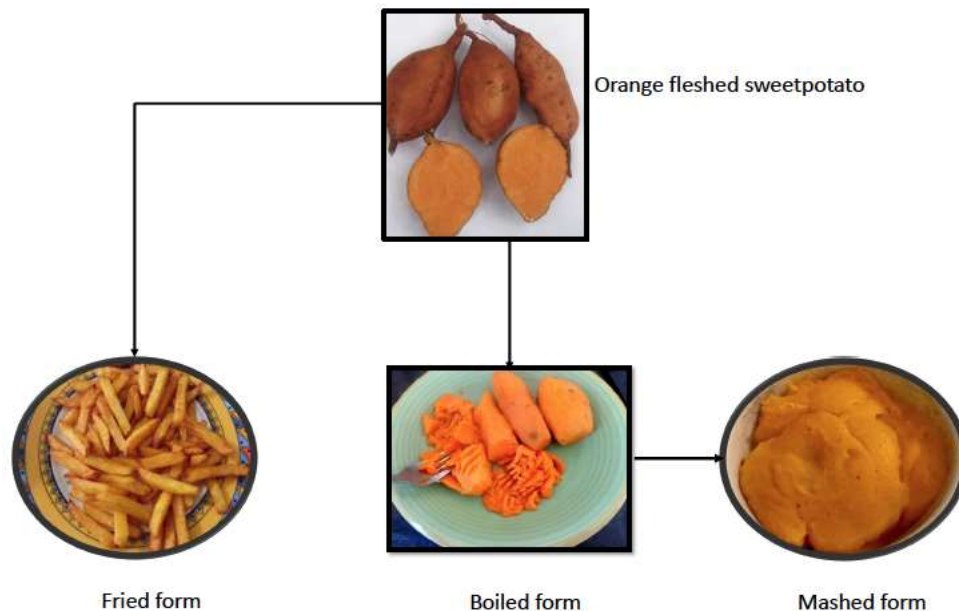


Figure 1. Different forms of OFSP preparations

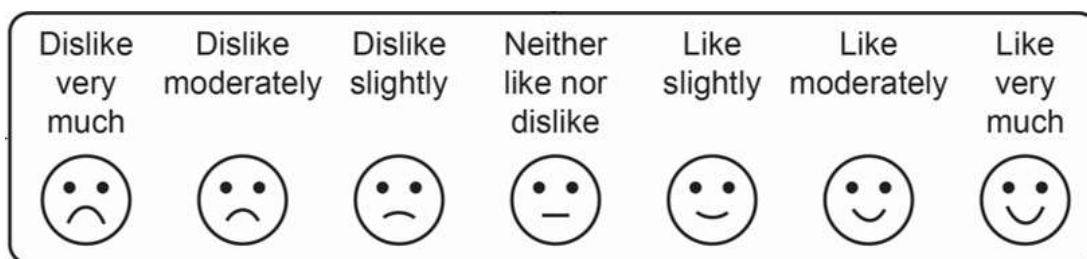


Figure 2. Seven-point facial hedonic scale.

Participant’s profile

Before starting the test, short interviews to profile each mother and their consumption of recently harvested OFSP from the farm were conducted using a simple questionnaire that included questions on their age, occupation, level of education, consumption of OFSP during the preceding 2 weeks, the origin of the OFSP usually consumed, usual preparation methods, and what they perceived as the personal benefits of consuming OFSP. Mothers completed, on behalf of their children, a similar questionnaire. The questions included the usual preparation methods for OFSP given to the child and what the mothers perceived as the benefits for their child from consuming OFSP.

Sensory evaluation

During the test, women were asked to choose the most common and their preferred methods of cooking OFSP (boiled or fried). The sensory test was carried out in an appropriate site by qualified personnel. Before starting test of the mothers, samples from the different preparation methods were randomly chosen, and boiled OFSP was given prior to the fried OFSP. Preparations were served

on plates as ready-to-eat food. After each test, the mothers were asked to assess their appreciation of the appearance, flavour, and texture using the 7-point hedonic scale (Figure 2) from 1 (“dislike a lot”) through 4 (“neither like nor dislike”) to 7 (“like a lot”) according to Tomlins et al. (2007). They were also asked to report boiled or mashed OFSP appearance, flavour, and texture for their children using the same hedonic scale.

Overall acceptability

Before the acceptability test, the emotional state of the children was assessed by the criteria defined by the WHO’s gross motor development study (Wijnhoven et al., 2004), which classified children’s emotional state in relation to 2 parameters: (1) sleepy or completely awake and (2) calm, restless or crying. If the child was not in an adequate emotional state during a waiting time of 30 min, he was excluded.

One hundred grams (100 g) of each of the two OFSP preparations (boiled and fried) were given to the mothers, and 50 g (boiled or mashed) were given to the children by their mothers 60 min after the last breastfeed. The mothers were instructed to continue feeding until their children ate all the food or refused

Table 1. β -carotene content and vitamin A intake of raw, boiled, and fried orange fleshed sweet potato (OFSP).

Parameter	OFSP form		
	Raw	Boiled	Fried
Total carotene contents ($\mu\text{g}/100\text{ g FW}^{\text{a}}$)	111.1	50.1	55.4
β -carotene contents ($\mu\text{g}/100\text{ g FW}$)	10000.0	4508.8	4988.1
Vitamin A intake ($\mu\text{g RAE}/100\text{ g FW}$)	275.0	137.2	124.9

^aFW: fresh weight

β -carotene contents were calculated as 90% of the total carotenoid contents

RAE: retinol activity equivalent; 1 retinol activity equivalent = 12 μg β -carotene with a bioavailable fraction = 33%

further food. Consumption time of each meal, as well as the quantity consumed by the women and children, was recorded (iBalance 2500 bowl scale, My Weigh, AZ, USA). For mothers, 50 ml of water was provided after the first meal, and there was a 30 min break between the first and second meal according to the approach used by Aaron et al. (2010). For sensory evaluation, after each meal test, the mothers were asked to assess their overall DOL using the 7-point hedonic scale. They were also asked to provide, with the help of interviewers, proxy ratings of their child's overall DOL. The sensory testing of children required an indirect approach in which the mothers interpreted the behaviour of the toddler as he/she tasted the food using nonverbal cues (for example, turning the head away, spitting out the food, pushing away or reaching for the spoon, and accepting the spoonful with an open mouth) and rated the child's acceptance using the same 7-point hedonic scale. The index of acceptability (IA) of women and their children was also calculated according to Fernandes and Salas-Mellado (2017) by

using the equation: $IA (\%) = \frac{\text{score} \times 100}{7}$ where score

represented appreciation reported by the mothers based on the 7-point hedonic scale. Food should have a minimum score of 70% IA to be considered acceptable (Spehar et al., 2002).

Statistics analysis

Data were computed using Epi-Info version 3.5.1, and statistical analysis of the data was conducted using Stata/Special 14 software (Stata Corporation, Texas, and USA). The results are presented for all mother-child pairs that completed the study and are expressed as the mean \pm standard deviation (SD) or as a percentage (%). For the consumption data and hedonic evaluation, independent Student's *t*-tests and paired *t*-tests were used to determine the differences between the types of food preparations. The relationship between the sensory descriptors and type of OFSP preparation was illustrated by the principal component analysis (PCA) plot prepared using R software version 3.4.3 (R Core Team, Vienna, Austria, 2018). All statistical analyses were performed using a significance level of 5%.

RESULTS

Maternal and child characteristics

A total of 80 mothers and 77 children completed the acceptability test. Three children were excluded because they were asleep during the test. The majority of mothers

were more than 20 years old and multiparous. Over 18% reported having an occupation and nearly 72% of them had attended school. The child's mean age was 12 months, and half of them were between 12 and 16 months of age. The gender among the children was quite balanced.

OFSP β -carotene content and vitamin A intake

The β -carotene contents of raw, boiled, and fried OFSP were 10000, 4508.8, and 4988.1 μg β -carotene/100 g, respectively (Table 1). Boiled and fried OFSP had lost 55 and 50% of their β -carotene content during processing, respectively, relative to the raw OFSP. The vitamin A (VA) contents in 100 g of raw, fried, and boiled OFSP were 275, 137, and 124 μg RAE/100 g, respectively, which account for 32.3, 16.1, and 14.6% of daily VA recommended safe intake (RSI) for lactating women. Among children aged 7-24 months, consumption of 100 g of boiled OFSP accounts for 31% of their daily VA RSI.

Attitudes of the mother-child pairs towards OFSP

The short interviews showed that nearly, 59% of the mothers usually consumed OFSP from 2 to 6 days per week, 35% once per day, and only 5% once per week (Table 2). Children typically consumed OFSP from 2-6 days per week (59%) and preferred the boiled (64%) and mashed (31%) OFSP. The most common preparation method was boiled (> 60%); however, OFSP was sometimes consumed fried (27%) or raw (10%). The OFSP consumed by the mothers and their children was mainly from the PRD's farm. The mothers generally mentioned the high vitamin A content of the OFSP as the primary health and nutritional benefit. Overall, 96% of the mothers were willing to buy OFSP and give it to their children (Table 2).

Sensory attributes

The scores for the sensory attributes for both boiled and

Table 2. Mother-child pairs profile on attitudes towards OFSP.

Parameter	Mothers (n=80)	Children (n=77)
OFSP consumption during the preceding 2 weeks	46.2 (37)	53.2 (41)
Usual OFSP consumption		
Every day	35.1 (13)	24.4 (10)
2-6 days per week	59.5 (22)	58.5 (24)
1 day per week	5.4 (2)	17.1 (7)
Form of OFSP consumption		
Raw	10.8 (4)	ND
Fried	27.0 (10)	4.8 (2)
Boiled	62.2 (23)	64.3 (27)
Mashed	ND	30.9 (13)
Source of OFSP		
Project farm	87.8 (36)	87.8 (36)
Market	7.3 (3)	7.3 (3)
Elsewhere	4.9 (2)	4.9 (2)
Benefits of OFSP		
Rich in vitamin A	60.0 (36)	63.3 (38)
Health/Nutrition	35.0 (21)	40.0 (24)
Rich in energy	5.0 (3)	ND
Increase milk production	3.3 (2)	ND
Willing to buy OFSP and give it to their children	96.0 (77)	ND

Data expressed in percentage (frequency).

ND: Not detected.

Table 3. Scores of acceptability and degree of liking (DOL) by mothers and children based on types of OFSP preparations.

Mothers	Boiled (n=80)	Fried (n=80)	p-value
Appearance	6.3 ± 0.9	6.9 ± 0.3	<0.001
Flavour	6.3 ± 0.8	6.8 ± 0.4	<0.001
Texture	6.3 ± 0.8	6.8 ± 0.4	<0.001
Overall acceptability	6.3 ± 0.7	6.9 ± 0.3	<0.001
IA (%)	90.5	98.0	<0.001
IA ≥ 70%	98.7	100	0.320
Children	Boiled (n=41)	Mashed (n=36)	p-value
Appearance	6.3 ± 0.8	6.5 ± 0.7	0.369
Flavour	6.3 ± 0.9	6.6 ± 0.6	0.176
Texture	6.3 ± 0.9	6.6 ± 0.5	0.131
Overall acceptability	6.2 ± 1.1	6.2 ± 1.2	0.984
IA (%)	88.2	88.1	0.984
IA ≥ 70%	96.6	93.8	0.591

Data are means ± standard deviation. IA= index of acceptability
p <0.05=means are significantly different.

fried OFSP were mostly greater than 5 and were evaluated as “moderately like” to “like very much” (Table 3). Nearly, 95% of the mothers liked moderately or liked a

lot the boiled OFSP, and 99% of them liked moderately or liked a lot the fried OFSP. Nevertheless, the DOL ratings for the flavour (P < 0.001), texture (P < 0.001), and

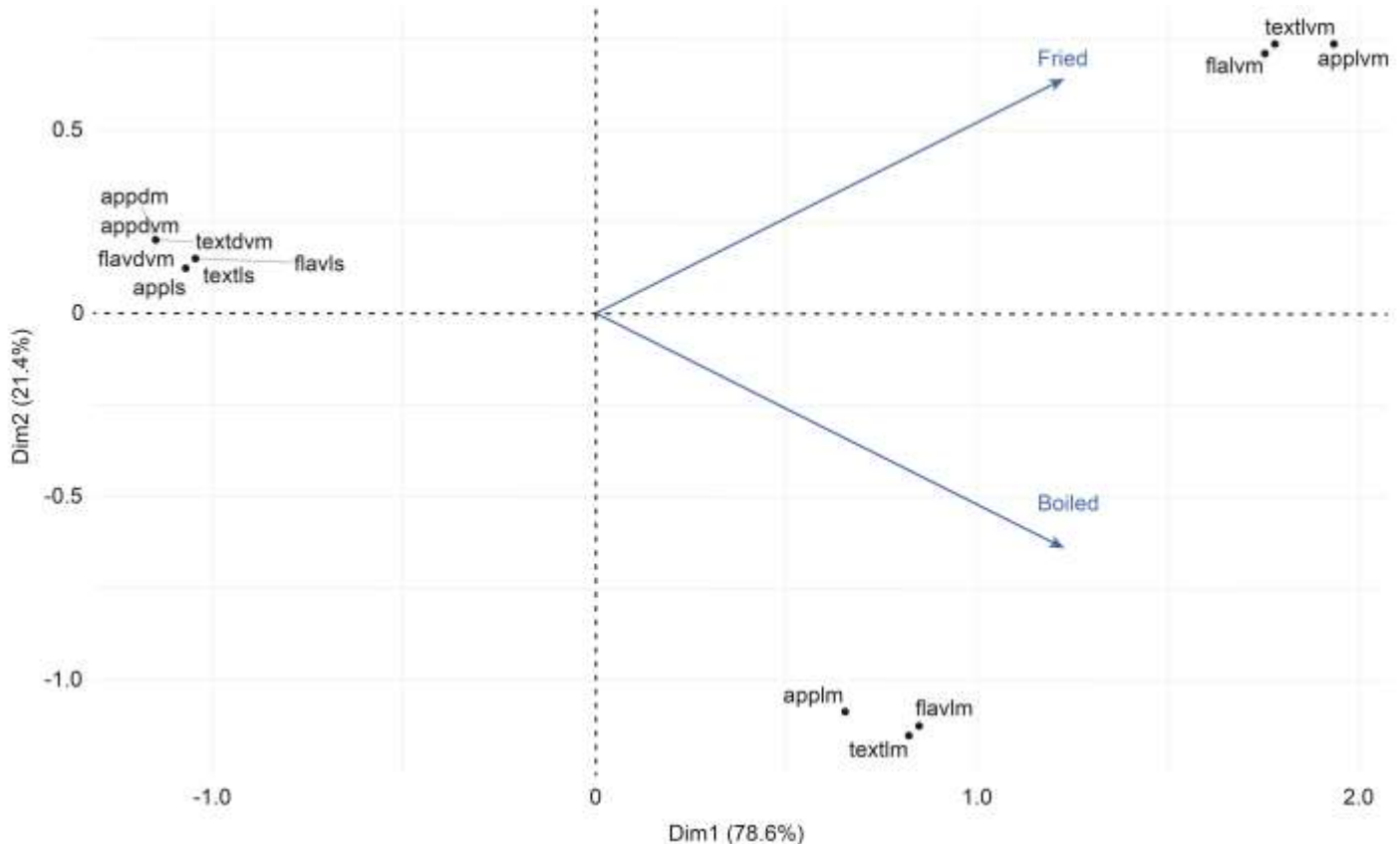


Figure 3. PCA plot of sensory descriptors and OFSP preparations among mothers.

appearance ($P < 0.001$) of fried OFSP were significantly higher than those of boiled OFSP. The relationships between the sensory descriptors and the OFSP preparations are illustrated in the PCA plots (Figures 3 to 4). The mothers reported very different sensory characteristics between the different OFSP preparations (Figure 3). Based on the paired t-tests, the sensory attributes of appearance, flavour and texture were better correlated with frying than with boiling. Frying (Figure 3; top right-hand quadrant) was most associated with “like very much” for texture, flavour, and appearance, while boiling (Figure 3; bottom right-hand quadrant) was more associated with “like moderately”.

All the children ≥ 1 year old received boiled OFSP and the others received mashed OFSP. For the sensory evaluation, the average scores for all attributes of the two types of preparations were generally greater than 5 (Table 3). Approximately, 91% of the mothers reported that their children liked moderately or liked a lot either boiled or mashed OFSP. Comparison using Student t-test, showed no significant difference between the boiled and mashed OFSP in their appearance, flavour, and texture parameters (Table 3). However, PCA (Figure 4) showed that the mashed OFSP (bottom right-hand quadrant) was more associated with “like very much”,

while boiling (top right-hand quadrant) was more associated with “like moderately”.

Intake parameters of the different OFSP preparations

No differences were observed between the OFSP preparation methods and the quantity of food consumed by the mothers (boiled = 96 ± 11 g; fried = 97 ± 10 g) and the children (mashed = 26 ± 15 g; boiled = 26 ± 15 g). However, there was a significant difference between feeding duration and eating rate among mothers for different preparations. The mean eating rate of the fried OFSP (11.5 ± 5.1 g/min) was significantly lower than that of the boiled OFSP (18.7 ± 7.4 g/min) ($P < 0.001$). In addition, the feeding duration for boiled OFSP (5.8 ± 1.9 min) was 4 min faster than that of fried OFSP (9.7 ± 3.4 min) ($P < 0.001$).

Overall acceptability of the different OFSP preparations

The mothers' overall acceptability of OFSP was high for both boiled and fried preparations with mean values that

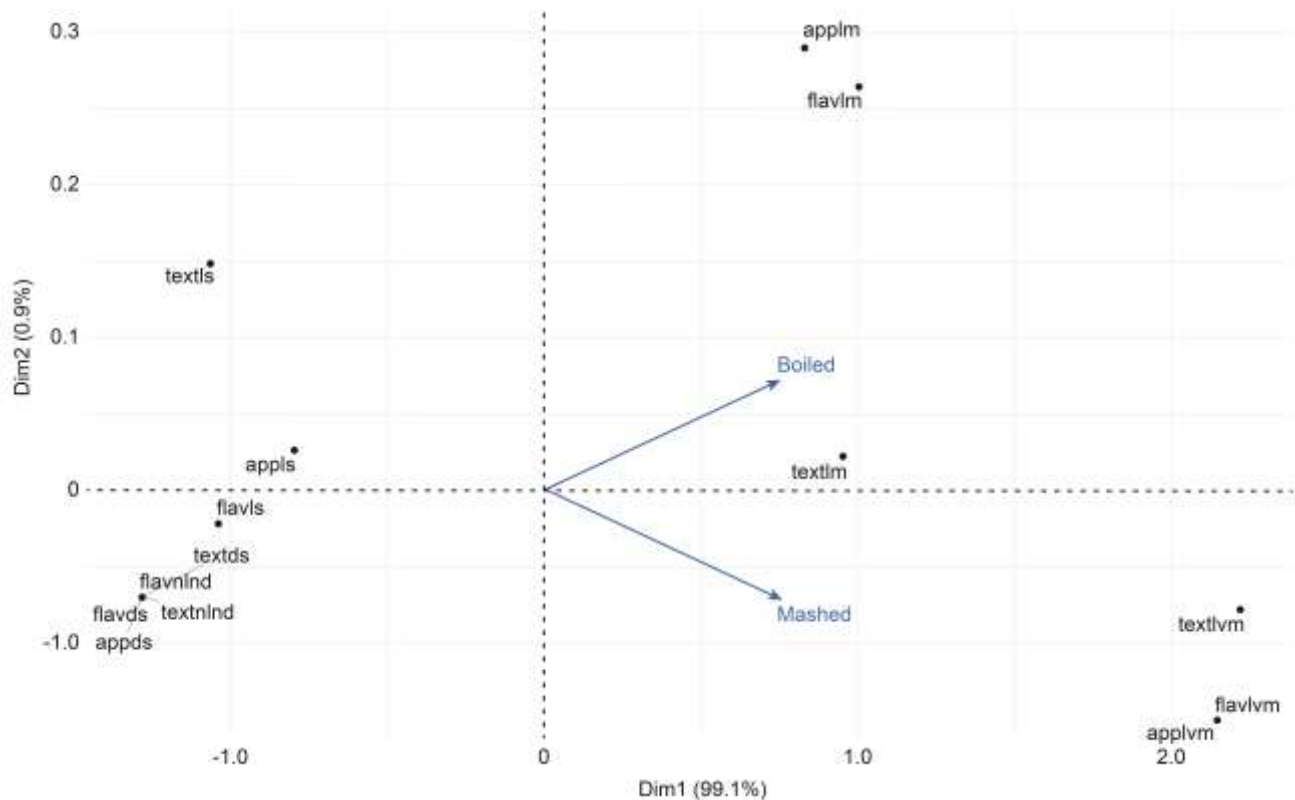


Figure 4. PCA plot of sensory descriptors and OFSP preparations among children.

were generally ≥ 6 (Table 3). However, there was a significant difference between the mean acceptability score of fried OFSP and boiled OFSP ($P < 0.001$). Based on mean overall DOL scores, all preparations were acceptable for consumption because the majority of mothers (>90%) reported a minimum IA score of 70%. Nevertheless, the IA values of fried and boiled OFSP were significantly different ($P < 0.001$), indicating that women generally preferred fried over boiled OFSP. Boiled and mashed OFSP were appreciated “moderately” to “very much” by the children with mean values of 6.2. The IA values were good (> 88%), indicating the children appreciated both forms of preparation of OFSP.

DISCUSSION

The β -carotene content in the raw OFSP cultivar is consistent with results reported for the Kande variety (11030 $\mu\text{g}/100\text{ g}$) by Kapinga et al. (2010) and are relatively comparable to the contents found in other OFSP varieties (Kidmose et al., 2007; Bengtsson et al., 2008; Burri, 2011). However, after cooking, boiled and fried OFSP had lost half of β -carotene contents, but their contents remained consistent with reported values for cooked OFSP (Kidmose et al., 2007; Bengtsson et al., 2008; Burri, 2011). Bengtsson et al. (2008) found that

average losses of 22% for both boiled and deep fat fried preparations of several Ugandan OFSP varieties. These low losses compared to those found in this study may be due to the effect of high temperatures during OFSP cooking which were not measured in this study. In methods such as deep frying, prolonged cooking cause large losses of β -carotene (Kotíková et al., 2016). On other hand, the nature of the OFSP samples used for total carotene analysis could be explained the variations. Indeed, in this study, carotene content was calculated on a fresh weight basis, which is how OFSP is consumed by the population, instead of the dry matter of OFSP, which is what is used by others, to analyse the β -carotene content (Kidmose et al., 2007; Bengtsson et al., 2008; Burri, 2011), as β -carotene retention is normally overestimated when it is calculated on a dry-weight basis (Kotíková et al., 2016).

The acceptability tests showed that the mothers can consume 100 g of OFSP, while on average, the children ate up to 25 g per meal. According to Burri (2011), after adjusting the carotenoid concentrations in OFSP for cooking loss, storage, and poor bioavailability, based on an estimated requirement of 400 RE/d, children aged 7-24 months needed to consume 23.4 to 131.2 g OFSP/d to meet their daily VA recommended safe intake (RSI), while lactating women, with an estimated requirement of 800 RE/d, needed to consume 46.8 to 262.5 g OFSP/d.

Thus, promoting a minimum consumption of 200 g of OFSP for mothers and 100 g for children, which corresponds to two and three meals per day, respectively, will provide a large portion of the VA RSI.

