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Nutritional Evaluation of Complementary Foods from Breadfruit, Breadnut and Groundnut

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ABSTRACT

Accessibility to nutritious and palatable high- protein infant diets made from local staple foods is essential for proper health and development of infants. Complementary foods of different ratios were formulated from breadfruit (*Artocarpus altilis*) pulp flour and breadnut (*Artocarpus camanis*) seed flour and roasted groundnut (*Arachis hypogeal*) which are locally available in Osun State, Nigeria. Four different infant diets were formulated: 100% Breadfruit (Basal diet), 80%:20% breadfruit-breadnut (BF-BN), 80%:20% breadfruit-groundnut (BF-GN) and 80%:10%:10% breadfruit-breadnut-groundnut (BF-BN-GN). The products were evaluated for their proximate composition (moisture, protein, fat, ash, crude fibre and carbohydrate) and used in animal feeding trials. The data obtained were compared with that of a commercial weaning food containing 16% protein, 9% fat, 5% ash and 61% carbohydrate (which serves as control). The protein, fat, ash, crude fibre and carbohydrate contents of BF-BN-GN diet were 18.29%, 10.20%, 4.60%, 3.58% and 57.43% respectively. There was significant difference ($p>0.05$) when this product is compared to the control diet. BF-GN and BF-BN diets also compared well with the control diet. The bioassay parameters: Protein Efficiency Ratio (PER) 3.96 and Net Protein Ratio (NPR) 4.65 for BF-BN-GN when compared with PER 4.20 and NPR 4.90 for commercial diet were favourable. The result for this work had shown that breadfruit and breadnut offered good potential in the formulation of complementary food. Both crops are available, nutritious and affordable and thus can be suitable in improving infant food formulation.

Keywords: *Breadfruit, Breadnut, Groundnut, Complementary foods, Formulation.*

INTRODUCTION

For the first few months of life, the human infant subsists on a diet of breast milk. Breastfeeding is acknowledged to be the optimal way of both feeding and caring for young infants (Baumslag and Michaels, 1995). Human breast milk provides the ideal food for human infants (WHO/UNICEF, 1998). It is also the cheapest means of feeding a child during the first six months of life. Breastfeeding is a caring practice (Dewey and Brown, 2003) and it is also a unique form of infant care that has been shown to be very important for infant development (UNICEF, 2000). During infancy, growth and maturation occurs rapidly and the demands of the body for nutrients are comparatively higher than any other period of life. It is well recognized that the period from birth to two years is the "critical window" for the promotion of optimal growth, health and development. In the first year of life, infants undergo periods of rapid growth when good nutrition is crucial (Davis, 2001). In fact, nutrition in the early years of life is a major determinant of healthy growth and development throughout childhood and of good health in adulthood (WHO, 2000).

Childhood malnutrition is very common in developing countries (FAO, 2004). This is because infants at this stage of development require a higher energy and protein in their diet so as to meet increasing demand for metabolism. Infant nutrition in the first two years of life has long-term consequences on the health and productivity of that individual. In developing countries, infants generally show satisfactory growth during the first six months of life when they are almost exclusively breastfed *(*Pelto *et al.*, 2003). Even with optimum breastfeeding children were stunted if they do not receive sufficient quantities of quality complementary foods after six months of age (FMOH, 2005). An estimated six percent or six hundred thousand under five deaths can be prevented by ensuring optimal complementary feeding (Davis, 2001).

Among many approaches needed to improve child survival and growth in developing countries is the provision of safe and nutritious infant foods (Pelto *et al.*, 2003). Infants at the early stage require energy and proteins in their diet so as to meet increasing demand for metabolism. The nutritional status of children less than 5 years of age is of particular concern, since the early years of life represent the period for optimal growth and development (Prechulek *et al.*, 1999; Happiness *et al.*, 2011). Their nutritional well-being reflects household, community and national investments in family health thereby contributing both directly and indirectly to overall country development and in particular, development of human resource (Central Bureau of Statistics, 1999).

Traditional infant porridges in Nigeria are usually made from local staples like Sorghum, maize, millet and oat resulting in gruels which have low nutritional value in terms of micronutrients and macronutrients (Dewey and Brown, 2003). The traditional infant foods have been implicated in the etiology of protein-energy-malnutrition (PEM) in children during weaning. This may be due to the low nutritive value characterized by low protein, low energy density and high bulk (Ajibola *et al.*, 2016).