In the present study, sensory evaluation and acceptability tests of different preparations (boiled, fried, and mashed) of Kandee OFSP to mimic the actual consumption of potatoes in Senegal, showed that boiled and fried OFSP have good sensory attributes among mothers. However, fried OFSP had better appearance, flavour, and texture than boiled OFSP, and this difference is probably due to the presence of fat in the preparation, as this ingredient positively contributes to both texture and flavour (Burri, 2011; Selvakumaran et al., 2017). Among the children, good sensory attributes were found for both boiled and mashed OFSP without any difference in their appearance, flavour, and texture parameters, indicating that after all, both preparations are boiled. These results suggested that mothers and their children have different perceptions of OFSP, especially with respect to the sensory properties, which is consistent with an earlier report by Tomlins et al. (2007).

All preparations were acceptable for consumption (Spehar et al., 2002), indicating a very good appreciation of this OFSP cultivar. Nevertheless, most mothers preferred fried OFSP over boiled OFSP, probably because, in Senegal, potato is traditionally consumed in boiled form as a vegetable rarely in fried form. Similar results suggesting that texture, flavour and taste can be used to predict consumption, overall acceptance and eating quality of OFSP, have been reported previously, and factors such as satiety and price are less important (Burri, 2011; Selvakumaran et al., 2017). In addition, the food matrix and the presence of fat influence carotenoid absorption and bioavailability (Bengtsson et al., 2008; Tumuhimbise et al., 2009). Among the children, the IA values obtained for both boiled and mashed OFSP were good (88%), indicating that both OFSP preparations were acceptable (Spehar et al., 2002). These results confirmed the values obtained by van Jaarsveld et al. (2005) in South Africa in school-aged children, and according to Tomlins et al. (2007) new OFSP varieties were generally accepted by young children.

Conclusion

This OFSP cultivar, which is being grown in the Kaffrine region for the first time, was acceptable to the mothers and their children <2 years old. The methods of cooking OFSP decreased the VA contents, but the levels of retinol activity remained acceptable. Boiled, fried, and mashed OFSP were well accepted, and high scores were reported for all the sensory attributes. Cooking OFSP with fat influenced positively OFSP consumption. The success of this cultivar and its acceptability are promising for the prevention of maternal and child VA deficiency in Kaffrine, Senegal.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Sensory evaluation of improved and local recipes for children aged 6 to 23 months in Bukoba, Tanzania

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Complementary foods are foods other than breast milk or infant formula (liquids, semisolids, and solids) introduced to an infant to provide nutrients as well as energy. To ensure sustainable consumption of the improved recipes, sensory evaluation is important to assess acceptability of the modified recipes among the targeted consumers or population. This study was to assess sensory attributes of the improved recipes for children in two rural villages of Tanzania. Consumer preference of eight recipes was assessed using a nine-point hedonic scale. The results revealed that the improved recipe of 'Katogo' dry beans with amaranth and palm oil and 'Katogo' fresh beans, pumpkin leaves and sunflower oil had the highest scores for colour (7.9 to 8.1), aroma (7.6 to 7.7), taste (7.6 to 7.8), texture (7.7 to 7.8) and overall acceptability (7.8 to 7.9) compared to local recipe of 'Katogo' steamed sardines and improved 'Katogo' with groundnut flour and pumpkin. Recipe 'Katogo' dry beans, amaranths and palm oil was the most preferred recipe compared to other recipes due to its colour, aroma, and taste, which resulted after adding red palm oil and amaranth. Likewise, the preference could have been contributed by the ingredients used in the recipes, which were similar to the local ones. This suggests that the modification improved the nutrient contents without affecting the preference. Porridge prepared using maize flour and orange fleshed sweet potatoes scored the highest for all attributes compared to porridge that had eggs in it (recipe egg porridge) and plain local maize porridge (recipe local maize flour porridge). Knowledge on nutrition education to enable community to accept other tastes than their own for better choice of healthy food is highly recommended.

Key words: Improved recipes, sensory evaluation, infant.

INTRODUCTION

Sensory evaluation is very important for assessing acceptability of developed, improved or modified foods. It

is a scientific discipline that is used to analyze and interpret reactions of consumers as related to the

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characteristics of products perceived through the senses of sight, smell, touch, taste and hearing (Svensson, 2012). Sensory attributes of products include colour, aroma, taste, texture (mouth feel), and overall acceptability (Svensson, 2012). Sensory evaluation is also important in assessing acceptability of complementary foods as perceived by mothers or care takers (Phang and Chan, 2009). Tests conducted in sensory evaluation are used to quantify the consumer preference or degree of liking/disliking of a product (Lawless and Heymann, 2010). This study evaluated the sensory attributes of five improved complementary foods formulated to increase the nutrient content of iron, vitamin A and protein in the foods. The purpose was to offer families the opportunity to feed their infants on improved recipes using low cost and locally available foods. The study used mother and/or caregivers and female and male representing the community as the panelist, because they are representative of consuming population. The advantage of using the aforementioned panelists is that they are the representative and/or end users and they give a real life assessment (Xazela et al., 2011).

Worldwide both vitamin A deficiency (VAD) and iron deficiency anaemia (IDA) affected over two billion people, among them 190 million preschool-age children are affected by IDA (Bailey et al., 2015; Detzel and Wieser, 2015). In Tanzania, about one third of children below five years of age are vitamin A deficient (TDHS-MIS, 2015-2016). Similarly, 60% of children below five years of age suffered from iron deficiency and of which 58% are anaemic and 45% of women of reproductive age are anaemic (TDHS-MIS, 2015-2016). The nutrition situation of children of age below five years in Kagera region, north western of Tanzania in the Lake Victoria basin is alarming. Report by NBS (2011) and TDHS-MIS (2015-2016) indicated that vitamin A deficiency is about 47% for children below five years and 58% are anaemic. In the same region, 40% of women aged 15 to 45 are anemic. The level of under nutrition in the country is partly exacerbated by minimal emphasis on the utilization of appropriate complementary foods to control micronutrient deficiency in many communities of Tanzania. Generally, the use of local foods formulated at homes is guided by the following principles: high nutritional value to supplement breastfeeding; use of local food items; low price; and acceptability; (Muhimbula et al., 2011; Pelto et al., 2003).

Local dishes consumed by people in Bukoba Rural district of Tanzania are '*Katogo*' (banana and beans), '*ugali*' (stiff porridge: a staple food in Tanzania prepared from cereals such maize, sorghum, cassava flour, etc.) with beans, cassava mixed with beans, sweet potatoes mixed with beans and porridge made from whole maize flour. All these dishes were low in vitamin A (Godson, 2014). Furthermore, the author observed that '*ugali*', boiled banana and boiled cassava were low in iron

content when compared with the recommended dietary allowance (RDA) (Godson, 2014). In addition, the most common complementary food is maize porridge (maize floury + water) prepared as a thin gruel.

Based on the findings by Godson (2014), it was necessary to improve the dishes in order to increase the nutrient contents of the complementary foods consumed by children and the rest of population categories in rural areas of Bukoba. Sensory evaluation of the improved recipes was important for assessing acceptability (culturally and socially) of the improved dishes. The purpose of this study was to assess sensory evaluation and consumer preference of the improved recipes for children below five years of age in the banana based farming systems of Kagera region, Tanzania.

MATERIALS AND METHODS

Study area

Dietary improvement was conducted in Izimbya ward of Bukoba district in Kagera region of Tanzania. The region is located in the North-Western Tanzania, west of Lake Victoria with a population of 2,458,023. Bukoba district has a population of 289,697 (NBS, 2011). Mothers ($n = 50$) with children aged between 6 and 23 months were randomly selected from Rugaze and Izimbya villages and participated in the recipe formulation/development exercise. They were from four sub-villages of Kakindo and Kyelima (Izimbya village) and Rugaze A and Kikagati (Rugaze village). Each village had 25 mothers and/or caregivers recruited for the exercise. In the same villages, sensory evaluation was conducted.

Sample size and sampling procedure

The study involved random selected 126 panelists, with mothers and/or caregivers (involved in recipe modification) and other stakeholders representing the consuming population (male and females from the study area). Women who participated in the food modification exercise were also invited to participate in the sensory evaluation. The sample was stratified randomly drawn from a village list of households after every 5 names.

Dietary improvement/modification

Mothers and/or caregivers were involved in the modification process and all the modified recipes were improved by using common household cooking methods commonly practiced in the community. During dietary modification, mothers and/or caregivers were asked to give options of improving the type of porridge intended to be fed to children as well as to propose means of improving the local banana diets. The ingredients used for preparation of improved and local recipes are shown in Table 1.

Sensory evaluation

Affective/Consumer test was conducted in two sites of Izimbya and Rugaze A villages in Bukoba rural district. The exercise was conducted for two days in each village during day time at the village office. The first day was used to prepare and assess acceptability of

Table 1. Local and improved recipes composition and preparation technique.

Recipe code and name	Ingredients	Processing method	Nutrient contents per 100g				
			Protein	Iron	pVACs	Bioaccessibility of pVAC %/100 g	Bioaccessibility of iron %/100 g
Local recipes							
OA: <i>Katogo</i> ' steamed sardines	Local 'Katogo' with banana, bean and steamed sardines (' <i>Luompo</i> ')	Beans were sorted and washed and put into sauce pan, about 1000 ml of water were added and boiled. Banana were peeled and cut into two pieces and then put into sauce pan without water and kept apart. Beans cooked for about 56 min (partially cooked) together with its soup; 500 ml of water was added into unwashed banana and put on fire for the mixture to be cooked. While beans and banana are boiling, sorting of sardines was done and washed once with cold water to remove other dirt mixed with sardines. ' <i>Luompo</i> ' (steamed sardines) after washing and removing the dirt onions were cut and mixed with washed sardines together with salt.	22.10	Banana and bean 1.18 Steamed sardines	43.68	nd*	nd*
		Mixture of onion and sardines were put on banana leaf and tied closely by using banana ropes and then were put on top of the boiling banana and steamed for 36 min, this type of (food) sardine is called ' <i>Luompo</i> '. When the mixture of beans and banana is about to be cooked, dilution of salt with cold water was done and diluted salt was added to the food to boil until well-cooked (7 min) before taking out of the fire. Total cooking time was 99 min (Godson, 2014)	9.63	2.83	nd*	nd*	nd*
OB: <i>Katogo</i> ' stewed sardines	Local 'Katogo' with banana, bean and stewed sardines	Beans were sorted and washed and put into sauce pan, about 1000 ml of water were added and boiled. Banana were peeled and cut into two pieces and then put into sauce pan without water and kept apart. Beans cooked for about 56 min (partially cooked) together with its soup; 500 ml of water was added into unwashed banana and put on fire for the mixture to be cooked for 36 min. While beans and banana are boiling, sorting of sardines was done and washed once with cold water to remove other dirt mixed with sardines.	22.10	Banana and bean 1.18 Stewed sardines	43.68	nd*	nd*
		Tomatoes and onions were washed, peeled and chopped into large pieces. Oil heated into sauce for 5.5 min, the chopped onion were added into the heating oil and fried for 6 min followed by addition of sardines (fried for 3 min), tomatoes (fried for 4.5 min), salt (fried for 7 min), water was lastly added into it and left to boil (10 min) so as to make sauce. Total cooking time was 128 min (Godson, 2014)	9.60	2.60	nd*	nd*	nd*
OI: Local maize flour porridge	Local porridge made from maize and water (' <i>Obushesha</i> ')	Three cups of water (750 ml) put to boil. Maize flour was placed in a bowl followed by water and stirred into a smooth paste. The smooth paste was added into the boiling water gently while stirring and cooked for 24 min (Godson, 2014)	nd*	nd*	nd*	nd*	nd*

Table 1. Contd.

Improved recipes							
OC: Banana purée 'Nshakala' dry bean	'Katogo' with 'Nshakala' (East African highland banana-EAHB) variety dry red kidney beans, amaranths, palm oil	Beans were sorted and washed, placed in saucepan, water was added to immerse beans, then soaked for 8-12 h, soaking water poured out to reduce phytates (Pereira et al., 2014; Saunders et al., 2013). Soaked beans were placed in saucepan, and immersed in water, covered with lid and boiled till tender (60 to 90 min). While beans boiling, red palm oil fruits were cleaned placed in a saucepan with 500 ml of water, covered and boiled for 10 min. Water was poured out and cooled. Red palm oil was pounded in a traditional mortar. About 500 ml of water were added and wringed to get red palm oil. Green banana were peeled using a sharp knife and cut into two pieces per finger then were immersed in water to avoid browning. Bitter tomatoes were cut into two halves and added to the saucepan with bananas. Onions and tomatoes were cut into small pieces and kept aside. The peeled and chopped green banana, onion, tomatoes, bitter tomatoes and red palm oil were added to the boiling beans at 40-70 min of cooking beans. Water was added to submerge the mixture and covered with banana leaves followed by a lid and cooked covered for 20 min (making the 60-90 min). Amaranth leaves were sorted and cleaned and kept aside. To reduce surface area for nutrient loss amaranths were cut into large pieces using a sharp knife. After 20 min amaranth were added to the boiling mixture. Salt dissolved (1 teaspoon) in ¼ cup of water and was added to the cooked food, then the ingredients were mixed by stirring and covered to cook for 7 min. The soup strained and kept aside. Food was mashed into a thick, smooth puree, the consistency depended on the child's age, and the strained soup was added back to the mashed food to avoid loss of nutrient. Total cooking time ranged from 67-97 min (using fire wood in two different villages) and 60 min (using electric cooker)	3.40	9.10	58.10	7.9	16.4
OD: Banana purée 'Nshakala' fresh bean	Katogo' with 'Nshakala' , fresh red kidney beans, pumpkin leaves, sunflower oil,	Fresh red kidney beans were removed from their pods, sorted, cleaned with water, and placed in a saucepan followed by addition of water. To reduce phytates, beans were boiled for 5 min and water poured out. Water was added to the beans and then covered with lid and boiled till tender (45 to 60 min). While beans boiling green banana were peeled using a sharp knife and cut into two pieces per finger then were immersed in water to avoid browning. Bitter tomatoes were cut into two halves and added to the saucepan with banana. Onions and tomatoes were cut into small pieces and kept aside. The peeled and chopped green banana, onion, tomatoes, better tomatoes and sunflower oil were added to the boiling beans at 25 to 40 min of cooking beans and water was added to submerge the mixture. The mixture covered with banana leaves followed by a lid and cooked covered for 20 min. While mixture boiling pumpkin leaves were sorted and cleaned and kept aside. To reduce surface area for nutrient loss, the leaves were cut into large pieces using a sharp knife. After the above 20 min pumpkin leaves were added to the boiling mixture. One teaspoon of salt dissolved in ¼ cup of water and was added to the cooking food. The ingredients were mixed by stirring and covered and cooked for 7 min. Soup were strained and put aside. Food mashed into a thick, smooth puree, the consistency depends on the child's age. To avoid loss of nutrient all the strained soup were added to the mashed food. Total cooking time ranged from 52-67 min (using fire wood in two different villages) and 45 min (using electric cooker)	2.80	6.10	44.5	12.2	18.4

Table 1. Contd.

OE: 'Katogo'l' matoke purée	'Katogo' with 'Nshakala' variety, pumpkin fruit, groundnuts flour,	<p>Groundnut were sorted and roasted to dry. Left to cool and pounded in a mortar and sieved to get fine groundnut flour.</p> <p>Green banana was peeled using a knife, cut into at least two pieces per finger and placed into a cooking saucepan with water to avoid browning. Pumpkin fruit cleaned with water, cut into small pieces and peeled with a knife. Pumpkin pieces were added into the peeled banana in a saucepan. Bitter tomatoes were into two halves added to the saucepan with banana and pumpkin pieces.</p> <p>Onions and tomatoes were washed, cut into small pieces and added to the mixture in a cooking saucepan and mixed well. Groundnut mixed with 1 cup of water and mixed to a thick puree. Then, puree added to the mixture and mixed well using a cooking ladle/spoon. Water was added to the mixture to submerge the ingredients. The mixture was covered using banana leaves followed by a lid and cooked for 20 min.</p> <p>One teaspoon of salt was dissolved into ¼ cup of water. After 20 min salt was added to the boiling mixture and cooked for 5 min.</p> <p>For children above 11 months the food was allowed to cool and served to the child or kept in a covered dish. Banana puree was prepared for children aged 6-11 months. Half a cup of soup from food was kept apart. One cup of food was placed in a bowl and mashed to make it soft. The remaining soup was added to the mashed food and mixed to get a thick puree.</p> <p>Total cooking time was 25 min (using fire wood in two different villages) and 25 min (using electric cooker)</p>	3.8	1.9	137	26.7	12.2
OF: Orange-fleshed sweet potato porridge	Porridge made from fermented maize flour, Orange- fleshed sweet potato (OFSP), groundnuts, and sugar	<p>One cup (250 ml) water and 100 g of maize flour were placed in a bowl and left for 24 h to ferment.</p> <p>Groundnut were sorted and roasted to dry, then pound in a motor to get fine groundnut flour.</p> <p>Unpeeled OFSP washed well with clean water and placed in a saucepan followed by 2 cups of water and covered with banana leaves followed by a lid. To retain nutrients OFSP boiled unpeeled for 30-45 minutes (Berti et al., 2014). Then, OFSP was hand peel, put in a dish and mashed until smooth. Water was added to the mashed OFSP to thick puree, stirred and kept aside. Four cups of water (1000 ml) were put to boil in a saucepan for 5 min. Groundnut flour was added into the fermented flour and stirred into a smooth paste. The smooth paste added into the boiling water slowly while stirring to avoid lumps. OFSP puree was added into the boiling mixture while stirring. The mixture simmered for 10 min. Sugar was added and stirred to mix well. Porridge removed, then cooled and served.</p> <p>Total cooking time ranged from 45-60 min (using fire wood in two different villages) and 30 min (using electric cooker).</p>	3.70	1.60	20.6	31.1	25.9

Table 1. Contd.

OH: Egg porridge	Porridge made from fermented maize flour, eggs, dry red beans, and sugar	<p>Beans were sorted, cleaned with water, and placed in a saucepan followed by addition of water to immerse beans and soaked for 8-12 h to reduce phytates. Soaking water poured out and coats were removed.</p> <p>Beans were placed in a saucepan followed by addition of water to immerse the beans. Beans covered with lid and boil till tender (60-90 min). Beans were mashed and ½ cup of water was added to the mashed beans and keep apart.</p> <p>Four cups of water (1000 ml) put to boil for 5 min. While waiting for water to boil, mashed beans were added into fermented maize flour and stirred into a smooth paste. The smooth paste was added into the boiling water gently while stirring and cooked for 10 min. One egg added to one portion of porridge for baby. This was done after beating the egg and poured it gently into the baby's portion while stirring. Sugar added as an optional.</p> <p>Total cooking time ranged from 75-105 min (using fire wood in two different villages) and 60 min (using electric cooker)</p>	3.00	3.50	1.1*	12.5	nd*
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PVACs: Pro-vitamin A carotenoids; For both recipes water added but not weighed 1.1* ~100 g of egg has 227.0 RE μ g which meet 56.8% RDA vitamin A (Miranda et al., 2015). nd*: not determined.

Source: Mbela et al. (2018).

improved recipes only. On the second day, both improved and local dishes were prepared and tested. This allowed having time for training panelists before the testing exercise for descriptive test in day two. The sensory evaluation involved 88 females and 38 males of age between 17 and 78 years making a total of 126 panelists. Adult panelists were used instead of children, because of their ability to objectively evaluate the sensory attributes of the improved recipes. Panelists were from Kakindo, Kyerima (Izimbya village) and Kikagati, Rugaze A and Rugaze B sub-villages (Rugaze village).

All the panelists were asked to complete the consent form before the taste. Three modified and two local banana-based and two modified and one local porridge-based recipes (Table 1) were tested. The improved and local recipes were subjected to sensory evaluation to test the preference. For improved recipes only, the food recipes were organoleptically evaluated for preference test using 85 (females = 58, males = 27) untrained panelists (Affective test). Izimbya village had 45 and Rugaze village had 40 participants. A panel of 41 (30=females, 11=males) of breastfeeding mothers and/or caregivers and male and female from different sub-villages were recruited to evaluate the preference of the improved and local diets for

descriptive tests. Izimbya village had 21 participants and 20 participants were from Rugaze A, Rugaze B and Kikagati villages.

The panelists were asked to express their degree for liking the sensory attributes using a 9-point hedonic scale, where 9 (like extremely) was the highest and 1 (dislike extremely) was the lowest score. Thus, 1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely (Muhimbula et al., 2011; Lim, 2011). Five ranking score was used to choose the most preferred recipes and the lowest score rated as most preferred and the highest score as least preferred (1=most preferred, 5=least preferred) (Meludu and Fakere, 2013). The samples were evaluated by the panelist for aroma (flavour), texture, colour and overall acceptability. All principles of good sensory practices were followed (Muhimbula et al., 2011). The coded improved and local recipes were presented to each of the panelist. Each panelist was given a serving plate, spoon, cup and a bottle of water to rinse their mouth after each taste to avoid being biased or influenced. The panelists were served with small amount of food sample. Conditions during tasting included rinsing the mouth with

water between each recipe to remove the taste of the previous food; panelists were separated during interview for them not to influence each other. The hedonic test was used to quantify the consumer preference of the recipe (Svensson, 2012).

Statistical analysis

Statistical analyses were performed using R statistical package (R Development core team, version 3.00, Vienna, Austria). Two way ANOVA was used for parametric test (hedonic data) and Friedman and Wilcoxon tests were used for non-parametric data (preference ranking). Tukey Honest Significant Difference (THSD) was used to separate means. Least Significant Rank Difference (LSRD) was used to separate median at $p \leq 0.05$. Data on descriptive test was analysed by Principal Component Analysis (PCA).

Ethical clearance and consenting

The community and individuals were informed about the

Table 2. Characteristics of consumer panelists 1 (N=41) and 2 (N=85).

Attribute	Category	Frequency (N)	Percentage
Panelists 1 (N=41)			
Age (Years)	20-35	24	58.5
	36-45	9	22
	Above 45	8	19.5
Sex	Male	11	26.8
	Female	30	73.2
Education level	No formal education	5	12.2
	Primary education	26	63.4
	Secondary education	8	19.5
	Post-secondary education	2	4.9
Occupation	Farmers	39	95.1
	Formally employed	2	4.9
Panelists 2 (N=85)			
Age (Years)	17-35	50	58.8
	36-45	22	25.9
	Above 45	13	15.3
Sex	Male	27	31.8
	Female	58	66.2
Education level	No formal education	9	10.6
	Primary education	61	71.8
	Secondary education	12	14.1
	Post-secondary education	3	3.5
Occupation	Farmers	78	91.7
	Formally employed	7	8.3

study. A written consent to participate in the study was obtained from the panelists. Ethical clearance was obtained from the National Institute for Medical Research (NIMR) permit number NIMR/HQ/R.8a/Vol.IX2202. Permission to conduct the study was obtained from regional, district and ward authorities.

RESULTS

Panel characteristics for tested local and improved recipes

Table 2 shows that the characteristics of consumer panel. For sensory evaluation of local and improved recipes, the panel was dominated by female panelists (73%) of age between 20 and 35 years (59%). About 95% were farmers, 63% had attained primary school education. The consumer panel for improved recipes only was also

dominated by female panelists (66%) of age between 17 and 35 years (59%). Most of them have attained primary school education (72%) and 92% of them were farmers.

Preference test for local and improved recipes

Local and improved porridge

Table 3 shows the results for mean hedonic scores for porridge. The scores differed significantly ($p < 0.05$) among panelists in terms of liking of colour, aroma, taste, texture and overall acceptability. Porridge prepared using maize flour and orange fleshed sweet potatoes (recipe OF) had the highest scores as compared to porridge that had eggs in it (recipe OH) and plain local maize porridge (recipe OI). The orange fleshed sweet potato porridge

Table 3. Mean hedonic values of porridge recipes (improved and local) (N=41).