Although a number of commercial infant foods exist, most families in the low and middle income earning groups cannot afford them. It is therefore expedient to formulate infant foods with adequate protein that will promote growth in children from cheap raw material using appropriate methods of processing that are adaptable to the community or home level (Dewey and Brown, 2003). In developing countries, some high-protein infant foods have been developed by supplementing cereals with legumes like soybeans, cowpeas and melons (Akpapunan and Sefa - Dedeh, 1995). Fashakin and Ogunsola (1982) formulated "Nut-ogi" (a mixture of corn gruel and peanut). Adepeju and Abiodun (2011) formulated cowpea-melon-ogi (a mixture of corn gruel with defatted melon seed and germinated cowpea) other useful combinations have been adopted by the food processing industries. However, the demand for carbohydrate sources (maize, sorghum or millet) is ever increasing owing to their increased utilization. Furthermore, the population is increasing and the requirement for cereals and cereal based foods is equally on the rise. In view of the high prices of commercial and traditional complementary foods therefore, the search for locally available, cheap and highly nutritious carbohydrate sources for complementary food formulation becomes imperative.

Breadfruit (*Artocarpus altilis*) and breadnut (*Artocarpus cummanis*) are tropical fruits. They are native to Malaysia and countries of the south pacific and Caribbean (Omobuwajo, 2007). They are now cultivated in west African countries. They belong to the Mulberry family Moraceae. The tree produces fruit twice a year, from March to June and July to September with some fruiting throughout the year (Omobuwajo, 2007). Their nutritive values especially carbohydrate, protein, fat and mineral contents is comparable with or even superior to some cereal food grains

(Adebowale *et al.,* 2008). Breadfruit and breadnut are considered by many locals as gifts from God. This is because people believe that the trees can be planted and left alone without proper care or management and will grow well and produce many edible fruits (Tuivavalagi and Samuelu, 2007). In west Africa, most especially Nigeria, breadfruit and breadnut consumption was stigmatized due to their association with slavery, food shortages and poverty (Roberts – Nkrumah and Badrie, 2005). In Nigeria, breadfruit is regarded as the poor man's substitute for yam because it is used in several traditional food preparations as a replacement of yam, but costs less than one-third the cost of procuring yam at the market (Adebowale *et al.,* 2008). Breadfruit is extremely cheap as two mature fruits each weighing about 1.5kg can be obtained for the #100:00k and can provides a meal for four adults. They can be boiled, fried, roasted or made into pottage. Most important food use of breadfruit is by boiling and pounding into a paste akin to pounded yam and eaten with soup. This is of special livelihood significance in Ile-Ife,Osun State, Nigeria during April – June/July, when the preferred yams and cocoyams are out of season and unavailable (Omobuwajo, 2007). Groundnut (*Arachis hypogeal*) also known as peanut, a member of the family legume. It is native to regions like South America, Mexico, and Central America. However it is successfully grown in other part of the world as well. Groundnut is known for its nutritional and health benefits. Five main nutrients required by the body to maintain and repair the tissues namely energy, protein, phosphorus, thiamin, and niacin, are found in good quantity in groundnut (Rai *et al.,* 1993; Freeman *et al.,* 1999). Groundnut is rich in vitamins and contains at least 13 different types of vitamin that include vitamin A, B, C and E, groundnut is rich in 26 essential minerals including calcium, Iron, Zinc, boron.

In the effort to curb problem of protein-energy –malnutrition (PEM) among infants in Nigeria, a lot of complementary foods have been formulated from locally available food materials(Ikujenlola and Fashakin, 2005; Ijarotimi, 2006; Abiose *et al*., 2015). Most of these complementary foods are still not accessible to many nursing mothers as a result of the high cost of the food materials. The present study was therefore aimed at producing complementary foods from breadfruit flour, breadnut flour (both are neglected food crops) and groundnut flours mixes and evaluates the nutritional quality of the formulated diets.

MATERIALS AND METHODS

Procurement and processing of the complementary Diets: Breadfruit, breadnut and groundnut were purchased at the Ile-Ife Central Market while the commercial baby food was purchased from a supermarket in lle -lfe. The vitamin and mineral were obtained from Pfizer Nig. Plc but mixed by the researcher. Thirty albino rats (Wister strain) of both sexes and weight between 29-38g were obtained from the Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife. All chemicals used were of analytical grade.

Preparation of Breadfruit Flour: Freshly harvested mature green and wholesome fruits were obtained from Ile-Ife, Osun State, Nigeria. The fruits were washed in clean water from the mains to remove adhering latex and dirt. Then the fruits were peeled manually using a sharp stainless knife and sliced. The core was separated and then pregelatinized. The pulp was dried in hot air oven at a temperature of 60 ^{o}C for 6 – 8 hours. The dried pulp was milled using hammer mill. The ground pulp were packed in polyethylene bags, sealed and stored in the refrigerator for further use. The flow chart for the production of breadfruit flour is shown in Figure 1.

Preparation of breadnut: Breadnuts seeds were sorted, washed and dried in a solar dryer at 55 ^{o}C for 4 days, dehulled and roasted in a gas oven at 120 ^{o}C for 20

minutes then milled using hammer mill and the resulting flour sieved. The flow chart is shown in Figure 2.

Preparation of Groundnut Flour: Groundnut was cleaned and graded after which it was steamed and dried. It was then roasted at 130 ^{o}C for 25 minutes in a gas oven, then dehulled and milled to obtain groundnut flour. The flow chart for the preparation of groundnut flour is illustrated in Figure 3.

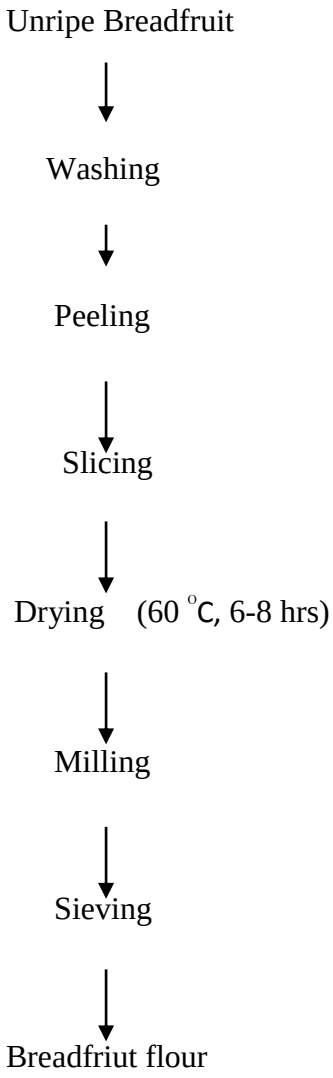

Fig. 1: Flow Chart for the Preparation of Breadfruit Flour

Source: Mayaki *et al*., (2003)

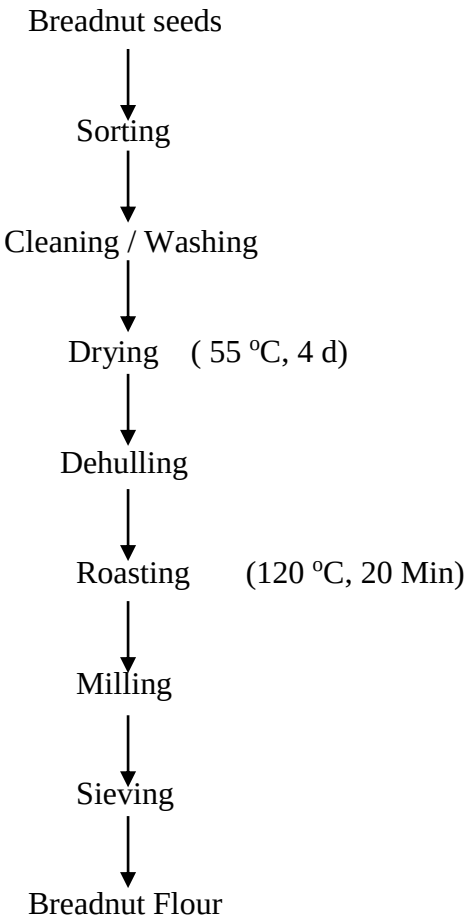

Fig.2: Production of Breadnut seed Flour

Source:Nelson-Quartey and Amagloh, (2007)