Recipe name	Recipe code	Color	Aroma	Taste	Texture	Acceptability
OFSP porridge	OF	8.3±0.76 ^a	7.9± 1.32 ^a	8.2±1.15 ^a	7.9± 1.40 ^a	8.9 ±0.92 ^a
Egg porridge	OH	6.4±1.60 ^b	6.4±1.60 ^b	6.6± 1.43 ^b	6.9± 0.74 ^b	6.7± 1.38 ^b
Popular maize flour porridge	OI	6.4±1.40 ^b	6.4±1.12 ^a	6.4±1.27 ^b	6.9 ± 1.21 ^b	6.4 ±1.33 ^b

Values are expressed as mean ±SD (N=41). Mean values with different superscript letters along the column are significantly different at p<0.05.

Table 4. Sensory attributes of local and improved banana recipes (N=41).

Recipe Name	Recipe code	Color	Aroma	Taste	Texture	Acceptability
'Katogo' steamed sardines	OA	6.4±1.23 ^c	6.9± 1.19 ^b	7.1±1.14 ^{bc}	6.6± 1.09 ^b	6.7 ±1.06 ^c
'Katogo' stewed sardines	OB	7.6±1.12 ^{ab}	7.2±1.04 ^{ab}	7.3± 1.08 ^{abc}	7.4± 0.74 ^a	7.5± 1.07 ^{ab}
'Katogo' dry beans	OC	8.1±1.04 ^a	7.7±1.10 ^a	7.8±1.00 ^a	7.8 ± 1.04 ^a	7.9 ±1.00 ^a
'Katogo' fresh beans	OD	7.9±0.75 ^a	7.6±0.85 ^a	7.6±0.7 ^{ab}	7.7± 0.59 ^a	7.8±8 0.83 ^a
'Katogo' nuts pumpkin	OE	7.0± 1.16 ^{bc}	7.0± 1.12 ^b	7.0±1.48 ^c	7.5 ±0.84 ^a	7.0 ±1.26 ^{bc}

Values are expressed as mean±SD (N=41). Mean values with different superscript letters along the columns are significantly different at p<0.05.

had higher scores for colour, aroma, taste, texture and overall acceptability than porridge with eggs and plain maize porridge. Thus porridge with orange fleshed sweet potato had high liking score by consumer compared to porridge with egg and plain maize flour porridge. There was a significant difference between the improved/modified orange fleshed sweet potato and the other two porridges (improved egg porridge and an improved local popular maize flour porridge) at p<0.05. However, there was no significant between the improved/modified egg porridge and local popular maize flour porridge at p<0.05.

Results of the mean hedonic scores for different recipes are shown in Table 4. The improved 'Katogo' of dry beans (recipe 3 OC) and 'Katogo' fresh beans (recipe OD) had the highest scores for colour, aroma, taste, texture and overall acceptability compared to local 'Katogo' steamed sardines (recipe OA) and improved 'Katogo' of groundnuts and pumpkin (recipe OE). These recipes had the lowest scores for colour, aroma, taste and overall acceptability. Furthermore, local 'Katogo' steamed sardines (recipe OA) recipe had the lowest texture score compared to local 'Katogo' stewed sardines (recipe OB) and improved 'Katogo' of dry beans (recipe OC).

Moreover, the Principal Component Analysis (PCA) biplot multivariate analysis (Figure 1) showed that, principal component 1 (PC1) accounted for 93.2% of variability in panelist (trained) liking of recipe and principal component 2 (PC 2) accounted for only 5.9%. The PC 1 was a contrast between recipes of improved 'Katogo' of dry beans (recipe OC) and 'Katogo' fresh beans (recipe OD); these were highly associated with attributes of aroma, colour, taste and overall acceptability compared to other recipes that had low scores in these attributes. Further

analysis (open ended questions) showed that 73% of the panelists liked recipe OC due to its ingredients, 15% liked it due to good aroma and taste and the rest (12%) did not like it.

There was significant difference in rank sum between 'Katogo' dry beans (recipe OC) and 'Katogo' stewed sardines (recipe OB) having lowest scores of 75 and 113, respectively (Table 5) and other recipes (recipes OA, OD and OE). This shows that the two recipes were most preferred compared to other recipes using a 1 to 5 scale (1 denotes most preferred and 5 lowest preferred).

In addition, PC 1 accounted for 99.6% of the systematic variation in liking and it is a contrast between OFSP porridge recipe associated with liking of all attributes on one side and other recipes with no attributes associated to it (Figure 2). This supported the ANOVA results, which showed higher liking scores of OFSP porridge recipe for all attributes.

Preference test

The rank sum values differed significantly (p<0.05) between the porridge recipes with OFSP porridge having lowest values of 49 (Table 6) than other recipes, which suggests that it was the most preferred recipe. No significant (p>0.05) variation was observed in preference between porridges with eggs and plain local maize.

Improved recipes only

Table 7 shows that the results for mean hedonic scores of improved recipes. There were significant (p < 0.05)

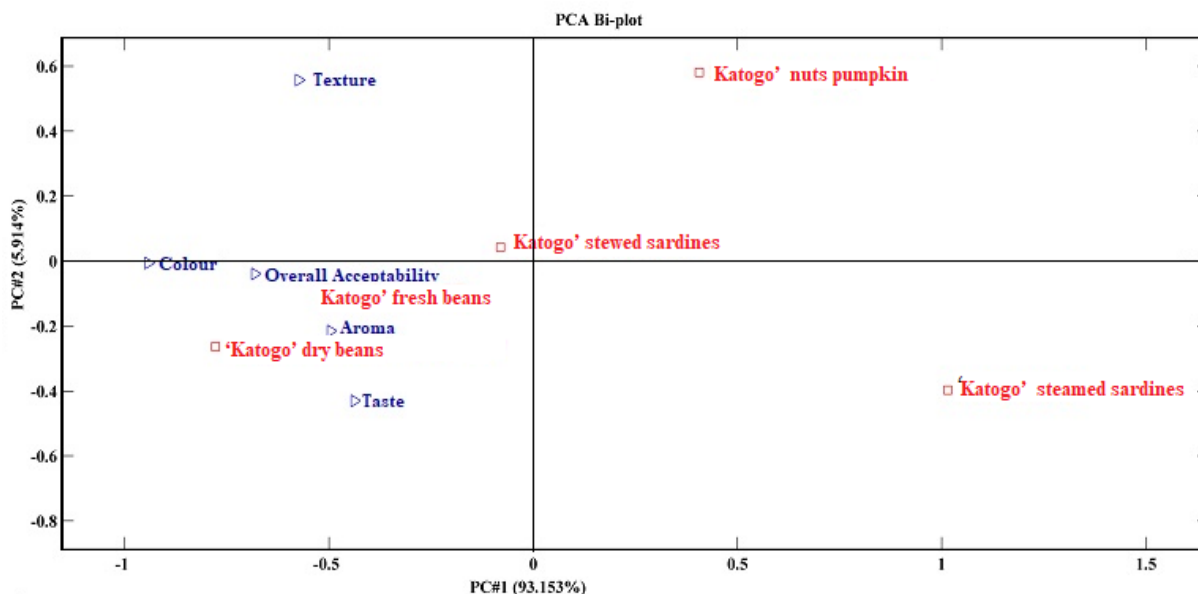


Figure 1. PCA Bi-plot showing systematic variation of recipes and liking of sensory attributes.

Table 5. Median and Rank sum values of local and improved recipes (N=41).

Recipe Name	Recipe code	Median	Rank sum
'Katogo' steamed sardines	OA	3	129 ^b
'Katogo' stewed sardines	OB	3	113 ^{ab}
'Katogo' dry beans	OC	2	75 ^a
'Katogo' fresh beans	OD	4	152 ^b
'Katogo' nuts pumpkin	OE	3	133 ^b

Friedman chi-square = 36.915, p-value = 1.875e-07 and Least Significant Rank Difference (LSRD) is 40. Mean values with different superscript letters along the column are significantly different at p<0.05. Scale (1=most preferred, 5= least preferred).

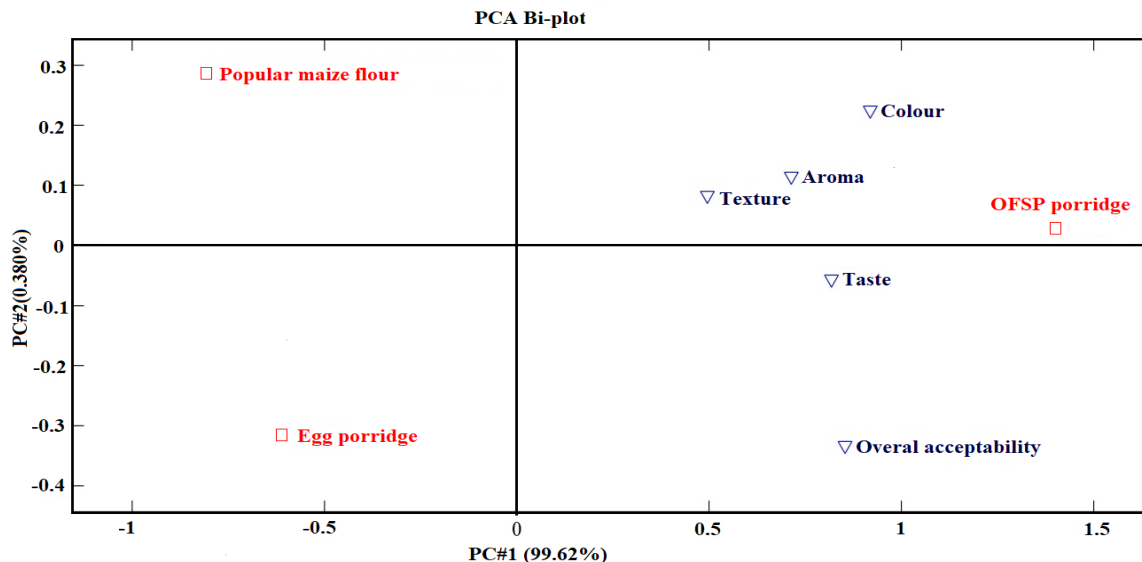


Figure 2. PCA Bi-plot showing systematic variation of porridge recipes and liking of sensory attributes.

Table 6. Median and Rank sum of porridge local and improved recipes (N=41).

Recipe Name	Recipe code	Median	Rank sum
OFSP porridge	OF	1	49 ^a
Egg porridge	OH	2	90 ^b
Local maize flour porridge	OI	3	101 ^b

Friedman chi-squared = 37.55, p-value = 7.017e-09 and the Least Significant Rank Difference (LSRD) is 21. Mean values with different superscript letters along the column are significantly different at p<0.05. Scale (1=most preferred, 5= least preferred).

Table 7. Sensory attributes of improved banana recipes (N=85).

Recipe name	Recipe code	Color	Aroma	Taste	Texture	Acceptability
'Katogo' dry beans	OC	8.3±0.92 ^a	8.1± 1.03 ^a	8.2±1.17 ^a	8.1± 1.30 ^a	8.2 ±1.25 ^a
'Katogo' fresh beans	OD	8.2±0.79 ^a	8.0±0.85 ^{ab}	8.0± 0.89 ^a	8.0± 0.88 ^a	7.9± 0.94 ^{ab}
'Katogo' nuts pumpkin	OE	7.5±1.05 ^b	7.8±0.98 ^b	7.9±1.10 ^a	8.1± 1.04 ^a	7.8 ±1.10 ^b

Values are expressed as mean ±SD (N=85). Mean values with different superscript letters along the column are significantly different at p<0.05.

Table 8. Median and Rank sum of improved banana recipes.

Recipe Name	Recipe code	Median	Rank sum
'Katogo' dry beans	OC	1	119 ^a
'Katogo' fresh beans	OD	2	203 ^b
'Katogo' nuts pumpkin	OE	2	186 ^b

Friedman chi-squared = 47.719, p-value = 4.344e-11 and the Least Significant Rank Difference (LSRD) is 31. Mean values with different superscript letters along the column are significantly different at p<0.05. Scale (1= most preferred, 5= least preferred).

Table 9. Mean hedonic values of the improved porridge recipes.

Recipe name	Recipe code	Color	Aroma	Taste	Texture	Acceptability
OFSP porridge	OF	7.6±1.9 ^a	7.8±1.65 ^a	8.2±1.19 ^a	7.8±1.38 ^a	8.2±1.25 ^a
Egg porridge	OH	7.3±1.55 ^a	7.2±1.58 ^b	7.4±1.64 ^a	7.5±1.70 ^a	7.5±1.66 ^b
Statistics						
t value	-	-1.0891	-2.3655	-4.0422	-1.1543	-3.332
P value	-	0.2791	0.020	0.0001168	0.2517	0.001284

Values are expressed as mean ±SD (N=85). Mean values with different superscript letters along the column are significantly different at p<0.05.

mean hedonic scores of colour, aroma and overall acceptability difference among improved recipes. 'Katogo' dry beans (OC) and 'Katogo' fresh beans (recipe OD) recipes had similar highest scores on colour, aroma, and overall acceptability compared to scores for the recipe with groundnut flour and pumpkin (OE). No significant variation (p>0.05) in taste and texture were observed among recipes.

Furthermore, there was significant (p<0.05) difference in rank sum between the recipes 'Katogo' dry beans (recipe OC) and other recipes. 'Katogo' dry beans had lowest score of 119 (Table 8). This shows that 'Katogo'

dry bean (recipe OC) was the most preferred recipe as compared to other recipes.

Improved porridge only

The variations between mean hedonic values of aroma and overall acceptability between two improved porridge recipes were significant. OFSP porridge recipe had higher scores compared to the recipe with eggs (Table 9). No significant variations were observed in attributes of colour, taste and texture between the two recipes.

Preference test (by Paired recipe Wilcoxon test) N=85

The rank sum values differed significantly ($p < 0.05$) between the porridge recipes with OFSP porridge having the lowest scores (110) compared to porridge with eggs (146). This shows that OFSP porridge was most preferred.

DISCUSSION

Organoleptic evaluation was carried out to determine acceptability and preference of the improved dishes as compared to the local complementary foods. Untrained panelist of mothers and/or caregivers and other stakeholders in the study area participated in this exercise. Affective/Hedonic tests were used to quantify consumer responses with regard to their preferences of the recipes (Svensson, 2012). Panelists were recruited to evaluate the preference of the improved and local diets for descriptive tests.

Generally, all the improved recipes were accepted by all panelists. However, '*Katogo*' dry beans (recipe OC) and '*Katogo*' fresh beans (recipe OD) were highly preferred among panelists because of taste, aroma (flavor), and colour attributes. This could have been contributed by the ingredients used in the recipes, which were similar to the local ones. The only difference was the addition of amaranth, palm oil and pumpkin leaves. This suggested that the modification improved the nutrient content without affecting the preference. The recipes were energy- and nutrient-dense; likely to be acceptable; affordable; and sustainable because they were made from culturally appropriate and daily consumed commodities. On other hand, there was an association of high liking scores with taste, aroma (flavor), and colour attributes for the same recipes. '*Katogo*' dry bean (recipe OC) was the most preferred recipe compared to other recipes. This could be due to its colour, taste and aroma that resulted after adding red palm oil and amaranth. The attractive colour was due to palm oil (golden red colour), which contributed to the colour of the food. Colour attribute scored higher than other attributes. The colour attribute is very important for consumer preference, the product might be having high nutritional value but without attractive colour such a product is likely to be not liked by consumers (Tirhas et al., 2015). Colour is a primary attribute, which attracts consumer attention and therefore influences acceptance (Tirhas et al., 2015). Despite contribution of palm oil to colour of the food, red palm oil is a potential source of vitamin A. Texture was an important attribute for consumer preference of the local recipe '*Luompo*' (recipe OA), which scored poorly because panelists disliked the texture. '*Katogo*' dry beans (recipe OD), '*Katogo*' fresh beans (recipe OD) and '*Katogo*' pumpkin seed flour

(recipe OE) recipes did not differ in scores for texture, because the consistency was almost the same.

Recipe OC ('*Katogo*' dry bean) and OB ('*Katogo*' stewed sardines) were significantly most preferred recipes compared to others recipes on a 1 to 5 scale. The second was a local diet that was preferred by panelist due to banana bean sardine mixture. This is due to the fact that this was the only animal (fish) source recipe, which is most expensive and therefore consumed occasionally (Kennedy et al., 2010).

Orange fleshed sweet potatoes (OFSP) porridge (recipe OF) scored the highest for all attributes including overall acceptability than other porridges. High score on colour of OFSP porridge could have been contributed by the orange colour of OFSP, which made the porridge to have an orange colour that appealed to panelists. A similar observation was made by Tirhas et al. (2015) where porridges prepared using OFSP were sensorially preferred. Colour is a primary attribute which attracted consumer attention and therefore influences the mind of the consumer (adult and children) when choosing food and therefore acceptance (Pobee et al., 2017). Therefore, to attract consumers, food colour is an important attribute to be considered when preparing food. The low score for recipe OH was attributed to the inclusion of egg which resulted in an aroma not quite familiar to the consumer. Basically the smell of eggs was repulsive and affected acceptability. A study conducted in Ghana (Pobee et al., 2017) observed that the porridge blended with an egg was scored low by panelists due unfamiliar aroma. The basic nutrition education given to mothers during discussions about dietary modification enhanced their knowledge on nutrition and was able to identify recipes that are less nutritious. Recipe IO scored 70% as being not nutritious. This was reason why the local porridge was least preferred compared to all other recipes.

Overall, mothers liked the new recipes and were willing to continue using them and prepare for their children because the local foods are culturally appropriate acceptable, affordable, feasible, and sustainable and are consumed on a daily basis (Ayoya et al., 2010). Five recipes were developed and most of the ingredients can be replaced by other foods from the same food group to make more recipes with similar nutritional values.

Conclusion

The results showed that the improved recipes as complementary foods which are nutrient-dense were accepted by the panelists. The improved banana recipes were preferred to local recipes. Thus, the three improved banana-based and two porridge-based recipes were in generally preferred by panelists. Mothers liked the new recipes and were willing to adopt and continue using

them to feed their children because they are prepared from local foods which are culturally appropriate and acceptable. Consumption of the improved complementary food by children aged 6 to 23 months could help solve the problem of vitamin A, iron and protein energy malnutrition. The community needs nutrition education for better choice of healthy foods for their children to eat. Sensory evaluation is an important component in developing complementary foods.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Effects of germination and defatting of the soybean and sesame on the mineral bioavailability and absorption of sweet potato based complementary diets

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The effect of germination of soybean and sesame seeds on the bioavailability of minerals of complementary foods was investigated using rats. Formulations of complementary diets 1 to 4 were produced from sweet potato, soybean and sesame flours at a ratio of 60:30:10, respectively. Formulated diets were significantly higher ($p<0.05$) in protein, fat and moisture content and lower in carbohydrate and crude fibre than in control. Diet 4 was significantly higher ($p<0.05$) in minerals (Ca, Zn and Fe) content than in control. The formulated diets were generally low in phytate and oxalate contents, while phytate content was much lower in the diets from germinated flour blends. Anti-nutritional factor (ANF): mineral molar ratio suggested good mineral absorption from the formulated diets. Mineral content of the tissues of rats fed with the formulated diets indicated good absorption within the rats; rats fed with the germinated flour blends had significantly higher ($p<0.05$) zinc, iron and calcium contents of tissues when compared. Diet 4 consistently received better rating among the samples.

Key words: Complementary food, sweet-potato, germination, anti-nutrient, micronutrient.

INTRODUCTION

Complementary feeding is said to be the process starting from when breast milk alone is no longer sufficient to meet the nutritional requirements of infants and therefore other foods and liquids are needed, along with breast milk (Dewey, 2001). The target age range for complementary feeding is generally taken to be 6 to 24 months of age, even though breastfeeding may continue beyond two years (Cohen et al., 1994; WHO, 2005). Due to their high demand and rapid growth, malnutrition starts in many infants, contributing to the high prevalence of

malnutrition in children less than two years of age (FAO, 2011). In many developing countries, complementary diets are derived mainly from local staples such as cereals and tubers, with animal proteins as supplements. However, since animal protein is expensive, attempts have been made to identify alternative sources of protein especially from plants (Ikujenlola et al., 2013).

The high content of anti-nutritional factors and poor availability of minerals in plant-based foods as well as losses during processing play a vital role in micronutrient

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deficiency (Solomon, 2005). Meanwhile, minerals such as iron, zinc and calcium play a vital role in growth, health and development of infants (Tizazu et al., 2011). The commercially standardized foods are generally very good and can help meet the nutritional requirements of young children in both developed and developing countries (Obiakor-Okeke et al., 2014). But as in most other developing countries, the high cost of nutritious proprietary complementary foods is always, if not prohibitive, beyond the reach of low income families. Such families often depend on inadequately processed traditional foods consisting of non-supplemented cereal porridges made from maize, sorghum and millet (Solomon, 2005). According to Gibson et al. (2010), complementary foods based on either root or tuber crops have been shown to be significantly lower in phytate (by 3 to 20%) than cereal and legume based foods. Also, cereal-based complementary foods form a relatively high viscous porridge which requires excessive dilution usually with water to reduce viscosity to be appropriate for infant feeding which leads to 'energy and nutrient thinning' (Amagloh et al., 2013). Therefore, at the household level sweet potato based complementary food has been suggested as a more suitable complementary food as it would be lower in starch, resulting in less viscous porridge as compared with a maize-based infant product in Ghana (Amagloh et al., 2012).

The nutrient potential of soybean and sesame seeds informed the supplementation with a sweet potato based complementary food. Several reports have been documented on sweet potato complementary foods (Sanoussi et al., 2013; Oyareku, 2013; Obiakor-Okeke et al., 2014; Amagloh et al., 2012, 2013), however, bioavailability and absorption of calcium, zinc and iron from a sweet potato-based complementary food blended with soybean and sesame seeds flours has not been reported. It was envisaged that germination would help to breakdown complex polysaccharides and reduce anti-nutrients present in soybean and sesame seeds, and thus improving digestibility and nutrient availability, while the defatting would help to reduce the incidence of rancidity, and therefore improve the keeping quality of the diets. The objective of this study, therefore, was to evaluate the effects of germination and defatting of the soybean and sesame on the proximate and mineral contents of sweet potato based complementary diets.

MATERIALS AND METHODS

Yellow fleshed sweet potato, soybeans and sesame seeds were purchased from Bodija Market in Ibadan. A commonly used proprietary infant cereal (Nestle Cerelac, South Africa) or children (6 to 24 months) was used as reference sample, and was purchased at a supermarket in Ibadan. The proximate composition of Nestle Cerelac was protein (15%), fat (10%), fibre (2%), ash (3%), and carbohydrate (68%). Male and female Wistar weanling rats of about 4 weeks old weighing between 40 and 55 g, purchased from the Faculty of Veterinary Medicine, University of

Ibadan, were used in the animal experimentation aspects of the study.

Preparation of sweet potato flour

The sweet potato flour was prepared using the method of Obiakor-Okeke et al. (2014) and sesame seed flour was produced using the method of Makinde and Akinoso (2013). Sweet potato roots were washed thoroughly with water to remove adhering soil particles, the washed roots were drained, peeled using sharp knives and uniformly chipped into slices of about 2 mm thickness to facilitate drying using kitchen plantain slicer. The sweet potato chips were thinly spread on aluminium foil lined oven cabinet (Genlab, QE/200, Widnes, Cheshire, United Kingdom) and dried at 65°C to a constant weight, and milled using the locally fabricated attrition mill, the obtained sweet potato flour was sieved using a 0.25 mm British standard sieve (Model BS 410) and kept in an airtight polyethylene bag until further processing.