Groundnut

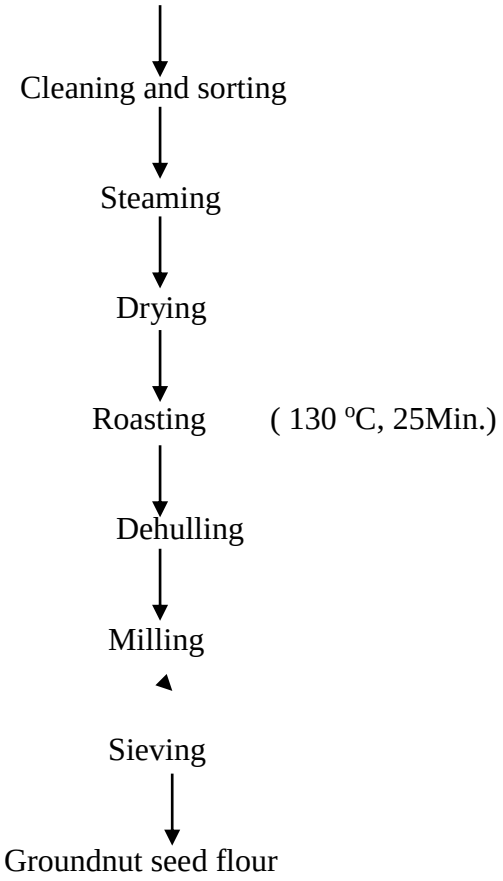

Fig. 3: Preparation of Groundnut Flour.

Source: Regena, (2010)

Formulation of the Experimental diets

Basal Diet: The breadfruit flour was mixed in a kenwood mixer for 10 minutes with sugar, vegetable oil, cod liver oil, mineral and vitamin mix to obtain basal diet. Similar processing method was followed for the other diets (Table 1).

The Experimental and control diets: The basal diet was mixed with individual protein source as recommended to achieve an Isonitrogenous diet at 10% protein level. The protein content of the experimental diets was reduced to the same level and they were named the isocaloric diets.

Breadfruit-breadnut diet: This diet was obtained by mixing 800gm of the basal diet with 200gm breadnut flour in a kenwood mixer for 10 minutes, packed in a plastic container, labeled breadfruit-breadnut diet, and stored in the refrigerator.

Breadfruit-groundnut Diet: This diet was obtained by mixing 800gm of the basal diet with 200gm groundnut flour in a kenwood mixer for 10 minutes tagged breadfruit-groundnut diet. It is then packed in a plastic container and stored in the refrigerator.

Breadfruit-breadnut-groundnut Diet: The diet was formulated by mixing 800gm of basal diet with 100g breadnut flour and l00g groundnut seed flour in the Kenwood mixer, packed, labeled as breadfruit-

breadnut-groundnut diet and stored in the refrigerator.

Commercial Diet: Commercial baby food, which is a cereal with vegetable based protein, was used in this work as a control for the formulated diets. The nutrient composition of the commercial baby food is shown in Table 2.

Table 1: Composition of the Experimental Diets

Ingredients	Basal diet (A)	Breadfruit-breadnut diet	Breadfruit-groundnut diet	Breadfruit-breadnut-groundnut diet
Breadfruit (g)	809	609	609	609
Breadnut (g)	-	200	-	100
Groundnut (g)	-	-	200	100
Vitamin premix (mg)	10	10	10	10
Mineral premix (g)	16	16	16	16
Vegetable oil (g)	100	100	100	100
Cod liver oil (g)	5	5	5	5
Sugar (g)	60	60	60	60

Table 2: Proximate analysis of the commercial baby food (Per 100g).