Preparation of soybean flour

Soybean flour was prepared using the method of Malomo et al. (2012). The soybeans were cleaned, picked manually and winnowing to remove all forms of foreign particles and defects. The soybeans were divided into two portions; the first portion was not subjected to any treatment. The second portion of soybeans where the soybean was washed with 0.7% sodium hypochlorite solution, drained and soaked in water for 6 h. After soaking, soybean was spread thinly in muslin-lined trays and allowed to germinate for 72 h, followed by drying in the oven at 65°C to 10% moisture content. During the period of germination, the seeds were intermittently sprinkled with water to facilitate germination. The dried germinated soybeans and non-germinated soybeans were milled using the locally fabricated attrition mill, the obtained flour samples were sieved using a 0.25 mm British standard sieve (Model BS 410) and kept in separate airtight polyethylene bag until further use.

Preparation of sesame flour

The sesame seeds were cleaned, manually picked and winnowed to remove all forms of foreign particles and defective seeds before processing commence; the sesame seeds were also divided into two portions, the first was not subjected to any treatment. The second portion of the sesame seeds were soaked in water for 6 h, drained and spread thinly in a muslin-lined tray, allowed to germinate for 72 h and sprinkled with water intermittently to facilitate germination. The germinated seeds were collected, dried in the oven (Genlab, QE/200, Widnes, Cheshire, United Kingdom) at about 40°C to a constant weight. The non-germinated seeds and the germinated seeds were milled using locally fabricated attrition mill, the obtained meal was kept in separate airtight polyethylene bags until further processing. The obtained meal from non-germinated sesame seeds and germinated sesame seeds were divided into two portions each, the first portions was kept as full-fat non-germinated sesame and full-fat germinated sesame seeds in airtight bags until further processing. While, the second portions were defatted by cold maceration using Unal and Yalcsn (2008) method, the meals from non-germinated sesame seeds and germinated sesame seeds were separately soaked in n-hexane at room temperature for 72 h, during which they were stirred intermittently. The supernatant was collected and the process was repeated three times using fresh solvent each time. The resulting defatted flours were air dried in hot air oven at 45°C (Genlab, QE/200, Widnes, Cheshire, United Kingdom) for 24 h and kept in airtight polyethylene bags for further use.

Table 1. Formulation of Experimental Diets based on different levels of Sweet Potato, (SPF) Soybean (SBF) and Sesame Seed (SSF) Flours.

Diet	Ingredient	Ratio
1	SPF : SBF : SSF	60:30:10
2	SPF : SBF _g : SSF _g	60:30:10
3	SPF : SBF : SSF _d	60:30:10
4	SPF : SBF _g : SSF _{dg}	60:30:10
5	Nestle Cerelac	Reference

g: Germinated; d: de-fatted; dg: germinated and de-fatted.

Formulation of experimental diets

The experimental diets were formulated from sweet potato flour (SPF), non-germinated soybean flour (SBF), germinated soybean flour (SBF_g), non-germinated sesame seeds flour (full-fat) (SSF), non-germinated sesame seeds flour (defatted) (SSF_d), germinated sesame seeds flour (full-fat) (SSF_g) and germinated sesame seeds flour (defatted) (SSF_{dg}) using the methods of Adenuga (2010) with some modifications which are shown in Table 1.

Chemical analysis of flours and formulated diets

Moisture content was determined using the method described by AOAC (2006 Method No 934.01). One gram of sample in pre-weighed crucible was placed in oven (100 ±5°C) for 24 h, cooled and reweighed. The percentage moisture was calculated as:

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where W_1 = weight of crucible, W_2 = weight of crucible after drying at 105°C and sample, and W_3 = weight of crucible and sample after cooling in an airtight desiccator.

Crude protein was determined using micro-Kjeldahl method as described by Pearson (1976); percentage protein was determined using nitrogen protein conversion factor, 6.25. Ash and fibre content were determined according to AOAC (2006 Method Nos. 942.05 and 978.10), respectively. Two grams of sample was added into pre-weighed crucible and incinerated in muffle furnace at 600°C for 18 h to light grey ash. Thereafter, they were removed and placed immediately in a desiccator to cool and weighed.

$$\text{Ash (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where W_1 = weight of cleaned, dried, ignited and cooled crucible; W_2 = weight of crucible and sample after incinerating at 600°C; W_3 = weight of crucible and sample after cooling in an airtight desiccator.

The method of Pearson (1976) was employed in determining crude lipid content, where 3 g of the sample was extracted using petroleum ether, dried and weighed to obtain percentage fat extracted. The total crude carbohydrate content of the diet samples was obtained by difference (AOAC, 2006, Item 85). Minerals (Ca, Zn and Fe) contents were determined using Atomic Absorption Spectrophotometer (PerkinElmer AAnalyst 200, Shelton, Connecticut, USA). The standard curve for each mineral was prepared from known standards and the mineral value of samples estimated against that of standard curve (AOAC, 1990).

Phytate and total oxalate contents of the diets were determined using the method of AOAC (1990), standard curve was prepared for each of the anti-nutrient and their concentration in the diet samples was extrapolated from the generated standard curve and expressed as mg/100 g sample.

Rat feeding programme

Twenty male and female Wistar-strain weanling rats weighing between 40 and 55 g were randomly distributed into 5 groups of 4 rats each during the feeding experimentation (2 males and 2 females per group). They were kept in well ventilated plastic cages with wire net lid. The rats were allowed to acclimatize on the animal pellets for 7 days before feeding with the experimental diets commenced. The animals were fed a known amount of each of the diet daily, fresh clean water was also served on a daily basis. Daily records of feed intake and weights of rats as well as mortality rate were kept. The experiment lasted 21 days after which the animals were sacrificed. The rats were anaesthetized and sacrificed by cervical dislocation. The liver, heart and kidneys were removed, drained of blood and weighed. The thigh muscle and femur was also harvested as a whole. The thigh muscle and femur was subjected to ashing in a furnace (Carbolite Gero, BWF 12/13, Derbyshire, UK) and dissolved in 10% HCl and made up to 100 mL standard flask with distilled water. The mineral (Ca, Zn and Fe) content of the tissue was analyzed using Atomic Absorption Spectrophotometer (PerkinElmer, AAnalyst 200, Shelton, Connecticut, USA) (AOAC, 1990).

Statistical analysis

Data obtained from chemical analysis, mineral content assay, were statistically analyzed using the analysis of variance (ANOVA), SPSS 20.0 version. Means were separated using Duncan (1955) multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

The proximate composition of the experimental diets is shown in Table 2. The formulated diets were significantly higher ($p < 0.05$) in protein, fat and moisture contents than the reference sample (Nestlé's Cerelac). Crude protein was significantly higher ($p < 0.05$) in diets 1 to 4 (21.37 to 21.94%) than in reference sample (14.70%). The moisture content of the experimental diet was significantly higher ($p < 0.05$) in diets 1 to 3 (5.35 to 6.6%) than in reference sample (4.36%) and diet 4 (4.75%) which are not

Table 2. Proximate composition (wet weight basis) of the experimental diets.

Nutrient (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Protein	21.37 ^b ±0.00	21.94 ^a ±0.06	21.85 ^a ±0.19	21.77 ^{ab} ±0.06	13.67 ^c ±0.30
Ash	2.00 ^c ±0.00	2.50 ^{bc} ±0.71	2.50 ^{bc} ±0.71	4.00 ^a ±0.00	3.50 ^{ab} ±0.30
Moisture	5.35 ^{ab} ±0.07	6.60 ^a ±0.85	5.50 ^{ab} ±0.71	4.75 ^b ±0.21	3.40 ^c ±0.14
Fat	17.09 ^a ±0.05	12.19 ^b ±0.03	12.00 ^b ±0.05	11.07 ^c ±0.09	3.64 ^d ±0.11
Fibre	1.25 ^c ±0.07	1.10 ^c ±0.14	1.40 ^{bc} ±0.40	1.65 ^b ±0.21	3.45 ^a ±0.07
Carbohydrate	52.94 ^d ±0.10	55.67 ^c ±0.02	56.74 ^b ±0.28	56.76 ^b ±0.15	72.35 ^a ±0.31

Values are means ±SD for three separate determinations. Figures in the same horizontal row with the same superscript are not significantly different ($P>0.05$).

Table 3. Mineral content of the experimental diets.

Mineral element (mg/100 g)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ca	254.00 ^d ±11.31	392.00 ^b ±8.49	308.50 ^c ±6.36	544.50 ^a ±34.65	406.00 ^b ±8.49
Zn	2.07 ^c ±0.08	2.64 ^{ab} ±0.01	2.12 ^c ±0.13	2.89 ^a ±0.23	2.51 ^b ±0.42
Fe	11.00 ^d ±0.57	13.00 ^{bc} ±0.28	13.78 ^{ab} ±2.09	14.50 ^a ±0.42	12.25 ^c ±0.21

Values are means ±SD for three separate determinations. Figures in the same horizontal row with the same superscript are not significantly different ($P>0.05$).

significantly different ($p>0.05$). Fat content (11.07 to 17.09%) was statistically higher ($p<0.05$) in diets 1 to 4 than in reference sample (9.50%).

The fibre and total carbohydrates contents were significantly lower ($p<0.05$) in the experimental diets than in reference sample, while the values obtained for ash content were comparable in diets 4 and 5 (reference sample), and lower in the diets which diet numbers. Fibre content (1.10 to 1.65%) was significantly lower ($p<0.05$) in diets 1 to 4 than in reference sample (3.00%). Total estimated carbohydrate, obtained by difference, was significantly higher ($p<0.05$) in the reference sample diet (65.24%) than the other experimental diets (52.94 to 56.76%). Ash contents of the reference sample (3.20%) was not significantly different ($p>0.05$) from those of the other diets (2.0 to 4.0%). Protein content of the experimental diets which is considerably high could be as a result of the soybean flour included in the diet owing to the fact that the seeds of soybean are rich in protein (Sanoussi et al., 2013; Onoja et al., 2014; Abioye et al., 2011).

The mineral element (Ca, Zn, and Fe) composition of the experimental diets is shown in Table 3. The calcium content of the diets showed significantly higher ($p<0.05$) content in diet 4 (544.50 mg/100 g) than the reference sample (406.00 mg/100 g) which was not significantly different ($p>0.05$) from the value of diet 2 (392.00 mg/100 g), while diets 1 and 3 (254.00 and 308 mg/100 g, respectively) were significantly lower ($P<0.05$). Zinc content was significantly higher ($p<0.05$) in diet 4 (2.89 mg/100 g) than in reference sample (2.51 mg/100 g). In

diet 2, the zinc content (2.64 mg/100 g) was comparable to that of the reference sample and values obtained for diets 1 and 3 (2.07 and 2.12 mg/100 g, respectively) were significantly lower ($p>0.05$) than the reference sample. Values obtained for Fe contents showed a significantly higher ($p<0.05$) content in diet 4 (14.50 mg/100 g) than in reference sample (12.25 mg/100 g), while diet 1 content (11.00 mg/100 g) was significantly lower. Mineral content of the diets was higher than what was observed by Solomon (2005). This high mineral content could be due to the inclusion of sesame seeds in the diet. According to Bamigboye et al. (2010), sesame seeds are a good source of iron, zinc and calcium as it contains about 3.83, 4.46 and 281.1 mg/100 g, respectively.

Phytate and oxalate values are shown in Table 4. Anti-nutrients were generally lower in the reference sample than in the experimental diets (Table 4). Phytate was significantly lower ($p<0.05$) in the reference sample (0.10 mg/100 g) than in the experimental diets (which ranges from 0.22 to 0.31 mg/100 g). Total oxalate content was not significantly different ($p>0.05$) between the reference sample diet (0.19 mg/100 g), and diets 1 and 2 (0.20 mg/100 g in both diets), while the content of total oxalate was observed to be significantly higher ($p<0.05$) in diets 3 (0.24 mg/100 g) and 4 (0.26 mg/100 g). Phytate and oxalate contents of the diets were generally low and this is comparable to data in the literature (Udensi et al., 2012), but higher as reported by Obiakor-Okeke et al. (2014) and Onoja et al. (2014). Low phytate content observed in the diets could be due to high proportion of sweet potato flour used for the formulation of the

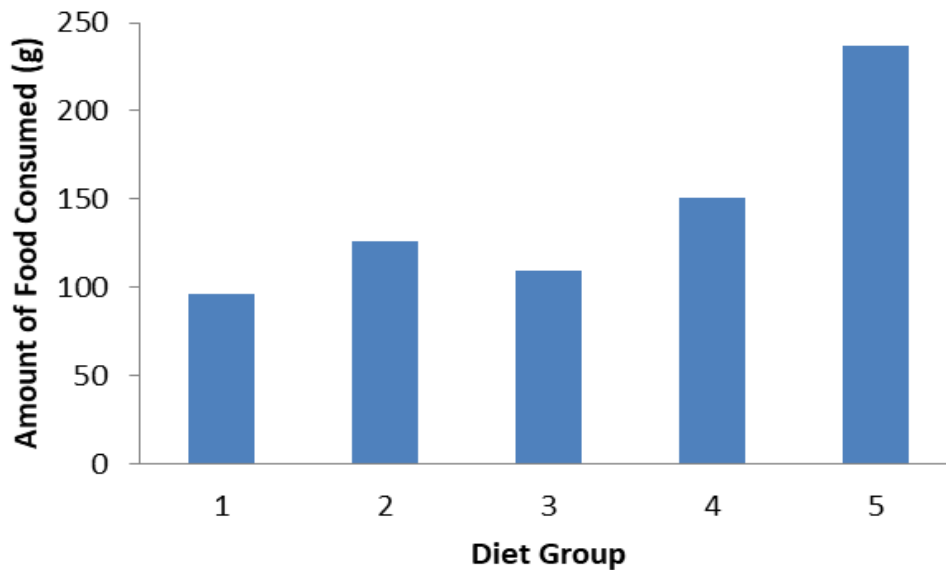
Table 4. Anti-Nutrients content of the experimental diets.

Anti-nutrient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5 (Reference)
Phytate (mg/100 g)	0.31 ^a ±0.00	0.24 ^b ±0.01	0.25 ^b ±0.00	0.22 ^b ±0.00	0.10 ^c ±0.00
Oxalate (mg/100 g)	0.20 ^b ±0.00	0.20 ^b ±0.24	0.24 ^a ±0.02	0.26 ^a ±0.02	0.19 ^b ±0.01

Values are means ±SD for three separate determinations. Figures in the same horizontal row with the same superscript are not significantly different (P>0.05).

Table 5. Anti-nutritional factor (ANF): mineral molar ratio.

ANF: Mineral	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Phytate: Ca	0.12 ^a	0.06 ^c	0.08 ^b	0.04 ^d	0.02 ^e
Phytate: Fe	2.82 ^a	1.85 ^b	1.81 ^b	1.52 ^c	0.82 ^d
Phytate: Zn	14.98 ^a	9.09 ^c	11.79 ^b	7.61 ^d	3.98 ^e
Oxalate: Ca	0.08 ^a	0.05 ^b	0.08 ^a	0.05 ^b	0.05 ^b

**Figure 1.** Food consumed by rats fed experimental diets for 21 days.

complementary diets. Both root or tuber crops have been shown to be significantly lower in phytate (by 3 to 20%) than cereal and legume based foods (Gibson et al., 2010).

The values obtained for the ANF, mineral molar ratio showed that phytate: Ca ratio was significantly lower ($p<0.05$) in the reference sample (0.02) than the other experimental diets (0.04 to 0.12) with diet 1 having the highest phytate: Ca value (0.12). Phytate: Fe and phytate: Zn values were all significantly lower ($p<0.05$) in the reference sample diet (0.82 and 3.98 respectively) than the other experimental diet (1.52 to 2.82 and 7.61 to 14.98, respectively) (Table 5). Values obtained for

oxalate: Ca show a significantly higher ($p<0.05$) values in diets 1 and 3 (0.08 in both). The anti-nutrient: mineral molar ratio obtained from this study is considerably low. The phytate: calcium, iron and zinc were comparable to those obtained from a sweet potato based complementary food (approximately 0.17, 1 and 15, respectively) which predicted better absorption as it contains at least half the phytate: mineral molar ratio of a maize based complementary food (Amagloh et al., 2012).

Food intake and growth rate from the animal experimentation is shown in Figures 1 and 2 respectively, while Table 6 shows the food intake, protein intake, weight gain, protein efficiency ratio (PER) and food

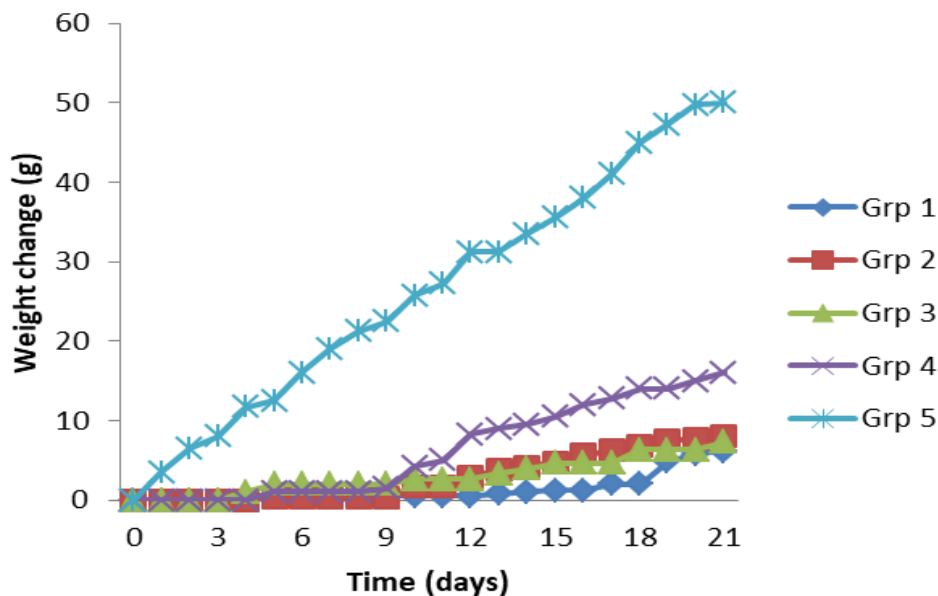


Figure 2. Change in weight (Growth Rate) of rats fed experimental diets for 21 days.

Table 6. Food intake, protein intake, weight gain and efficiency ratios of the diets.

Variable	Group 1	Group 2	Group 3	Group 4	Group 5 (Reference)
Food intake (g)	96.41 ^e	126.00 ^c	109.01 ^d	150.50 ^b	237.00 ^a
Protein intake (g)	20.60 ^e	27.64 ^c	23.82 ^d	32.76 ^b	34.84 ^a
Weight gain (g)	6.17 ^c	8.00 ^c	7.29 ^c	16.00 ^b	50.00 ^a
PER	0.30 ^c	0.29 ^d	0.31 ^c	0.49 ^b	1.44 ^a
FER	0.06 ^c	0.06 ^c	0.06 ^c	0.11 ^b	0.21 ^a

Table 7. Mineral content of the animals tissue (Thigh).

Mineral element (mg/100 g)	Group 1	Group 2	Group 3	Group 4	Group 5
Ca	600.00 ^b ±14.14	659.00 ^a ±11.31	550.75 ^c ±3.89	579.05 ^c ±12.66	516.50 ^d ±4.95
Zn	10.70 ^a ±0.42	11.08 ^a ±0.21	8.62 ^c ±0.31	9.51 ^b ±0.07	9.10 ^{bc} ±0.14
Fe	1.65 ^c ±0.12	4.85 ^a ±0.10	1.82 ^c ±0.13	2.63 ^b ±0.16	5.08 ^a ±0.18

Values are means ±SD for three separate determinations. Figures in the same horizontal row with the same superscript are not significantly different (P>0.05).

efficiency ratio (FER) per rat.

Table 6 shows that the group of rats fed the reference sample diet has a significantly higher ($p<0.05$) food intake (237.00 g), protein intake (34.84 g), weight gain (50.00 g), PER (1.44) and also FER (0.21) than the group of rats fed the other experimental diets. The growth rate of rats fed the experimental diets was low as compared to that of proprietary formula. As observed in literature, proprietary formula promotes optimum growth amidst the rats (Ibironke, 2014). Low PER and FER were observed in the experimental diets. The values obtained were similar to

PER of 0.66 to 0.99 obtained by Ijarotimi and Olopade (2009), and lower than 1.77 to 1.90 reported by Ibironke (2014) and Shiriki et al. (2015). This low PER was expected as protein of plant origin have low biological value (Solomon, 2005).

Table 7 shows the mineral content of the tissue collected from the rats fed the experimental diets. The Ca content of the tissue from group 2 rats (659.00 mg/100 g) was significantly higher ($p<0.05$) than that of rats fed with the reference sample (516.50 mg/100 g). The Zn content of the tissue was significantly higher ($p<0.05$) in groups 1

and 2 rats (10.70 and 11.08 mg/100 g, respectively) than in those fed with the reference sample (9.10 mg/100 g) and lower in group 3 rats (8.62 mg/100 g). The iron (Fe) content of the tissue obtained from the rats, showed no significant difference ($p>0.05$) in the reference sample group rats (5.08 mg/100 g) and group 2 (4.85 mg/100 g) which were both significantly higher ($p<0.05$) than those of the other groups (1.65 to 2.63 mg/100 g). Non-heme iron which was present in plant food is poorly absorbed (Lean, 2006), this explains the higher iron absorption in the proprietary formula fed rat. Generally, absorption of mineral was high which made the mineral available for body metabolism and physiological functions.

Conclusions

Nutritious complementary food could be produced from sweet potato based diet supplemented with germinated and defatted soybean and sesame seed flours which had low anti-nutrients, increased bio-availability of minerals and absorption rate. The low anti-nutrient content and increased bioavailability of the complementary food make the products suitable for infants and children. These products could be produced at the cottage level to alleviate the prevalent malnutrition problems.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Oxalate, phytate and nitrate content in African nightshade, spider plant and amaranths at different stages of maturity

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African indigenous green leafy vegetables play important role in income generation and subsistence; they are the cheapest and most readily available sources of important minerals and vitamins. On the other hand, they contain anti-nutritional factors that reduce availability of vital nutrients. This study was conducted to determine oxalates, phytates and nitrates content in commonly consumed *Amaranthus cruentus*, Spider plant (*Cleome gynandra*) and African night shade (*Solanum villosum*) at 21, 28 and 35 days age of the plant harvest. Vegetables were planted on plots and harvested at 21, 28 and 35 days. At each stage, about 600±2 g of the edible part was harvested and standard chemical analyses procedures were followed to determine oxalate, phytate and nitrate contents. Using Statistical Product and Service Solutions (SPSS 20) data were analysed and results presented as simple means, ranges and standard deviations. Analysis of variance (ANOVA) with 5% level of significance was done to determine differences in the levels of nutrients between the vegetable varieties and three maturity stages. African nightshade Nduruma BG 16 had lowest oxalate concentrations (28.7 ± 0.1 mg/100 g) at maturity stage I while African nightshade Olevolosi SS 49 had the highest value (60.9 ± 0.9 mg/100 g) at maturity stage III. There was no particular trend for phytates in all maturity stages and in all varieties but amaranths Madiira AM 38 had exceptionally the highest values (0.7 ± 0.0 mg/100 g) at stage III of maturity. The highest nitrate content was 85.6 ± 1.8 mg/100 g in Olevolosi SS 49 at maturity stage I, whereas the lowest value was 45.3 ± 1.3 mg/100 g in amaranths Madiira AM 38 at stage III. There was a slight variation in antinutrients composition of the studied vegetables and the composition was generally very low.

Key words: Antinutrients, indigenous vegetables, maturity stage, Tanzania.

INTRODUCTION

African indigenous green leafy vegetables (GLV) play important role in income generation and subsistence;

they are the cheapest and most readily available sources of important minerals and vitamins (Schippers, 2000).

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They are important commodities for poor households because their prices are relatively affordable when compared with other food items. In Tropical Africa where the daily diet is dominated by starchy staples, indigenous GLVs are the cheapest and most readily available sources of important minerals and vitamins (Dzomeku et al., 2011). In addition, indigenous vegetables have the added advantage of possessing other desirable traits such as aroma and flavor which make them quite acceptable in the local communities. These vegetables are often easier to grow, resistant to pests and diseases and do not require intensive management (Moyo et al., 2013). Furthermore, they are a rich source of vitamins and other components that contribute to antioxidant activity in the diet and contain non-nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases (Moyo et al., 2013). On the other hand, green leafy vegetables contain antinutrients that compromise digestion and absorption of vital nutrients (Uusiku et al., 2010). These components occur in combinations and may act synergistically or may have contraindicating effects with each other. Understanding the role and contents of these components is crucial for managing micronutrient deficiencies and chronic diseases of lifestyle.

Green leafy vegetables are harvested by rural communities from crop fields at different stages of plant growth (Modi et al., 2006). For most of green leafy vegetables, there is a preferred stage of plant development when flavor and palatability are favorable for human consumption. The maturity stage of a conventional vegetable is universally defined, and a crop is normally harvested and consumed at a known stage of plant development, irrespective of environmental conditions for plant growth. Unlike conventional crops such as staple cereals, information about the stage of plant development to define harvest maturity for indigenous green leafy vegetables is scarce. Moreover, studies have indicated that levels of nutrients and toxic substances in vegetables are influenced by stages of plant development (Khader and Rama, 2003).

Anti-nutritional factor is known to interfere with metabolic processes such that growth and bioavailability of nutrients are negatively influenced (Agbaire and Emoyan, 2012). Phytate and oxalates have the ability to form chelates with metallic ions such as Cd, Mg, Zn and Fe to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract thus decreasing their bioavailability. Ladji et al. (2004) reported that oxalates causes irritation and swelling in the mouth and throat. They further noted that phytate inhibits the functions of some digestive enzymes. Oxalate binds to calcium to form calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body and aiding the formation of kidney stones. Addition of a source of calcium to vegetables containing high levels of

soluble oxalate has shown to reduce intestinal available oxalate content in such foods (Radek and Savage, 2008). Phytate is the major phosphorus storage compound in African leafy vegetables (Schlemmer et al., 2009). Although it is an antioxidant, it has been shown to inhibit absorption of minerals. Phytate chelates multivalent metal ions such as calcium, iron and zinc, thus it is a strong inhibitor of iron mediated free radical generation hence the diet high in phytate content reduces bioavailability of zinc and iron. A number of studies indicate that green leafy vegetables contain various amounts of phytic acid (Agbaire, 2012; Agbaire and Emoyan, 2012; Nkafamiya et al., 2010). While some studies reveal that domestic thermal processing methods can significantly reduce phytate content in vegetables (Imaobong et al., 2013; Yada and Sehgal, 2003); other studies report that phytate content increases or remains unchanged when heat processed (Embaby, 2010). Nitrates form part of the essential chemistry of soils and plants. Thus plant roots are able to absorb nitrate directly from the soil. Nitrate contamination in vegetables occurs when crops absorb more than they require for their optimal growth. Nitrate content of vegetables may range from 1 to 10,000 mg/kg (Renseigné et al., 2007). The nitrate content varies with plant species, cultivars of the same species, and even genotypes with different ploidy (Grzebelus and Baranski, 2001; Sunaga et al., 2005). Thus, selection of genotypes that accumulate fewer nitrates may contribute significantly to reduction in the nitrate consumption by humans through vegetables and the subsequent risk of nitrate poisoning. The main concern for the public health is the link between nitrates and stomach cancer due to the fact that nitrates may lead to formation of carcinogenic nitrosamines (Renseigné et al., 2007; Walker, 1990).

Consumption of GLV is recommended as a strategy of preventing and alleviating minerals and vitamin deficiencies in both rural and urban societies, hence it is important that vegetables are harvested and consumed when the levels of micronutrients is at maximum; and when the quantities of anti-nutritional factors is at the minimum. However, there is limited information about the stage of plant development at which antinutrients are at minimum levels with balanced flavor, palatability and other nutrients. The need for exploration of anti-nutritional information in traditional leafy vegetables is significant in overcoming micronutrient deficiencies in order to contribute to health and nutritional security in Africa. It is in this view that the research is designed to determine oxalates, phytates and nitrates content in commonly consumed *Amaranthus cruentus*, Spider plant (*Cleome gynandra*) and African night shade (*Solanum villosum*) at 21, 28 and 35 days age of the plant harvest.

MATERIALS AND METHODS

This study was conducted at Sokoine University of Agriculture

(SUA) situated in Morogoro urban district. The district is characterized with temperatures ranging from 16 to 33°C and average annual rainfall ranging from 821 to 1505 mm (URT, 2016). The field sites were the crop experimental plots located at SUA.

Description of the vegetables under study

Five varieties of indigenous green leafy vegetables namely *A. cruentus* (Madiira 1 EX Zim and Madiira II AM 38), *S. villosum* or African night shade (Nduruma BG 16 and Olevolosi SS49) and Spider plant (*C. gynandra*) were used in the study. Seeds of these vegetables were purchased from Horticulture Training Institute of Tengeru (HORTI – Tengeru) Arusha and from the horticulture unit of Sokoine University of Agriculture (SUA), Morogoro.

Planting, management and collection of samples

The seeds were sown at the crop experimental plots at SUA, Morogoro Tanzania. Before sowing the seed, the land was cleared, ploughed, harrowed and fertilized using poultry manure. It is a common practice for farmers to use poultry manure for vegetable growing in Morogoro urban because it is of low cost and results to relatively more yield. Seeds of the five vegetables varieties were sown on a plot in three replications. The area was divided into three rows for the three replications and each row was divided into five beds, one for each vegetable variety. The seeds were sown in 30 cm inter and intra spacing. The density of the plants was 45 plants per bed. The vegetables were watered twice daily (mornings and evenings) and weeding was done weekly.

The three maturity stages were 21, 28 and 35 days from sowing the seeds. At each stage, about 600 ± 2 g of the edible parts (leaves and young stems) were harvested by uprooting the whole plant and picking the edible leaves and shoots. The picked leaves were placed in dark colored polythene bags and transported to the food laboratory for chemical analyses. The edible portions of the samples were washed with running tap water, drained to remove excess water, cut into small pieces (about 2 mm) using domestic sharp knife and cutting board and homogenized. Samples were oven dried at 60°C until constant weight was obtained.

The dry samples were removed from the oven and immediately ground into a fine powder using motor and pestle. The powdered samples were placed in transparent polythene bags, labeled and stored ready for oxalate, phytate and nitrate analyses.

Determination of oxalate content

The titration method as described by Baker (1952) was followed. 2.0 g of powdered sample was heated in 50 ml distilled water. 0.3 M HCl was then added to the heated sample. The cold filtrate was treated with 3 drops of methyl red indicator and NH_4OH solution before heating the mixture to 100°C. The mixture was left to cool. After cooling, the filtrate was heated further before the addition of 10 cm³ of 10% CaCl_2 solution and allowed to stand overnight. The mixture was filtered by using Whatman paper No. 1. After filtration, the precipitate formed was washed to remove traces of Ca^{2+} before dissolving in H_2SO_4 solution. The solution formed was brought to boil by heating before warm titrating with 0.05 M KMnO_4 solution until a faint pink colour persisted for at least 30 s. The oxalate content was then be calculated by taking 1 ml of 0.05 M KMnO_4 as equivalent to 2.2 mg oxalate using the following formula:

$$O = T_s \times M_d \times M_o \times 100 / W_s$$

where O = Oxalate concentration in mg/100 g, T_s = Volume of potassium permanganate used for sample, M_d = number of moles

of potassium permanganate reacted, M_o = number of moles of oxalate reacted, and W_s = sample weight.

Determination of phytate content

Phytic acid contents of the vegetable samples were determined by method as described by Wheeler and Ferrel (1971). About 0.2 g of each powdered sample was weighed into a 125 ml Erlenmeyer flask. The phytic acid was extracted using 50 ml 3% Trichloroacetic acid (TCA) for 30 min with occasional swirling by hand for 45 min. The suspension was centrifuged and 10 ml aliquot of the supernatant was transferred to a 50 ml conical flask. Four milliliters of FeCl_3 solution was added to the aliquot by lowering rapidly from the pipette. The content was heated in a boiling water bath for 45 min. After 30 min, two drops of 3% sodium sulphate were added in 3% TCA extract and continued to be heated. The supernatant was centrifuged for 15 min and decanted. The precipitate was washed twice by dispensing well in 20 to 25 ml of 3% TCA, heated in boiling water bath for 10 min and centrifuged. Washing with water was repeated. The precipitate was dispersed in 27 ml of water and 3 ml of 1.5 N NaOH with mixing. The volume was brought to approximately 30 ml with water and heated in boiling water bath for 30 min. The precipitate was filtered through a moderately retentive paper Whatmann No. 2. The precipitate was washed with 70 ml hot water and the filtrate was discarded. The precipitate was dissolved from the paper with 40 ml 3.2 N HNO_3 into a 100 ml volumetric flask. The filter paper was washed with several portions of water and the washed papers were collected in the same flask taking care not to exceed the 100 ml volume. The flask was cooled at room temperature and diluted to volume with water. A 5 ml aliquot was transferred to another 100 ml volumetric flask and diluted to approximately 70 ml. 20 ml of 1.5 M KSCN was added and diluted to volume and color was read immediately within 1 min at 480 nm. A reagent blank was run with each set of samples.

Phytate content in sample was calculated using the following formula:

$$\text{Phytate content in } \mu\text{g}/100 \text{ g sample} = (C \times E / S \times A_v) \times 100$$

where C = phytate concentration from standard graph, E = total extraction volume, S = analytical sample taken, and A_v = analytical volume

Determination of nitrate content

Determination of nitrate contents was done by using spectrophotometric method as outlined by Gaya and Alimi (2006) where 10 g of each sample was taken into a 250 cm³ beaker and 2.5 ml of 4% NaOH was added. The content of the beaker was warmed at 80°C for 25 min with occasional shaking. The resulting solution was filtered through a fluted filter paper into 100 cm³ volumetric flask and made up to the mark. An aliquot of 4 cm³ was taken into a test tube cooled in ice. 1 cm³ of 5% Ag_2SO_4 solution was added followed by subsequent addition of 7 cm³ of 98% H_2SO_4 and 0.1 cm³ of 5% phenol 25 solutions. The solution was allowed to stand for 20 min while shaking occasionally. The resulting mixture was extracted in 50 cm³ separating funnel by adding toluene and shaking for 5 to 10 min. The lower aqueous layer was discarded. The organic phase was washed twice with 10 ml of distilled water by shaking for 2 min and each time discarding the aqueous phase. The organic phase was extracted again by shaking for 1 min with 10 cm³ of 10% Na_2CO_3 solution and collected in a test tube. Absorbance was read at 407 nm. Since 4 cm³ of the 100 cm³ filtrate was used for analysis.

The amount of nitrate (mg/g) in the vegetable was calculated by

Table 1. Oxalate content (mg/100 DM) in different vegetables at different stages of maturity.

Vegetable	Stage of Maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
African nightshade (<i>Solanum villosum</i>)(Nduruma BG 16)	28.7±0.1 ^a	38.2±1.5 ^c	59.6±0.3 ^f
African nightshade (<i>Solanum villosum</i>) (Olevolosi SS 49)	42.9±0.2 ^d	55.9±1.2 ^e	60.9±0.9 ^f
Spider plant (<i>Cleome gynandra</i>)	38.4±1.7 ^c	48.3±1.5 ^d	30.5±0.5 ^a
<i>Amaranthus cruentus</i> (Madiira Ex Zim)	39.9±0.8 ^c	54.9±0.5 ^e	60.3±2.0 ^g
<i>Amaranthus cruentus</i> (Madiira AM 38)	34.6±1.3 ^b	38.1±0.3 ^c	31.1±1.2 ^c

Data presented as arithmetic means ± SD (n = 3). Means within the vegetable variety in row/column with different superscript letters are significantly different at p<0.05.

Table 2. Phytate content (mg/100 DM) in different vegetables at different stages of maturity.

Vegetable	Stage of Maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
African night shade (<i>Solanum villosum</i>) (Nduruma BG 16)	0.04±0.0 ^{ab}	0.2±0.0 ^{ab}	0.3±0.1 ^{cd}
African night shade (<i>Solanum villosum</i>) (Olevolosi SS 49)	0.2±0.0 ^{bc}	0.3±0.0 ^{cd}	0.4±0.0 ^{cd}
Spider plant (<i>Cleome gynandra</i>)	0.02±0.0 ^a	0.3±0.0 ^{bcd}	0.3±0.0 ^{bcd}
<i>Amaranthus</i> (Madiira Ex Zim)	0.2±0.0 ^{bcd}	0.4±0.0 ^{cd}	0.5±0.0 ^d
<i>Amaranthus</i> (Madiira AM 38)	0.02±0.0 ^{ab}	0.2±0.0 ^{abc}	0.7±0.0 ^e

Data presented as arithmetic means ± SD (n = 3). Means within the vegetable variety in row/column with different superscript letters are significantly different at p<0.05.

the formula:

$$\text{Nitrate} = \frac{C \times 100}{W_s \times 4}$$

where C = concentration of nitrate in the sample as from calibration graph (mg cm⁻³) and W_s = weight of the sample used (g).

Statistical analysis

The data obtained was analysed using Statistical Product and Service Solutions software (Version 20). Results were presented as simple means, ranges and standard deviations. Analysis of variance (ANOVA) with 5% level of significance was done to determine any significant differences in the levels of nutrients between the vegetable varieties and the three maturity stages (21, 28 and 35 days).

RESULTS

Oxalate content

Table 1 shows the oxalate concentration in the study vegetables at three stages of maturity. Oxalate content increased significantly (p<0.05) with plant age in *S. villosum* (Nduruma BG 16), *S. villosum* (Olevolosi SS 49) and Amaranthus (Madiira Ex Zim). In Spider Plant (*C. gynandra*) and Amaranthus (Madiira AM 38), oxalate content increased from stages I to II and then decreased

at stage III. In both cultivars of *S. villosum* (Nduruma BG 16 and Olevolosi SS 49), the changing pattern of oxalate was the same, that is, it increased significantly with plant age and similar oxalate levels at stage III. However, this was not the case in the two cultivars of *A. cruentus* (Madiira AM 38 and Madiira EX Zim) where oxalate content increased continuously with age in Madiira EX Zim but in Madiira AM 38 it increased from stages I to II and then decreased at stage III.

Phytate content

The phytate concentration in all vegetables varieties increased from stages I to III as shown in Table 2; the increase being very high in Amaranths (Madiira AM 38) from stages II to III of maturity. The increase in phytate content in Spider plant (*C. gynandra*) from stages II to III was not significantly different. All varieties had similar phytate contents at the first harvesting stage that is at 21 days.

Nitrate content

In all five varieties, the nitrate content decreased with maturity but the differences were not always statistically different (p<0.05) except in in *S. villosum* (Olevolosi 49). The highest level of nitrate concentration at any maturity stage was observed in *S. villosum* (Olevolosi 49) while

Table 3. Nitrate content (mg/100 DM) in different vegetables at different stages of maturity.

Vegetable	Stage of maturation		
	Stage I (21 Days)	Stage II (28 Days)	Stage III (35 Days)
African night shade (<i>Solanum villosum</i>) (Nduruma BG 16)	66.8±1.4 ^{bc}	64.5±0.8 ^b	63.0±1.4 ^b
African night shade (<i>Solanum villosum</i>) (Olevolosi SS 49)	85.6±1.8 ^g	79.9±0.3 ^{ef}	75.4±1.8 ^{de}
Spider plant (<i>Cleome gynandra</i>)	81.2±1.6 ^{fg}	78.6±0.8 ^f	64.3±3.7 ^b
<i>Amaranthus cruentus</i> (Madiira Ex Zim)	81.4±1.1 ^{fg}	66.2±1.7 ^{bc}	63.0±1.7 ^b
<i>Amaranthus cruentus</i> (Madiira AM 38)	71.2±0.1 ^{cd}	66.4±0.6 ^{cd}	45.3±1.3 ^a

Data presented as arithmetic means ± SD (n = 3). Means within the vegetable variety in row/column with different superscript letters are significantly different at p<0.05.

the lowest levels were observed in *A. cruentus* (Madiira AM 38). The nitrate content in *C. gynandra* was significantly different between stages II and III only. Nitrate content was slightly lower in Nduruma BG 16 as compared to Olevolosi SS 49, the difference being significant at all maturity stages (Table 3).

DISCUSSION

This study aimed to determine variation in oxalate, phytates and nitrates in five varieties of indigenous vegetables harvested after 21, 28 and 35 days. Generally, oxalate levels increased with maturity stage but in Spider Plant (*C. gynandra*) and *Amaranthus* (Madiira AM 38) oxalate content increased from stages I to II and then decreased at stage III. These results resembled the findings reported by Kitchen and Burns (2006) who worked on dark green bloomsdale spinach and found that total oxalate content was maximum at 32 days after planting and decreased subsequently as the plants developed towards the vegetative phase. Musa et al. (2011) also reported an increase in total oxalate content in *A. cruentus* grown in Nigeria from 4.40 ± 0.19 mg/100 g at market maturity stage (vegetative stage) to 5.27 ± 0.24 mg/100 g at heading (reproductive stage). This suggests that harvesting the vegetables after 35 days could have reduced oxalate concentration. Variation was observed in oxalate content across the cultivars. Oxalate content was much lower than reported in other vegetables like sweet potato leaves and amaranths consumed in Tanzania, Kenya and in Nigeria (Musa and Ogbadoyi, 2014; Mwanri et al., 2011; Mziray et al., 2001). Studies show that chemical composition of plants is affected among other things the variety and even the cultivar (Singh et al., 2001). Therefore, the differences observed in oxalate concentration in the vegetables in this study might be attributed to varietal differences as well as the part of plant consumed.

Processing methods such as pressure cooking and open pan cooking can help to reduce oxalate levels significantly. A study by Virginia et al. (2012) on effects of cooking and processing on oxalate content in green leafy

vegetables and pulses revealed that blanching, pressure cooking for 10 min and open pan cooking of green leafy vegetables reduced their oxalate concentration from 88.8 mg/100 g fresh samples to 48.4, 57.2 and 60.13 mg/100 g, respectively. Other studies reported reduction of oxalates in boiling and drying of vegetables (Essack et al., 2017; Ilelaboye et al., 2013; Mwanri et al., 2011); which means the amount of oxalate present in studied vegetables may not be harmful for human consumption since the contents were relatively low and these vegetables are usually boiled before consumption.

There was variation in phytate content in different varieties but no significant difference was observed at 21 days. Phytate content were lower than reported for other vegetables commonly consumed in Africa. For example, studies by Gupta et al. (2005) found 1.95 mg/100 g in *Amaranthus tricolor* at market maturity stage. In addition, higher levels of phytic acid were observed in *Amaranthus* (4.12 mg/100) and *Solanum* (7.39 mg/1000) species at market maturity and in several leafy vegetables consumed in Nigeria (Agbaire, 2012; Agbaire and Emoyan, 2012). Significant variation of phytic acid content among different varieties were also reported by Hossain and Becker (2001) who found that three varieties of *Sesbania* seeds (*S. aculeate*, *S. rostrata* and *S. sesban*) had 0.28, 0.16 and 0.39 g/100 g phytic acid content, respectively. Phytic acid varies not only among varieties but also among different cultivars of the same variety (Purvika et al., 2012). Phytate content in green leafy vegetables can be reduced by various processing methods including blanching and cooking. A study by Ilelaboye et al. (2013) reported that phytate content in *Amaranthus hybridus* was reduced from 191 mg/100 in raw vegetables to 81.65 mg/100 by blanching and 56.67 mg/100 g by cooking.

Generally, nitrate content decreased with maturity stage. The decreasing trend in nitrate content in vegetables with maturity was also reported by Musa et al. (2011) who found that nitrate content of *A. cruentus* decreased from 17.71±2.42 g/kg at market maturity to 7.62±1.00 g/kg at vegetative stage. The amount of nitrate in plants is determined mainly from its genetically based metabolism, the age of the plant, environmental factors

and the amount of available nitrate in the soil (Masclaux-Daubresse et al., 2010; Rachmilevitch et al., 2004). Nitrate content was higher in *S. villosum* (Olevolasi SS 49) and lowest in Nduruma BG 16 variety. The variation of nitrate according to varieties were reported by other researchers who found higher levels in arugula (4354.9 mg/kg) as compared to kale (603.0 mg/kg), but all were below the maximum recommended levels for human consumption (Brkić et al., 2017). Strategies to reduce amount of nitrate in diets include selecting varieties with low nitrate, avoiding excessive nitrate fertilizers, avoid application of nitrogen fertilizer shortly before harvesting the vegetables and harvesting vegetables in afternoons and sunny period (Renseigné et al., 2007).

It is important to note that poultry manure used in this study was not analysed so its chemical composition is not known. However, it is known that nitrogen composition in organic manure increase nitrogen in the soil which ultimately may affect nitrate concentration in vegetables since excess nitrogen tends to accumulate as nitrate. Compared to dairy and goat manure, poultry manure was reported to contribute relatively more soil nitrogen (Maerere et al., 2001). Therefore, nitrate concentration observed could be overestimated due to nitrogen content of the poultry manure used. But for the sake of comparisons made in this study, it is important to bear in mind that all the experimental plants were treated the same.

Conclusions

Given the findings of this study, it is therefore not possible to generalize the 'best' stage to harvest and consume these three African indigenous green leafy vegetables based on the plant contents of three known anti-nutrient factors (oxalate, phytate and nitrate). Mixing of the plant parts harvested at varying stages when preparing these vegetables for consumption, may offer a good opportunity of minimizing intake of such compounds and for that case, any danger associated with them. Variation of antinutritional compounds across the varieties confirms the importance of diversification for improved nutrient intake. In addition, the three antinutrients investigated were generally in low levels so consumption of vegetables to increase micronutrient intake is encouraged.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Dietary practices, habits and physical activity levels of the Swahili community, Kenya in relation to obesity and chronic diseases of lifestyle

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Chronic diseases of lifestyle like diabetes mellitus and hypertension appear at greater rates in populations which consumed high fat, high calorie diets and engaged in low or no physical activity. Physical inactivity and unhealthy diets are major contributors of overweight and obesity which are risk factors for lifestyle diseases. This study aimed at determining the relationship between dietary practices, habits, physical activity and the prevalence of obesity, diagnosed diabetes and hypertension in the Swahili community of Old Town and Kisauni districts in Mombasa County, Kenya. A cross-sectional study design was used. Cluster random sampling was done to pick 207 households. Data was collected on food consumption, dietary habits and physical activity. Focus Group Discussions and Key Informants Interviews were used to collect qualitative data. Results indicate that dietary habits of the Swahili community involved preparation and consumption of high fat, sugar and coconut milk-based foods. Most (75.8%) of the interviewed members of this community had low physical activity. Their dietary habits and low physical activity levels were associated with obesity, diabetes and hypertension ($p < 0.05$, 95% CI). Physical inactivity levels were associated with obesity [Odds Ratio, 0.49; 95% Confidence Interval, 0.27 - 0.88]. There is need to acquire sustainability of consumption of healthy diets and physical activity through education and provision of physical activity facilities to prevent obesity, a major risk factor for diabetes and hypertension.

Key words: Dietary practices, dietary habits, physical inactivity, obesity, hypertension, diabetes, Swahili, Kenya.

INTRODUCTION

Chronic diseases of lifestyle are a group of diseases that shared similar risk factors as a result of exposure, over many decades, to unhealthy lifestyle. The unhealthy

lifestyle involves lack of regular physical activity and consumption of diets rich in highly saturated fats, sugars and salt (Steyn et al., 2006).

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The burden of these diseases is increasing globally and possesses a major public health concern, a large part of which is preventable (WHO, 2017). They are a major contributor to the burden of disease in developed countries and are increasing rapidly in developing countries (Lopez et al., 2006). The locus of the burden resides in the developing world, as it has been projected that, by 2020, about three-quarters of mortality due to heart disease, stroke, and diabetes will occur in developing countries and exceed by 2030 (WHO, 2017). Chronic diseases of lifestyle have an impact on the burden of disease in sub-Saharan Africa (Aikins et al., 2010). WHO projections showed that these diseases will be responsible for a significantly increased total number of deaths in Africa, South-East Asia and the Eastern Mediterranean, where they will increase by over 20% (WHO, 2011). In Kenya, these diseases account for 27% of the total deaths and over 50% of total hospital admissions (MOH, 2015). The major ones are cardiovascular conditions, cancers, diabetes, and chronic obstructive pulmonary diseases with their sequel and shared risk factors (MOH, 2015).

The chronic diseases of lifestyle are largely due to risk factors such as high blood cholesterol, high blood pressure, obesity, physical inactivity and unhealthy diet (Steyn et al., 2006). These risk factors can be classified as modifiable and non-modifiable (WHO, 2011). Nutrition is a major modifiable determinant of chronic lifestyle diseases (McNaughton et al., 2012). Scientific evidence supports the view that alterations in diet and physical activity have affected health throughout life (McNaughton et al., 2012; Reiner et al., 2013). The dramatic changes occurring in people's diets around the world are referred to as nutrition transition. People consumed more fats, more animal-based products, and more sugar, as well as more processed foods and less fiber (Kearney, 2010). Lifestyle diseases are linked to high consumption of such foods (Bourne et al., 2002). Unhealthy diets, especially those which have a high content in fats and free sugars and physical inactivity are among some of the leading causes of these diseases including cardiovascular diseases (CVDs), type 2 diabetes and certain cancers (Whatnall et al., 2016).

Nutrition patterns are influenced by many factors, including individual preference, culture, traditions and beliefs (Nordstrom et al., 2013). People are normally proud of who they are and where they come from. The foods that people feed throughout their childhoods most often stay with them throughout their lives like favorite food types. Even in the midst of globalization where many aspects of healthy living are a concern, people hold onto the cultural cooking practices that are known to be the best and have helped them to shape up to the people they are and what they believe about life in general (Morgan, 2000). However, this does not mean that all types of cooking are healthy. In the US for example, in

Georgia, Mississippi and Alabama, majority of the populations are overweight or obese. This has a lot to do with the famous Southern cooking that has been traditionally based on staples like fatback, fried potatoes, fried chicken, fried cabbage, fried catfish, fried eggs and a lot of other fried items (Balarajan, 2001). Besides widespread obesity rates, there are also increased levels of lifestyle diseases (Balarajan, 2001). Overweight and obesity predisposes affected individuals to the chronic diseases of lifestyle (Crino et al., 2015). The Swahili community of the Kenyan coast has specific cooking methods and diets based on their culture. They use coconut milk and a lot of sugar in their cooking. Their staple food has a lot of Indian influence, thus most of their cooking is rich in spices (MOT, 2015). Popular Swahili cuisine includes *wali*, that is, rice cooked in coconut milk, which is served with a thick meat stew or fish, and *pilau*, a spicy variety of *wali*. Goat meat and chicken curries are traditionally popular during special occasions. They also eat a lot of different grains, vegetables and fruits including beans, peas, tomatoes, potatoes, okra, kale, spinach, mangoes, coconut and bananas (MOT, 2015).

Physical activity is defined as any bodily movement produced by skeletal muscles that require energy expenditure (Singh, 2013). It is a key determinant of energy expenditure, and thus is fundamental to energy balance. Physical inactivity, that is, a lack of physical activity is an independent risk factor for lifestyle diseases. It is associated with increased levels of obesity, breast cancer, colon cancer, osteoporosis, stress, anxiety and depression. It is one of the major underlying causes of mortality in the world (Booth et al., 2012). It is estimated to cause 1.9 million deaths globally. Lifestyle diseases associated with physical inactivity are the greatest public health problem in most countries around the world (WHO, 2010). Many studies across sub-Saharan Africa have revealed the impact of a sedentary lifestyle on emerging chronic disease risk factors (Forrest et al., 2001; Aspray et al., 2000; Sobngwi et al., 2004). Steyn et al. (1999) showed an independent association between low levels of physical activity and having diabetes in a poor, peri-urban community near Cape Town, South Africa. It has also been shown that moderate amounts of physical activity are associated with health benefits, and can help reduce various chronic diseases related to lifestyle (Blair et al., 2001).

Studies have shown that there is a relationship between risk factors for lifestyle diseases and their prevalence (Nazri et al., 2008; Gothankar, 2009). A study on prevalence of overweight, obesity and self-reported chronic diseases among residents in Malaysia reported a relationship between overweight, obesity and chronic diseases of lifestyle (Nazri et al., 2008). In another study on prevalence of obesity and its associated comorbidities amongst adults, Gothankar (2009) reported an

association between obesity, hypertension and diabetes. These studies determined the relationship between obesity and lifestyle diseases. Physical inactivity and unhealthy diets which are documented as major contributors of overweight and obesity were not looked into. This study was to investigate dietary practices, habits and physical activity in the Swahili community of the Kenyan coast in relation to the prevalence of overweight, obesity, diabetes and hypertension.

METHODOLOGY

Location of the study

The study was conducted in Mombasa County of Kenya. It is situated in the Southeastern part of the former Coast province. It covers an area of 229.9 km². Water mass accounts for 65 km². It borders Kilifi to the North, Kwale to the South and West and the Indian Ocean to the East. The district lies between latitudes 3°-80' and 4°-10' South of the Equator and between longitudes 39°-80' East of the Greenwich Meridian. This County is an island linked to the mainland by a road and a railway bridge to the northwest and a further road bridge to the north coast. A vehicular ferry links the island with the south coast at Likoni (Berry, 2014).

Experimental design

The study design assumed both quantitative and qualitative approaches and was a one-time cross-sectional study involving men and women aged 30 to 70 years old. Children as well as men and women whose age did not fall between 30 and 70 years were excluded from the study.

The study also assumed a comparative approach to determine any significant differences that existed between the Old Town and Kisauni districts' respondents.

Diabetes mellitus and hypertension were documented based on self-reports and showing of clinic cards by already diagnosed individuals. Body mass index (BMI) was used to classify people into underweight, normal weight, overweight or obese. The BMI was calculated as a ratio between weight in kilograms and height of the respondent in meters squared (kg/m²) and compared with internationally recommended cut-off points for status (Kolimechkov, 2014). A person with a BMI of 30 or more is generally considered obese. A person with a BMI equal to or more than 25 is considered overweight (Kolimechkov, 2014).

Study population

The study population was the Swahili community. This community has been described as an ethnic-mixed group of people speaking closely related forms of Bantu speech, living on islands and coastal areas of East Africa. Historically, the Swahili developed as Arab and Persian traders established business contacts and married local women on the East African coast around AD 700 (MOT, 2015). The resulting people were Islamic Bantu-speaking fishers, traders and woodwork artisans, living in city-states varying from governorships to republics. The people's features vary from light-skinned Arab to Bantu. They have close association with Arabic and Islamic cultures (MOT, 2015). The Kenyan Swahilis are the Bajun, Fundi, Ozi, Pate, Vumba, Mvita (from Old Town, Mombasa), Shela, Amu (the dialect of Lamu Island) and Siyu, which are

communities resulting from mestization among the ancient Arabs and Persians. In Mombasa, the Swahili maintain close relationships with Arabs, some native to Kenya and some Yemeni. They have a wide range of socio-economic activities, religious and cultural values. They are matriarchal and family or clan oriented. They are renowned as sailors, traders and artisans. They are a welcoming, hospitable people and enjoy meeting people from other places and cultures (MOT, 2015).

Target population

The target population consisted of men and women aged 30 to 70 years old from the Swahili community in Old town and Kisauni districts of Mombasa County. Old town is an historical home of the Swahili community. The town's inhabitants were mostly of the ancient Arabs (UNESCO, 2011). Kisauni on the other hand is sub-county (district) within Mombasa County. Old town has a high population of the Swahili people, therefore the community here possessed more Swahili culture and characteristics hence had slightly different dietary habits and practices compared to Kisauni district community, which had slightly deviated from the typical Swahili culture due to adopting other ethnic groups' cultures (UNESCO, 2011).

Sampling and sample size

The sampling frame consisted of men and women residing in Old town and Kisauni districts, as the two study clusters. Both of these two districts were purposively selected for the study. The unit of analysis was the individual households, represented by the selected members of the households, who were administered with and responded to the survey questionnaire. A man and woman were randomly picked from each household. Simple random sampling was used where the members of the household picked papers from a hat and one man and one woman were selected. The sample size was calculated using the Fisher's formula recommended by Mugenda and Mugenda (2003). A sample size of 207 households was achieved. Household lists of Old town and Kisauni districts were obtained from the local government offices. Proportionate sampling was used to get the specific number of households from each of the two study sites. From Kisauni district, $12,747/26,650 \times 207 = 99$ households and Old Town district, $13,903/26,650 \times 207 = 108$ households.

Dietary assessment

Two methods of dietary assessment were used in this study; a Food Frequency Questionnaire (FFQ) and 24-h recall. More than one method of assessing dietary intake is recommended as one method is susceptible to biases (Previdelli et al., 2016).

Physical activity assessment

The Global Physical Activity Questionnaire (GPAQ) was developed by WHO for physical activity surveillance in countries (WHO, 2004). It collected information on sedentary behavior and physical activity participation in three settings or domains which include: activity at work, travel to and from places and recreational activities. The GPAQ was validated by a WHO expert group working on physical activity measurement. Around 50 developing countries are now using the GPAQ for physical activity data collection (WHO, 2004). This study assessed physical activity in the three settings or

Table 1. Mean age of the interviewed household members.

Study area	Gender	Mean age (years)	SD	Range
Kisauni district (n=99 households)	Females	43	5.24	31-57
	Males	45	5.23	35-58
	Total (Females and Males)	44	5.37	31-58
Old town (n=108 households)	Females	44	5.13	32-57
	Males	47	4.85	35-58
	Total (Females and Males)	45	5.31	32-58
Total (Kisauni and Old Town)	-	45	5.34	31-58

domains. The data was analysed using EpiInfo™ CDC software (WHO, 2004). The interviewed household members' mean physical activity was described using an indicator called metabolic equivalents (METs) then categorized in 3 levels of physical activity as low, moderate and high. This calculation and categorization was done using the GPAQ guide (WHO, 2004).

Focus group discussions (FGDs)

Ten volunteers from each of the study sites were selected and invited to a Focus Group Discussion (FGD), to respond to some structured questions that sought to elicit information on Swahili dietary habits and practices with particular emphasis on type of foods consumed and their preparation methods. Information derived from the FGD was used to complement that from household survey. Women were purposively selected to be involved in these discussions because they were involved in food preparation.

Key informants interviews (KIIs)

KIIs were used to seek in-depth information over and above what was collected from focus group discussions and household survey. The key informants, mainly the village elders, were identified by the community members based on their positions in the community and interaction with the community members.

Observation

Observations were made on the Swahili community's way of life, their dietary habits, cookery ingredients and food preparation methods during data collection. These observations were used to verify the information given by the respondents.

Ethical considerations

An introductory letter was obtained from Egerton University Graduate School before carrying out the study. This facilitated the acquisition of ethical approval from the University's Ethical Review Board. A study permit from the National Council of Science and Technology authorizing the carrying out of the study among Swahili people in Mombasa County was also acquired. Permission was also sought from the administration offices to carry out the study in

the County. Individuals' informed consent was obtained before interviewing them and this was after explaining to them the purpose of the study and how the results from the study will be used. They were also assured of strict confidentiality of all the information collected in the study. This was done by ensuring that their names were not included in the questionnaires.

Data analyses

Following the coding and computer entry of the data, both descriptive and inferential analyses of the data were undertaken, using the Statistical Package for the Social Sciences (SPSS) version 20 (SPSS Inc., USA). Appropriate descriptive analysis was used to generate frequency distributions and tables and inferential analyses to indicate relationships between various independent and dependent variables, including bivariate and multivariate analysis to measure the strength of relationships between the variables. The Chi-square test at 95% confidence intervals and a p-value <0.05 was used to test for differences between the Old town and Kisauni districts findings. Descriptive analysis was undertaken for the Focus Group Discussion and the Key Informant interviews to complement the analyses from the questionnaire survey findings. Nutri-survey program was used to analyze the 24-h dietary recall data. GPAQ data was cleaned and analyzed using EpiInfo™ CDC software. The interviewed household members' mean physical activity was described using an indicator called metabolic equivalents (METs) then categorized in 3 levels of physical activity as low, moderate and high. This calculation and categorization was done using the GPAQ guide.

RESULTS

General household characteristics

A total of 414 participants (198 from Kisauni district and 216 from Old town) were included in the study. Out of this, 99 women and 99 men were from Kisauni district and 108 women and 108 men from Old town district. The mean age of the household members that were interviewed in Kisauni district was 44 years whereas in Old town it was 45 years (Table 1). The mean number of household members in Kisauni district was 3.4 whereas

Table 2. Mean daily macronutrient intakes of the Interviewed Household members.

Item	Kisauni district (n=33 households)	Old town (n=36 households)	Sig. (P= 0.05) (t-test)
Energy (kcal)	2578.5 ± 314.6	2987.5 ± 352.5	P<0.05
Carbohydrates (g)	341.2 ± 41.6	347.5 ± 41.0	P<0.05
Carbohydrates (% of energy)	54	55	-
Fat (g)	88.8 ± 10.8	94.6 ± 11.2	P<0.05
Fat (% of energy)	31	33	-
Protein (g)	97.6 ± 11.9	78.1 ± 9.2	P>0.05
Protein (% of energy)	15	12	-

in Old town it was 3.8.

Food consumption and dietary practices and habits

Nutrient intake

The nutrient intake of the individuals studied indicated comparable intakes between both Kisauni district and Old town. Percentage of calories coming from fat and carbohydrates was slightly higher in the community members from Old town than those in Kisauni district. This difference was non-significant. The percent calories coming from fat was 31% for the group from Kisauni district and 33% for the group from Old town, 15% came from proteins for the group in Kisauni district and 12% for the group in Old town and rest (54%) from carbohydrates for Kisauni district and 55% for Old town (Table 2). The individuals in Kisauni district had mean energy intake of 2578.5 kcal and those in Old town had mean energy intake of 2987.5 kcal. It was reported during the FGD in Kisauni district, that apart from the typical Swahili dishes, the community in Kisauni district consumes foods from other communities like *sima* (*posho* made from maize flour) and fish. One woman said, '*Mbali na vyakula vya kikwetu, huwa twala sima na samaki.*' This is translated as, 'Other than our typical foods we eat *sima* and fish.' It was also reported that, 'Foods like *sima*, *sukuma wiki* (kales) and *githeri* (mixture of maize and beans) are not common in the Swahili culture.'

Dietary practices and habits

Dietary practices and habits were assessed using a FFQ. The frequency of some of the Swahili foods that were mostly consumed is shown in Table 3. These foods include: *pilau* (Figure 1) and *biriyani*, which are spicy varieties of rice and served with *kitoweo* (beef or chicken stew), *mahamri* (doughnut-like snacks flavoured with coconut milk and spices; Figure 2), *mbaazi* (pigeon peas

cooked with coconut milk and served with thick coconut paste) and coconut meat stew (Figure 3). Other Swahili foods consumed included: *samaki wa kupaka* (fish cooked in coconut milk), *uji wa mchele* (porridge made from rice flour and coconut milk), *katlesi* (fried mashed potatoes coated with egg white), *viazi vya jeera* (spicy mashed potatoes), *tambi* (pasta), *sharbatu ya tende* (milk shake prepared using coconut milk and dates), *viazi vya tamu* (sweet potatoes cooked in coconut milk), *vibibi* (pancakes cooked with coconut milk), *kaimati* (pastries cooked using coconut milk and coated with sugar; Figure 4), *viazi vya karai* (deep fried potatoes coated with wheat and gram flour paste; Figure 5) and *mkate wa tambu* (baked pasta bread flavored with coconut milk; Figure 6). These foods are high in fats and sugars. The high fat and sugar content was observed in the streets of Mombasa County as the foods and snacks were being prepared for sale. Some the foods are mostly consumed during special occasions and religious occasions like Muslim fasting period (Ramadan), *maulid* and *idd* as reported by the women in the FGDs and Key Informant Interviews (KIIs). These occasions occurred once or twice in a year but consumption of these foods may contribute to obesity because the foods are prepared and consumed in large quantities during this period. A significant difference was indicated in coconut milk intake between Kisauni and Old town districts. Members of the Swahili households in Old town district had a higher intake of coconut milk than those in Kisauni district. Coconut milk was obtained primarily by pressing the grated coconut's white kernel or by passing hot water through grated coconut, which extracts oil and aromatic compounds.

The women participating in the FGDs were very responsive when describing the Swahili diet and dietary habits. They said, "The Swahili diet is high in fat, species and sugars. They mentioned *wali*, *pilau*, *biriyani*, *mahamri* and *mbaazi* as the Swahili foods that are commonly consumed. "Foods like *tambi*, *kaimati*, *sharbatu ya tende*, *uji wa mchele*, *samaki wa kupaka* among others are consumed during special occasions like Ramadan," they said. It was also reported that three meals and two

Table 3. Swahili foods consumption in the Swahili households.

Study area	Foods	Number of times (%)				
		1/day	5-6/week	2/week	1/week	Almost never
Kisauni	<i>Wali</i>	0	63.6	35.4	1.0	0
	<i>Pilau</i>	0	4.0	79.8	15.2	1.0
	<i>Biriyani</i>	0	2.0	17.2	73.7	5.1
	<i>Kitoweo</i> (beef)	0	35.4	61.6	2.0	1.0
	<i>Kitoweo</i> (chicken)	0	0	14.1	57.1	28.8
	<i>Mahamri</i>	2	24.4	56.6	14.1	3.0
	<i>Mbaazi</i>	0	0	9.1	73.7	17.2
	<i>Coconut</i>	0	70.9	11.1	5.1	1.0
	<i>Ugali</i>	0	0.7	18.9	21.8	58.6
	<i>Sukuma wiki</i>	0	0.4	16.4	40.1	43.1
Old town	<i>Wali</i>	0	67.1	32.4	0.5	0
	<i>Pilau</i>	0	0.9	81.9	16.7	0.5
	<i>Biriyani</i>	0	2.8	26.9	67.9	2.8
	<i>Kitoweo</i> (beef)	0	53.7	44.9	0.9	0.5
	<i>Kitoweo</i> (chicken)	0	0	20.4	70.4	9.5
	<i>Mahamri</i>	0.9	38.0	55.1	4.6	1.4
	<i>Mbaazi</i>	0	0	17.6	74.1	8.3
	<i>Coconut</i>	0	82.2	15.3	6.5	1.4
	<i>Ugali</i>	0	0	0	0.4	99.6
	<i>Sukuma wiki</i>	0	0	0	1.8	98.2
Total (Kisauni and Old town)	<i>Wali</i>	0	65.5	33.8	0.7	0
	<i>Pilau</i>	0	2.4	80.9	15.9	0.7
	<i>Biriyani</i>	0	2.4	23.2	70.5	3.9
	<i>Kitoweo</i> (beef)	0	44.9	52.9	1.4	0.7
	<i>Kitoweo</i> (chicken)	0	0	17.4	64.0	18.6
	<i>Mahamri</i>	1.4	31.4	55.8	9.2	2.2
	<i>Mbaazi</i>	0	0	13.5	73.9	12.6
	<i>Coconut</i>	0	79.7	13.3	5.8	1.2
	<i>Ugali</i>	0	0.35	9.45	11.1	79.1
	<i>Sukuma wiki</i>	0	0.2	8.2	20.95	70.65

snacks are served in Swahili households and dining out was not common in this community. They preferred eating their meals at home. Food preparation and cookery methods were also discussed. The following were the representative responses given on this subject: "The ingredients used for preparation of most Swahili dishes include: cinnamon, cardamom, black pepper, mixed spices, tamarind among other spices." "Most of the foods are deep fried." "The fat used for frying is vegetable oil commonly known as *uto*."

Dietary habits of the Swahili community generally involved preparation and consumption of high fat, sugar and coconut milk foods. These foods were frequently consumed in majority of the households.

Physical activity levels

About 75.8% of the interviewed household members in both Kisauni and Old town districts had low levels of physical activity (Table 4). Majority (81.3 and 70.8% in Kisauni and Old town, respectively) of the individuals had low levels of physical activity. A significant difference between physical inactivity in Kisauni district and Old town district was indicated. Individuals in Kisauni district had lower physical activity levels than those in Old town. This was noted during the FGD in this district where it was reported that, '*Kwa waswahili hakuna mazoezi mtu akitaka kwenda pahali kidogo mpaka tuktuk ama matatu*'. This is translated as 'In the Swahili community people do



Figure 1. Pilau.



Figure 2. Mahamri.



Figure 3. Mchuzi wa nazi: Coconut meat stew.



Figure 4. Kaimati.



Figure 5. Vazi vya karai.



Figure 6. Mkate wa tambu.

Table 4. Physical activity levels of the interviewed household members.

Study area	Physical activity level	Percentage
Kisauni district	High	1.5
	Moderate	17.2
	Low	81.3
Old town	High	3.7
	Moderate	25.5
	Low	70.8
Total (Kisauni and Old town)	High	2.7
	Moderate	21.5
	Low	75.8

not engage in physical activity, they use vehicles to travel short distances'. Old town district is situated in an area that has no grounds for physical or recreational activity; the houses are very crowded. Despite the availability of grounds for physical or recreational activity in Kisauni, members of this district did not engage in physical activity. The women in both areas were mainly homemakers and those that worked had businesses that involved sitting down, cooking and selling the Swahili foods and snacks hence minimal movement. The men on the other hand said that they were not physically active. Few of them said they went to the gym, for swimming and some jogged hence 2.7 and 21.5% of the interviewed household members had high and moderate physical activity level, respectively.

Prevalence of overweight and obesity in the Swahili community of old town and Kisauni district

There was a high (87.2%) prevalence of overweight and obesity among the interviewed household members in both Kisauni and Old town districts as shown in Table 5. The mean BMI in both study areas was above 25, hence the high prevalence overweight and obesity. About 48.5 and 35.9% of the interviewed household members in Kisauni district were overweight and obese, respectively compared to 37.5 and 52.3%, respectively in Old town district. There was a significant difference between overweight and obesity levels in Kisauni and Old town districts. Old town had more individuals that were obese.

Prevalence of diagnosed diabetes mellitus and hypertension in the Swahili community

About 11.1 and 39.9% of the interviewed household members in Kisauni district had diagnosed diabetes and hypertension, respectively compared to 14.8 and 44.0%, respectively in Old town district (Table 6).

Association between the risk factors for chronic diseases of lifestyle (Unhealthy dietary habits and practices, physical inactivity, overweight and obesity)

Dietary habits of the interviewed household members in Kisauni and Old town districts were significantly associated with their physical activity levels, weight status and diagnosed diabetes and hypertension ($p < 0.05$, 95% CI). Physical activity levels in Old town district were significantly associated with overweight and obesity ($p < 0.05$, 95% CI, 0.27 - 0.88). Overweight and obese individuals had low physical activity levels.

Logistic regressions indicated that, in Kisauni district, the odds that someone who was physically inactive is 1.28 times likely to be obese than the individuals who were physically active whereas in Old town district, the odds are 0.49 times. Logistic regressions between dietary intake and obesity did not show statistical significance association between both.

Obesity among the individuals in Kisauni ($p < 0.05$, 95% CI, 2.17 - 15.80) and Old town ($p < 0.05$, 95% CI, 1.37 - 7.50) districts was statistically significantly associated with diagnosed diabetes. Obesity as assessed by BMI in this community was associated with hypertension and diabetes. In Kisauni district, the odds that someone who was obese (BMI > 30) is 5.87 times likely to be diabetic than the individuals who had a BMI < 30 whereas in Old town the odds are 3.20 times. On the hand, in Kisauni district the odds that someone who was obese (BMI > 30) is 1.67 times likely to be hypertensive than the individuals who had a BMI < 30, whereas in Old town the odds are 1.02 times.

DISCUSSION

The slightly higher macro-nutrient intake in Old town can be explained by consumption of typical Swahili foods which are high in sugars and fat. The Swahili community

Table 5. Prevalence of overweight and obesity in Kisauni and Old town districts.

Study area		N	Female (%)	Male (%)	Total (%)
Kisauni	Total no. of individuals	198	-	-	100%
	Underweight	2	2.0	0.0	1.1
	Normal weight	29	17.2	12.1	14.7
	Overweight	96	46.5	50.5	48.5
	Obese	71	34.3	37.4	35.9
	BMI: mean (SD)	-	28.69 (3.58)	28.68 (5.20)	28.7 (4.45)
Old town	Total no. of individuals	216	-	-	100%
	Underweight	4	3.7	0.0	1.9
	Normal weight	18	11.1	5.6	8.3
	Overweight	81	36.1	38.9	37.5
	Obese	113	49.1	55.6	52.3
	BMI: mean (SD)	-	30.18 (3.55)	29.73 (5.79)	29.95 (4.80)
Total (Kisauni and Old town)	Total no. of individuals	414	-	-	100%
	Underweight	6	2.9	0.0	1.4
	Normal weight	47	14.0	8.7	11.4
	Overweight	177	41.1	44.4	42.8
	Obese	184	42.0	46.9	44.4
	BMI: mean (SD)	-	29.23 (5.53)	29.47 (3.63)	29.35 (4.67)

SD: Standard deviation.

Table 6. Prevalence of diagnosed diabetes mellitus and hypertension in Old town and Kisauni districts.

Study area		Female (%)	Male (%)	Total (%)
Kisauni	Diagnosed diabetes	11.1	10.1	11.1
	Diagnosed hypertension	33.3	42.4	39.9
Old town	Diagnosed diabetes	14.8	15.7	14.8
	Diagnosed hypertension	40.7	50.9	44.0
Total (Kisauni and Old town)	Diagnosed diabetes	13.0	13.0	13.0
	Diagnosed hypertension	37.2	46.9	42.0

from Kisauni district on the other hand had diversified its diet by consuming foods from other communities which are not as high in fat and sugars like their typical foods. The high and frequent consumption of coconut milk in Old town is also attributed to the Swahili community's dietary habits and practices (MOT, 2015).

In the study on dietary habits of the sub-urban Saudi Arabian community which is similar to the Swahili community, Bader and Khalid (2008) reported that around half of the participants eat meals regularly, two snacks between three main meals. More than three quarters (78%) do not eat meals from outside, and the types of fat and oil used were mostly of vegetables

source (83.9%). Historically, the Swahili people developed as Arab and Persian traders established business contacts. They have close association with Arabic and Islamic cultures (MOT, 2015). This explains the similarity in dietary habits between the Saudi Arabian and Swahili communities (MOT, 2015).

The physical inactivity can be attributed to most of the individuals not participating in physical activity during leisure time and engaging in occupational and domestic activities that do not involve a lot of physical activity. The culture and religion, as stated in the focus group discussions, require the women to stay home as the men go out to work which translated into physical inactivity

among the women who just had minimal movements around the house. Generally, in the urban settings, people tend to do less physical exercise. Public transport is mostly used thus walking is minimal. An increase in the use of passive modes of transport has been associated with declining physical activity levels (WHO, 2010). People do less labor-intensive work and watch television even in the poorer urban settings, compared to those living in rural settings (Miranda et al., 2016). Mombasa County is an urban area where majority of the people use public transport and are involved in jobs that are less labour-intensive hence sedentary behavior during occupational activities (MOT, 2015).

Old town had more obese individuals, which could be attributed to the physical inactivity and high caloric intake especially from coconut milk. Over 95% of coconut oil is fat and the fat content of scraped coconut is around 34% and of coconut milk around 24% (Amarasiri et al., 2006). The community in Kisauni district on the other hand consumed other foods that were not typical of this community which may be the explanation of their lower caloric intake. However, their physical activity level was low hence the cases of overweight and obesity among individuals in this district. Physical inactivity and unhealthy diets are major contributors to overweight (Whatnall et al., 2016).

The association between physical inactivity and obesity as well as between obesity and lifestyle diseases in this study is also reported in other studies. For instance, the physical inactivity of the Nigerian civil servants, studied by Forrest et al. (2001), was correlated to increased weight, BMI and waist-to-hip ratio in men. Similarly, Sobngwi et al. (2004) in Cameroon as well as Aspray et al. (2000) in Tanzania concluded that physical inactivity was associated with obesity in the people they studied in urban and rural settings. In a study on physical activity among middle-aged West African women, more walking was associated with a three-unit lower BMI (Sobngwi, 2004). Therefore, increased physical activity among members of the Swahili community would lead to a decline in the cases of overweight and obesity. Similar findings are reported in the study among the Saudis where Mohammed et al. (2002) reported that there was an association between obesity in the Saudi population and physical inactivity. He also reported that mean BMI is increased by decreased physical activity. In a study on prevalence of obesity and its associated comorbidities, Gothankar (2009) reported that there was a significant association between BMI (≥ 25) and diabetes ($p < 0.05$) and BMI and hypertension ($p < 0.05$). Other results in a study on overweight, obesity, central adiposity and associated chronic diseases in Cuban adults, Diaz et al. (2009) reported that obesity was significantly more frequent in diabetics and hypertensive individuals. The association between hypertension and obesity has been documented in other countries. In Zimbabwe, Mufunda et

al. (2000) found this strong association. Mohammed et al. (2002) also reported a significant association between high blood pressure and obesity among Saudi men and women. Joubert et al. (2007) on the other hand found that increased body mass index (BMI) was associated with hypertensive diseases and osteoarthritis.

Conclusion

Unhealthy dietary habits and practices and physical inactivity are major risk factors for obesity and chronic diseases of lifestyle like diabetes and hypertension among members of the Swahili community.

The findings of this study are important in providing a baseline that would help stakeholders come up with timely dietary and lifestyle interventions that would be used in the prevention of obesity and lifestyle diseases. Such interventions like sustainability of consumption of healthy diets require financial investments, which are small when compared with the resources needed for the treatment and management of these diseases as well as the losses due to morbidity and mortality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Microbiology, heterocyclic amines and polycyclic aromatic hydrocarbons profiles of some grilled, roasted and smoked foods in Lagos and Ogun States, Nigeria

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Dietary intake of polycyclic aromatic hydrocarbons (PAH) and heterocyclic amines (HCA) has posted a great health risk as they have been identified as a most potent human carcinogen. Microbial quality of food is also of concern as they contribute to food poisoning and infection. Sixty food samples comprising roasted yam, plantain, grilled and smoked fish and meat were randomly sampled from Lagos and Ogun State, Nigeria, and the PAHs, HCAs contents and microbial load were determined. Isolates were subjected to antibiogram assay. The pH of the samples ranged between 5.08 and 7.49, titrable acidity was in the range of 0.50 and 1.20. *Staphylococcus*, *Bacillus*, *Micrococcus*, *Proteus*, *Pseudomonas*, *Citrobacter* and *Klebsiella* sp. had been identified. Antibiogram revealed that the isolates were multi-resistant and most resistant to ceftazidime, cloxacillin and tetracycline and more susceptible to ofloxacin. PAHs were detected in some grilled, roasted and smoked samples and with the highest concentrations 314.85 and 139.97 µg/g Dibenzen[e,a,h]anthracene established in roasted yam and smoked fish samples. Only 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was detected in grilled fish and meat samples. This study therefore established the presence of chemical and microbial contaminants in some of the food items investigated. It recommended that strict sanitary practices and appropriate cooking methods be enforced during food preparation.

Key words: Antibiogram, carcinogens, hazard analysis and critical control point (HACCP), heterocyclic amines, polycyclic aromatic hydrocarbons, Nigeria.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemically inert, hydrophobic compounds consisting of three or more condensed aromatic rings soluble in organic solvents which are ubiquitous in the environment as a result of incomplete combustion of

organic materials during industrial processing and various human activities (Ishizaki et al., 2010; Wretling et al., 2010). Although widely present in the environment (soil), they can be introduced into the food chain by plants and animals. Various food preparation conditions like drying,

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Table 1. Samples analyzed and their respective codes.

S/N	Samples	Codes
1	Roasted yam	A-E
2	Roasted plantain	F-J
3	Grilled fish	K-N
4	Smoked fish	O
5	Grilled meat	P-S
6	Smoked meat	T

smoking, frying contribute greatly to their introduction into foods (Zelinkova and Wenzl, 2015). Smoked fish has been identified as a major food source of PAHs (Tongo et al., 2016). Polycyclic hydrocarbons and heterocyclic amines have been identified as a most potent human carcinogen (Jamin et al., 2013; Purcaro et al., 2013). Due to their mutagenic and carcinogenic effects, PAHs have been listed on the priority pollutant list of several agencies including the Agency for Toxic Substances and Diseases Register (ASTDR), the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (USEPA) (Yusuf et al., 2015).

Based on their structures, HCAs can be divided into two classes (Turesky and Le, 2011). The first class of HCAs; 2-Amino-3-methylimidazo[4,5-f]quinoline (IQ-) are produced at temperatures below 300°C during heat processing of creatinine/creatine free amino acids mixtures and sugars. The second class, non-IQ-type HCAs is formed by pyrolysis of amino acids and proteins, at temperatures above 300°C (Woziwodzka et al., 2013). Meat and fish products that have been cooked at a temperature of 150°C and more have been identified as potential carriers of mutagenic and/or carcinogenic HCAs (Kizil et al., 2011). The increased consumption of meat has also increased the risk for colorectal cancer (Wada et al., 2017). Colorectal cancer is the third most frequently diagnosed cancer in both men and women with an estimated 51,370 deaths occurring annually (Ollberding et al., 2012). Gibis (2016) reported that trial animals being fed with 0.03 or 0.04% HCAs in their food over a period of 2 years developed tumors after several months with the tumors appearing mainly in the animals' livers or intestines.

The microbial composition of smoked foods is also of concern. Microbial contamination of food is even more complicated than chemical contaminations (Sheen, 2012). Various studies have established the presence of coliforms, *Shigella* sp., *Salmonella* sp., *Staphylococcus* sp. and *Bacillus cereus* in smoked fish (Akinwumi and Adegbehingbe, 2015) and meat (Edema et al., 2008). These organisms are known pathogens; for instance, *Shigella* has been identified as one of the most important agents of diarrhea by World Health Organization (WHO, 2005). Shigellosis has been reported as an

important health problem around the world, occurring predominantly in young children, mainly in developing countries like Nigeria (Partha, 2014). *Salmonella* sp. has also been identified as one of the leading causes of foodborne illnesses around the globe (Faleke et al., 2017). Between the years 2013-2016, of the 24,029 cases of gastrointestinal infections identified by Foodnet, *Salmonella* and *Shigella* had the highest cases besides *Campylobacter* spp. (Marder et al., 2017).

The pH is essential as regulatory agencies may impose certain pH limits on food products; also the pH value of foods contributes to the microbial quality as the pH values of food limits the range of microorganisms which the particular food item may support (Oladipo et al., 2010). This study therefore was carried out to determine safety of commonly consumed pre-cooked food items in Lagos and Ogun States, Nigeria by determining the microbial load and chemical profiles (heterocyclic amines and polycyclic aromatic hydrocarbons contents) which are critical indicators of food safety.

MATERIALS AND METHODS

Sample collection

A total of sixty samples were employed for this study. They included ten samples each of roasted yam, roasted plantain, grilled fish, smoked fish, grilled meat and smoked meat (Table 1). Samples were purchased from food vendors in Lagos (6°27'14.65" N 3°23'40.81" E) and Ogun (7°00'0.00" N 3°34'59.99" E) states, Nigeria. The samples were appropriately labeled and transported in sterile plastic bags and cold chain 4°C to the microbiology laboratory in the Department of Biological Sciences, Covenant University, Ota, Nigeria for analyses.

pH and titrable acidity

A 10 ml aliquot of the sample homogenate in distilled water was measured and titrated against 0.1 mol l⁻¹ NaOH to an endpoint which was determined by pH at 7.0 (Chen and Liu, 2000). The sample homogenates were prepared by weighing twenty-five grams of each sample and homogenized in sterile stomacher using sterile distilled water at a ratio of 1:10. The titratable acidity was expressed as the volume consumed in millilitres of 0.1 mol l⁻¹ NaOH per 100 ml sample. The pH was also determined using a calibrated standard pH meter model PHS-2S, (SHANGHAI JINYKE

REX, CHINA).

Microbial assessment of samples

Coliform test and count

Determination of total coliforms was carried out according to the method of Sangadkit et al. (2012).

Twenty-five grams of each food was homogenized with 225 ml of sterile peptone water (0.1% w/v) for 2 min and then serial 10 fold dilutions were prepared with sterile peptone water. Sample suspension (1 ml) at appropriate dilution was pipetted onto the surface of a Petrifilm™. *Escherichia coli* /Coliform count (EC) plate, the cover film on the plate was slowly applied and incubated at 35°C for 24 h. Plates were examined and read based on the manufacturer's instruction, red colonies surrounded by trapped gas were recorded as coliforms and blue colonies with trapped gas were *E. coli*. Duplicate trials were performed per dilution, while *E. coli* and coliforms were re-confirmed using EMB agar and IMVIC tests. A positive *E. coli* control was included in the experiment for validation (Sangadkit et al., 2012).

Total aerobic plate count (TAPC)

Twenty-five grams of each sample was weighed into sterile plastic McCartney bottles. Samples were homogenized in sterile Stomacher lab blender and 225 ml peptone water for 1:10 dilution. Further, tenfold dilutions of the sample homogenate were made (Sangadkit et al., 2012). Plate count agar was used to determine the total aerobic bacteria by pour plate method. Colonies were counted after 48 h incubation at 37°C

Fungal count

This was carried out following the procedures of the International Commission for the Microbiological Specification for Food (ICMSF, 2002). Tenfold serial dilution for each sample was prepared in 0.1% peptone water and 1% glucose broth and subsequently plated onto standard Saboraud dextrose agar (SDA) for viable fungal counts. The SDA plates were incubated at 27± 2°C for 72 h. The colony forming units (cfu) were counted on plates having between 30 and 300 colonies. The enumerations of the viable fungal count were carried out in duplicate on each sample and the isolated fungi were stored at 4°C for further characterization.

Characterization of microbial isolates

The preliminary identification of the bacterial isolates was based on cultural, morphological, and biochemical test; Gram staining, motility, sugar fermentation, hydrogen sulfide production, urease, catalase and coagulase production, oxidase and bacitracin test, methyl red vogues Proskauer (MRVP), utilization of citrate, glycerol and indole production, starch and aesculin hydrolysis, arginine dihydrolase and gelatin liquefaction. Further identification of bacterial isolates was based on standard bacteriological procedures and employing the Biomerieux® sa API system. Identification of fungal isolates was based on cultural characteristics, morphological and microscopic features with reference to a standard atlas and mycological identification keys (Tsuneo, 2010).

Antimicrobial sensitivity test

The antibiotic sensitivity testing was carried out on Mueller-Hinton

agar, using the disc diffusion method as described by Bauer et al. (1966). Results were interpreted in accordance with the 2002 guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2002). The antibiotics used were Ceftazidime (30 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Cloxacillin (25 µg) Amoxycillin (25 µg), Cotrimoxazole (25 µg), Nitrofurantoin (50 µg), Gentamycin (10 µg), Nalidixic acid (15 µg), Ofloxacin (5 µg), Augmentin (25 µg), Tetracycline (10 µg).

Determination of polycyclic hydrocarbons (PAHs)

This was carried out following the method as described by Adetunde et al. (2012) whereby PAHs in all samples were extracted using solvent extraction by ultra-sonication and the extracts were analyzed for the 16 USEPA PAHs using high-performance liquid chromatography (HPLC) Millenium 2010 HPLC system (Millipore Corp., Milford, MA). The supernatant was decanted following centrifugation of extracts at 2500 rpm for 10 min. The supernatant was cleaned-up using the Whatman nylon filter membrane with further clean-up using the solid phase extraction (SPE) cartridges. The filtered extracts were loaded onto the cartridges after the sorbent of the SPE cartridges was first conditioned with N-hexane. The analytes were eluted with dichloromethane and then blown down to dryness and the extract reconstituted in acetonitrile. The PAHs was quantified using an Agilent 1100 model HPLC system with a vacuum degasser, a temperature controlled column oven, a quaternary pump, and a UV diode array detector. A monomeric type octadecyl silica column, Supelcosil LC PAH 2 cm × 4.6 mm i.d enclosing 5 µm particles at 25 ±1°C ambient temperature on a flow rate 1.0 ml/min was used for the separation of the PAHs. The standard used for quantification was phenanthrene.

Determination of heterocyclic amines (HCAs)

This was carried out using the method of Felton et al. (1994). Briefly, 20 g each of the pulverized samples were homogenized with 60 g 1 M NaOH. Four-gram aliquots of the homogenized mixture were used, for which two were spiked with a mixture of heterocyclic amines in 50 µl methanol. A packet of the Extrelut diatomaceous earth was used to mix each sample thoroughly and poured into an Extrelut 20 column. Following the methods as described by Gross and Grüter (1992), the extractions were made by collecting 40 ml dichloromethane through attached PRS columns and with subsequent washing and extraction onto C18 columns, separating polar and apolar extracts. The eluted polar and apolar mixtures were evaporated to dryness at 50°C, re-dissolved in 50 µl of methanol containing 5 µg/ml caffeine as an internal standard. The optimized HPLC separation by Gross and Grüter (1992) was employed, on a TSK gel ODS80TM column (TosoHaas, Montgomeryville, PA, 250 mm × 4.6 mm I.D.) with a mobile phase of triethylamine phosphate, 0.01M, pH 3.6 (solvent A) and acetonitrile (solvent B). The ternary buffer (pH 3.2), was not employed because it is not necessary for the separation of the heterocyclic amines used in this study. A linear gradient (5-15% B from 0-10 min; 15-25% B from 10-20 min; 25-55% B from 20-30 min) was used. The Millenium 2010 HPLC system (Millipore Corp., Milford, MA) with a 996 photodiode array detector and a Hewlett-Packard 1046A Programmable Fluorescence Detector was used to analyze the samples. The UV absorbance spectra were compared to library spectra acquired from standard solutions to identify chromatographic peaks.

Statistical analysis

Results are presented as mean and standard deviations,

Table 2. Mean pH, titratable acidity and major tentative isolates from samples marketed in Lagos and Ogun States, Nigeria.

Sample	Mean pH \pm SD	pH range	Mean TA \pm SD	TA range	Major tentative isolates
Roasted Yam	6.66 \pm 0.74	5.74 – 7.23	0.90 \pm 0.28	0.65-1.20	<i>S. aureus</i> , <i>Bacillus</i> sp. <i>Micrococci</i> , <i>Pseudomonas</i> sp.
Roasted plantain	5.28 \pm 0.24	5.08 – 5.56	0.64 \pm 0.20	0.50 -0.90	<i>Staphylococcus</i> sp <i>Pseudomonas</i> , <i>Bacillus</i> sp.
Fish samples	6.83 \pm 0.55	6.39 – 7.49	0.73 \pm 0.15	0.55-0.85	<i>Bacillus</i> sp., <i>Pseudomonas</i> sp.; <i>Proteus</i> sp., <i>Staphylococci</i> <i>Citrobacter</i> sp.
Meat samples	7.09 \pm 0.32	6.79 – 7.44	0.65 \pm 0.15	0.50 -0.80	<i>S. aureus</i> ; <i>Bacillus</i> sp., <i>Klebsiella</i> sp. <i>Pseudomonas</i> sp.; <i>Proteus</i> sp.

Key: TA= Titrable acidity, SD- Standard deviation, n= 10.

Table 3. Mean microbial count cfu/g sample marketed in Lagos and Ogun States, Nigeria.

Sample	Total aerobic plate count		Fungal count		Coliform count	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Grilled fish	8.39 \times 10 ⁶ \pm 6.57 \times 10 ²	2.7 \times 10 ³ - 2.1 \times 10 ⁶	1.17 \times 10 ³ \pm 8.26 \times 10 ¹	0 - 7.1 \times 10 ⁴	-	NG
Smoked fish	9.15 \times 10 ⁷ \pm 2.43 \times 10 ³	4.9 \times 10 ⁴ - 4.0 \times 10 ⁸	8.36 \times 10 ⁵ \pm 1.04 \times 10 ¹	0 – 2.3 \times 10 ⁶	5.42 \times 10 ⁶ \pm 4.00 \times 10 ²	0 - 4.8 \times 10 ⁷
Grilled meat	5.06 \times 10 ⁶ \pm 5.66 \times 10 ³	1.8 \times 10 ⁵ - 1.3 \times 10 ⁷	1.86 \times 10 ⁴ \pm 1.84 \times 10 ¹	0 - 3.7 \times 10 ⁴	5.40 \times 10 ⁶ \pm 3.06 \times 10 ²	0 - 6.0 \times 10 ⁷
Smoked meat	2.00 \times 10 ⁷ \pm 1.41 \times 10 ³	2.2 \times 10 ⁴ - 2.0 \times 10 ⁷	3.82 \times 10 ⁵ \pm 2.00 \times 10 ¹	0 - 4.7 \times 10 ⁵	NG	NG
Roasted plantain	1.62 \times 10 ⁶ \pm 1.73 \times 10 ³	1.7 \times 10 ⁴ - 4.2 \times 10 ⁶	2.18 \times 10 ⁴ \pm 9.38 \times 10 ¹	0 - 3.5 \times 10 ⁴	NG	NG
Roasted Yam	6.05 \times 10 ³ \pm 7.47 \times 10 ¹	2.3 \times 10 ² - 7.7 \times 10 ³	NG	NG	NG	NG

KEY: NG= No growth less than 10.

antibiograms were presented as percentages of susceptibility. One way analysis of variance was employed to compare mean microbial and pH compositions at 0.05% level of significance using SPSS statistics version 20 (SPSS Inc., USA) for the microbial, pH, titratable acidity and PAH compositions.

RESULTS

pH, titratable acidity and identified isolates

The pH was in the range of 5.08- 7.74. The lowest and most acidic pH was obtained from plantain samples while the highest pH was obtained from fish samples. Titratable acidity was between 0.50 and 1.20. Biochemical profiles revealed that the most prominent bacterial isolates were members of the genera *Staphylococcus* and *Bacillus* (Table 2).

Total aerobic plate count, coliform count and fungal count

The total aerobic plate count was observed to be highest in smoked fish and meat samples. In most of the samples, no coliform was detected (Table 3). However, some fish and meat samples had mean coliform counts of 5.42 \times 10⁶ \pm 4.00 \times 10² and 5.40 \times 10⁶ \pm 3.06 \times 10², respectively. There were no fungal growths in all the yam samples, however, smoked fish and meat samples had

the highest fungal load of 8.36 \times 10⁵ \pm 1.04 \times 10¹ and 3.82 \times 10⁵ \pm 2.00 \times 10¹ (Table 3).

Antibiogram for predominant bacteria isolates to commercial antibiotics

The results of antibiotics sensitivity test obtained from the isolates (most predominant organisms isolated from each sample) revealed that the isolates were multi-resistant to the antibiotics, with *Pseudomonas*, *S. aureus* and *Micrococcus* sp. having the highest rates of multi-resistance. The isolates were most resistant to Ceftazidime and Cloxacillin (90.91% resistance) and to Tetracycline (88.24%). The isolates are, however, 88.24 and 76.47% susceptible to Ofloxacin and Gentamycin respectively (Table 4).

PAHs and HCAs in tested foods in Lagos and Ogun States, Nigeria

Table 4 (Figure 1a and b) showed that of the 16 USEPA PAHs samples, twelve of the PAHs were detected either singly or in combinations in the samples. Acenaphthalene was detected in all the samples, and except for smoked meat samples, Indeno (1,2,3-C, D) perylene was detected in all other samples. Acenaphthene and Benz (ghi) perylene were found present in four of the six sample

Table 4. Antibiogram of predominant isolates from samples to commonly used antibiotics.

Sample	Organism Isolated	CRX	CAZ	CTR	ERY	CXC	AMX	COT	NIT	GEN	NAL	OFL	AUG	TET	%S	%R
Roasted Yam	<i>S. aureus</i>	R	R	R	S	R	S	R	-	S	-	S	R	R	36.36	63.64
	<i>Micrococcus</i>	R	R	S	R	R	R	R	-	R	-	S	S	R	27.27	72.73
	<i>Bacillus</i> sp.	S	S	S	R	S	S	R	-	S	-	S	S	S	81.82	18.18
	<i>Pseudomonas</i> sp.	-	-	-	-	-	R	R	R	R	S	R	S	R	25.00	75.00
Roasted plantain	<i>Pseudomonas</i> sp.	-	-	-	-	-	R	R	R	S	R	R	R	R	12.50	87.50
	<i>Bacillus</i> sp.	R	R	S	S	R	S	R	-	S	-	S	S	R	54.55	45.45
	<i>Staph.</i> sp.	S	R	S	R	R	R	S	-	S	-	S	S	R	54.55	45.45
Fish samples	<i>Proteus</i> sp.	-	-	-	-	-	S	R	S	S	S	S	S	R	75.00	25.00
	<i>S. aureus</i>	R	R	R	R	R	R	S	-	R	-	S	R	R	18.18	81.82
	<i>Pseudomonas</i>	-	-	-	-	-	R	R	R	S	S	S	R	R	37.50	62.50
	<i>Bacillus</i> sp.	R	R	R	R	R	R	S	-	S	-	S	R	R	27.27	72.73
	<i>Micrococcus</i>	R	R	S	R	R	S	R	-	S	-	R	R	R	27.27	72.73
Meat samples	<i>S. aureus</i>	R	R	R	R	R	S	S	-	S	-	S	S	R	45.45	54.55
	<i>Staph.</i> sp.	R	R	R	R	R	S	R	-	R	-	S	R	R	18.18	81.82
	<i>Bacillus</i> sp.	R	R	R	R	R	R	R	-	S	-	S	R	R	18.18	81.82
	<i>Klebsiella</i> sp.	-	-	-	-	-	R	R	S	S	S	S	R	S	62.50	37.50
	<i>Pseudomonas</i> sp.	-	-	-	-	-	R	R	R	R	R	S	R	R	12.50	87.50
% S		18.18	09.09	45.45	18.18	09.09	41.18	23.53	33.33	76.47	50.00	88.24	35.29	11.76	36.33	63.67
%R		81.82	90.91	54.55	81.82	90.91	58.82	76.47	66.67	23.53	50.00	11.76	64.71	88.24	63.67	36.33

KEYS: CRX= Cefuroxime; CAZ= Ceftazidime; CTR= Ceftriaxone; ERY= Erythromycin; CXC= Cloxacillin; AMX= Amoxicillin; COT= Cotrimetrazole; NIT=Nitrofurantoin; GEN= Gentamycin; NAL= Nalidixic acid; OFL= Ofloxacin; AUG= Augmentin; TET= Tetracycline; R= Resistance with no zone of inhibition; - = Not tested for i.e. not applicable because it's a Gram positive or Gram negative disk. *Staph.*= *Staphylococcus* (S).

types. The following HCAs content of the samples were analyzed; 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx), 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 2-amino-3,4-dimethylimidazo[4,5-f]quinolone (MeIQ) and 2-(Diphenyl methoxy)-N,N-dimethylethanolamine Hydrochloride.

However, only 2-amino-1-methyl-6-

phenylimidazo[4,5-b]pyridine (PhIP) was detected in fish and meat samples(Figure 2).

DISCUSSION

PAHs were detected in all the food samples (Table 5; Figure 1a and b). Of public health concern is the fact that with the exception of Benz (ghi) perylene, all the PAH detected in this study

are listed in the PAH priority list identified by the US EPA, the US Agency for toxic substances and disease registry (ATSDR), and the European food safety authority (EFSA), due to their carcinogenicity or genotoxicity and/or ability to be monitored (EFSA, 2008; ATSDR, 2011; Keith, 2015). PAHs have been implicated in cardiovascular diseases and in developmental impacts including poor fetal growth and neurological development, reduced immune functions, and low

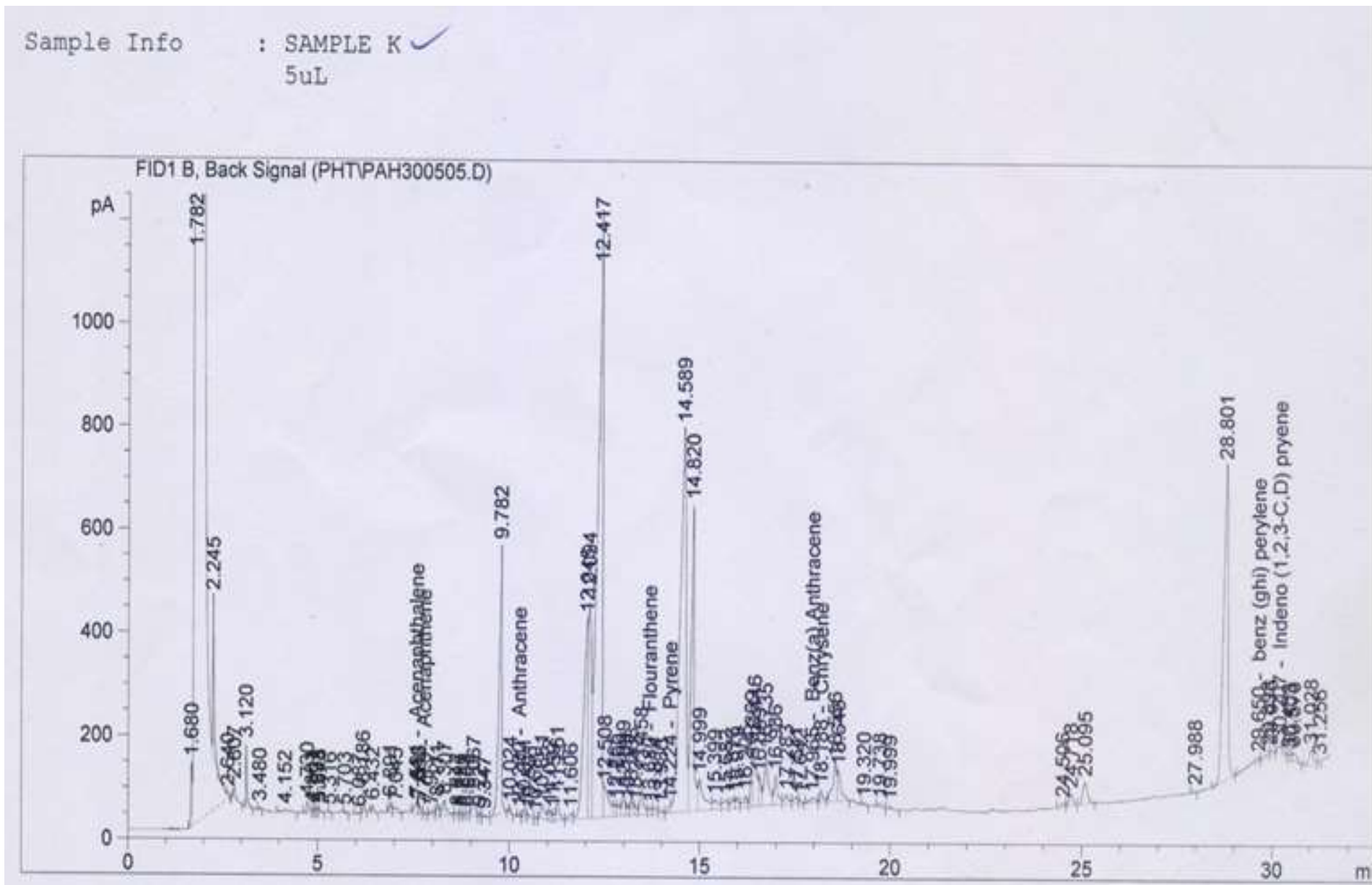


Figure 1a. PAH detected in grilled fish marketed in Lagos and Ogun States, Nigeria.

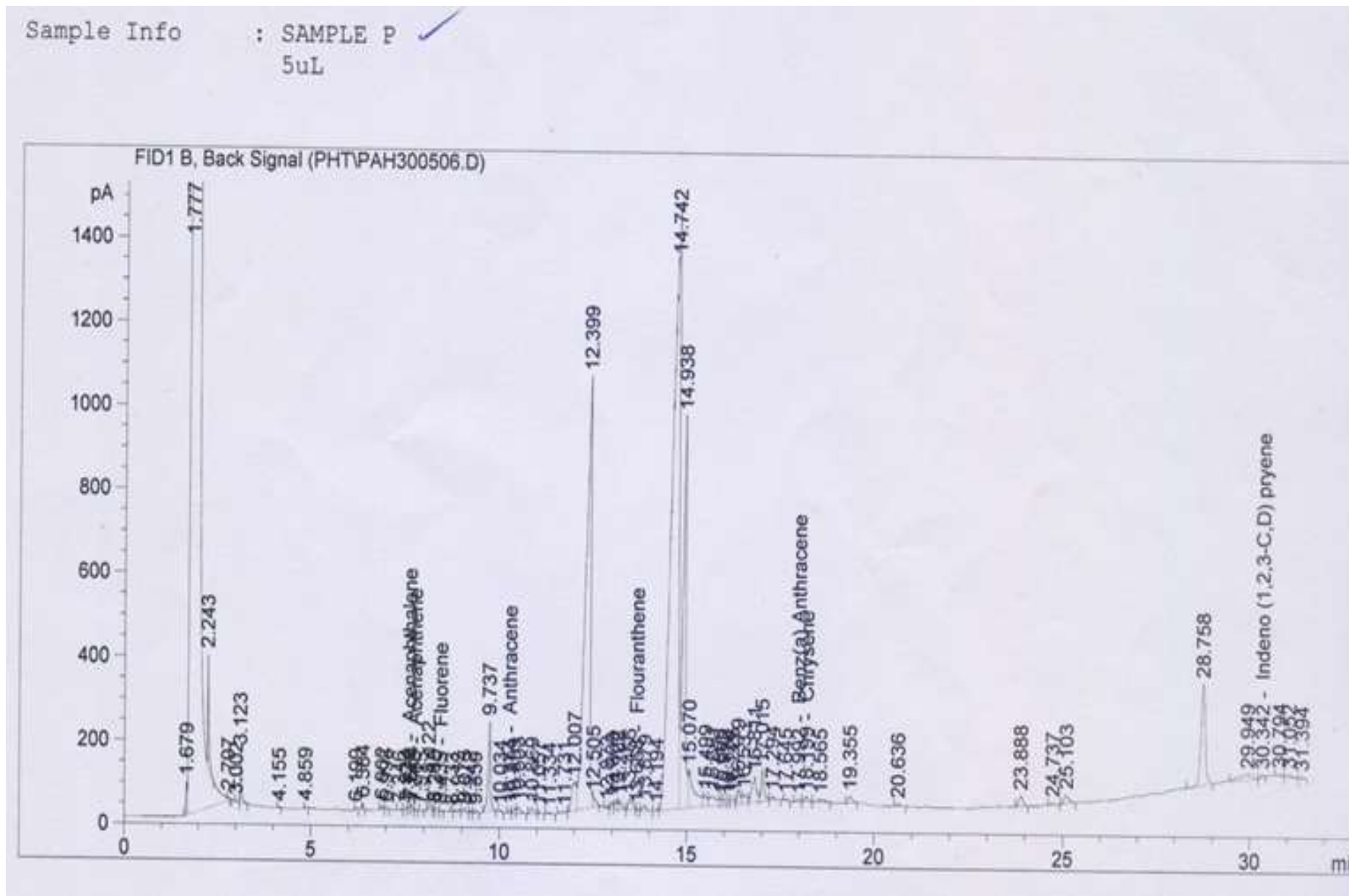


Figure 1b. PAH detected in grilled meat marketed in Lagos and Ogun States, Nigeria.

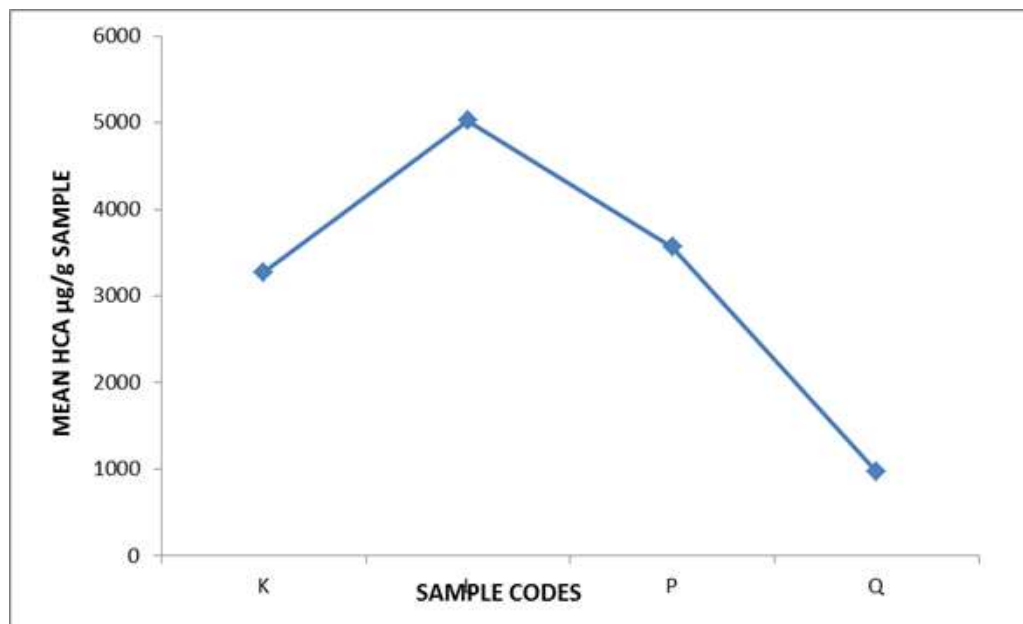


Figure 2. Heterocyclic amines (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in fish and meat samples marketed in Lagos and Ogun States, Nigeria.

Table 5. Mean PAH (µg/g) ±SD detected in some selected food samples marketed in Lagos and Ogun States, Nigeria.

PAH	Grilled Meat	Smoked Meat	Grilled Fish	Smoked Fish	Roasted Yam	Roasted plantain
Acenaphthalene	3.2195±0.22	5.5803±0.61	12.3354±2.80	5.1154±1.11	2.1124±0.08	2.1210±0.01
Acenaphthene	8.8282±1.41	1.0749±0.01	1.3648±0.05	5.5930±0.25	ND	ND
Fluorene	3.0856±0.43	2.5764±0.51	ND	ND	ND	ND
Anthracene	2.5703±1.32	ND	2.3737±1.30	1.4614±1.03	ND	ND
Flouranthene	1.3009±0.55	129.9589±5.33	6.3791±2.01	ND	ND	ND
Pyrene	ND	3.6232±0.02	5.2949±0.66	ND	ND	ND
Benz (a) Anthracene	4.1581±2.00	ND	9.2124±3.12	ND	ND	ND
Chrysene	8.3204±2.11	42.3288±3.22	13.5833±1.34	5.5787±0.81	ND	ND
Dibenz (a,h) Anthracene	ND	ND	ND	139.9682±6.05	314.8474±8.02	35.9105±3.25
Benz (ghi) perylene	ND	ND	20.8664±4.23	68.1131±3.33	45.4627±2.55	23.2140±1.12
Indeno(1,2,3-C,D) pryene	2.8484±0.45	ND	10.6661±1.22	78.7444±5.47	23.9248±3.62	10.2423±1.20
Naphthalene	ND	7.6208±2.24	ND	ND	ND	ND

Key: ND = Not detected, Detection limit= 0.5µg/g, No. of replicates= 2.

intelligence quotient "IQ" (Wormley et al., 2004; Sram et al., 2005; Korashy and El-Kadi, 2006; Winans et al., 2011; Suades-González et al., 2015). PAHs are known to be ubiquitous in the environment and are products of wood and biofuel combustion, their presence in roasted, smoked, and grilled foods could, therefore, be explained. The high temperature and direct exposure to the heat involved in these cooking methods have been reported to increase the production of carcinogenic PAHs within the food (Bansal and Kim, 2015). In all the food samples analyzed, Benzo[a]pyrene (BaP) listed as group 1 carcinogen and identified as the most potent carcinogenic polycyclic aromatic hydrocarbon (ECHA, 2016; IARC, 2017) was not detected. BaP is of significant public health importance. Wretling et al. (2010) reported that the presence of high levels of BaP in smoked meat and fish samples. Previous reports have also established the presence of high concentration of PAHs in roasted food items (Adetunde et al., 2012; Ogbuagu and Ayoade, 2012; Ishizaki et al., 2010). The highest concentration of PAHs was Dibenzene[a,h] Anthracene with a concentration of 314.85 and 139.97 µg/g detected in roasted yam and smoked fish samples (Table 4). This result is in contrast with the results of Martorell et al. (2010) where the least concentration of PAHs was seen in milk, tubers and fruits and the highest concentration in meat and meat products.

The present results also established the presence of HCAs in fish and meat samples. This had previously been reported by Zeng et al. (2018) and Khan et al. (2017). Of the six HCAs, only 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) was detected and in higher concentrations when compared with the daily intake of HCAs in fish and meat as reported by Augustsson *et al.* (1997). This increased concentration of HCA may be due to the cooking methods employed (smoking and grilling). In their study, Oz and Kotan (2016) detected PhIP in 9 of the 30 samples (30%). PhIP has been described as one of the most common HCAs in meats subjected to heat treatment. Szterk (2015) in an analysis of grilled beef, however, reported aminoimidazoazaarenes4,8-DiMeIQx as the most prevalent. The presence of PhIP in fish and meat samples analyzed calls for concern as it has been established as reasonably carcinogenic (IARC, 1997; Cross and Sinha, 2004; USDHHS, 2016).

This investigation revealed that the food items sampled were in the acidic and neutral pH. The pH of food items most readily contributed to microbial spoilage of the food. As reported Batt (2016), food items around neutral pH and above are most susceptible to microbial spoilage. This could be explained by the diverse microbial species isolated from the fish and meat samples. Sample pH in this study ranged from 5.08- 7.74.

The presence of *Staphylococcus* and *Bacillus* sp. occurred in all the foods sampled. *Bacillus* sp. have previously been isolated from food products with mean

pH values 7.4 ± 0.2 and 7.1 ± 0.1 (Chettri and Tamang, 2015). The presence of *Bacillus* spp. in the meat samples corroborates Batt (2016) where it was reported that *Clostridium*, *Bacillus*, and lactic acid bacteria were among the Gram-positives which spoil meats. *Bacillus* spp. are spore formers hence may withstand food processing and harsh conditions (Chukwu et al., 2016, 2017). The presence of toxigenic species in food has been associated with food poisoning outbreaks (Geng et al., 2017). *Staphylococcus* spp. has also been implicated in staphylococcal foodborne disease caused by ingesting food containing staphylococcal enterotoxins (Wakabayashi et al., 2018). *Staphylococcus* sp. is known to be normal human flora; improper handling of food particularly the post-processing of samples in this study may have contributed to the presence of *Staphylococcus* sp. (Oniciuc et al., 2017). Coliforms were absent in all the samples besides some fish and meat samples. The presence of coliforms could be from the food handlers, food processing environment or from water used for processing, it could be also from the aquatic environment from which the fish was harvested. Faecal coliforms may be introduced into the water bodies through anthropogenic activities like inappropriate disposal of human wastes, sewage discharges or agricultural runoffs (Malham et al., 2014). Rubini et al. (2018) have previously isolated faecal coliforms from sea water. The improper handling of the meat during slaughter and processing may have also contributed to the presence of coliforms. Some coliforms are thermotolerant and are able to withstand the heat treatment during food processing (Paruch and Maehlum, 2012).

The antibiogram revealed that most of the microbial isolates were multi-resistant to the common antimicrobial agents, This poses a serious public health concern because should such a multi-drug resistant organism cause infection, empirical treatment will be difficult (Yah et al., 2007; Jakee et al., 2009). The multi-resistance of isolates could be explained that these organisms may have been subjected to several harsh environmental conditions and exposed to sub-lethal doses of antimicrobial substances, causing them to adapt and mutate into resistant strains (Adegoke et al., 2016). Inappropriate use of antimicrobials, lack of health care personnel with continual health education on antimicrobials and poor quality drugs have also been reported as a major cause of antimicrobial agents resistance specifically in developing countries (Yah et al., 2007; Jakee et al., 2009). Several mechanical, epidemiological and genetic factors may lead to the development of drug resistance (Yah et al., 2007; Bennett, 2008; Canton, 2009; Hung and Kaufman, 2010).

Conclusion

Microbial contaminants, coliforms and chemical

contaminants (PAH's and HCA) were detected in some of the samples marketed in Lagos and Ogun States, Nigeria the quality assessment of food greatly contributed to the overall health of consumers and the incidences of outbreaks of foodborne diseases are largely a product of the microbial quality of food items. Besides this, even more, important is the chemical composition of the food. PAHs and HACs have been identified as human carcinogens. In 2017, National cancer institute reported about 8.2 million cancer-related deaths worldwide. As food items are the most common entry route for PAHs and HCAs, it has, therefore, become necessary to ensure adequate food preparation techniques.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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