Protein	16.0
Ash	2.0
Crude fibre	5.0
Fat	9.0
Moisture	4.0
Carbohydrate	64.0

Source: Nestle Nigeria Plc, 2014

Experimental Animals: In this study, thirty weanling albino rats (Wistar strain) of both sexes weighing 29-38 g were obtained from Faculty of Health Sciences, Obafemi Awolowo University, Ile- Ife. They were weighed and randomly distributed in the

metabolic cages and fed normal (pellet) diet for a period of 7 days for proper acclimatization to the environment before the commencement of the experiments. After the acclimatization period, the animals were then reweighed and grouped into six groups of five rats each per group such that the differences in their mean weights were ±2gm. A group of five animals served as control for the experimental groups, were sacrificed and tissues from liver, kidney and *plantaris* muscle of the hind-leg were removed, weighed and frozen until nitrogen was determined from which protein was calculated. The remaining animals were placed on the experimental diets and water *ad libitum* over a period of twenty eight days. During this period dietary intake per day and weight of the animals were recorded.

Analysis of the formulated diets and experimental animals

Animal Tissues Evaluation

At the end of the experiment, each rat was anaesthetized and sacrificed. Tissues from the heart, kidney and *plantaris* muscle were removed and weighed. The values were subsequently expressed in g/kg of body weights. These were freeze dried and used for nitrogen determination through which net protein ratio (NPR) and protein efficiency ratio (NPR) were calculated.

Proximate Analysis: Proximate analyses (Protein, fat, ash, crude fibre, moisture and carbohydrate by difference) of commercial (control) and the experimental diets were carried out using AOAC (2005) methods.

RESULTS AND DISCUSSION

The proximate compositions of the ingredients and dietary samples in grams per 100g of diet (Mean ± SEM) were shown on Table 3. In terms of protein, all the diets compared well with the control diet. Their protein contents meet the normal required standards for infant diet (FAO/WHO, 1992) which must not be less than 15.00%. Breadfruit-breadnut diet had a value of 14.79% which is very close to the required standard value; the value observed for basal diet is as expected since the diet was not fortified with a protein source. With the simple technology involved in the formulation of Breadfruit-breadnut-groundnut diet, it is more likely to prevent against protein energy malnutrition in infants compared to the basal diet. Both groundnut and breadnut seeds increased the protein content of breadfruit by three and two times respectively since breadfruit averagely contains 6.02g/l00g (Adepeju and Abiodun, 2011). The high fat content obtained in breadfruit-groundnut diet may be due to the fact that groundnut seed is an oil seed. Fat contributes to energy density, one of the primary requirements in the formulation or improvement of infant food (Brown, 1991). All the formulation containing groundnut had higher fat content indicating that at least 10% of legumes should be incorporated in infant food formulation to improve fat content and energy density. The fat content in the diet meets the normal required standards recommended for infant diet (FAO/WHO, 1992). The value obtained for the basal diet (3.28%) is far below the recommended value of 10% and cannot therefore meet the nutritional requirements of a growing baby.

The crude fibre content of the breadfruit-breadnut-groundnut, breadfruit-breadnut and breadfruit-groundnut are 3.58%, 3.05% and 3.31% respectively. All the products had fibre contents within the proposed range of less than 5% for infant (*Codex Alimentarius*, 2000). These values are low as compared with that of the control diet. A very low level of fibre content in weaning food has been recommended (Ajibola et al., 2016). The low fibre content will encourage high digestibility and absorption of the diets by the infants. For moisture, ash and carbohydrate contents, the moisture content of all the diets were higher than that of the control diet. However these values still fall within the expected range for weaning diet

which must not exceed 10 %. The high value of moisture content observed in the formulated diets may be due to the type of drying technique used for the production of the constituents' breadfruit, breadnut and groundnut flours in the diet preparation as well as packaging and storage conditions. The cabinet drier was used. Moisture content is an indication of product shelf life, a very important measure of product quality: the lower the moisture content, the longer the expected shelf life. Roasting breadnut and groundnut reduced the moisture content considerably indicating the suitability of the roasted seeds for developing products with a longer shelf life. The ash content of the diets were lower than that of the control diet but falls within the recommended value for weaning food which must not exceed 5%.The carbohydrate content of the diets was obtained by difference. It indicated the caloric or energy values of the diets and was found to be 57.43 g, 57.79 g, 63.60 g, and 61.00 g for Breadfruit-breadnut-groundnut, breadfruit-groundnut, breadfruit-breadnut and Commercial diet respectively. Infant diet is expected to be appreciably high in carbohydrate content. The basal diet had a carbohydrate content of (88.82%) indicating its low values for protein and fat. Breadfruit-groundnut diet had the highest value of energy. This is as expected since groundnut is an oil seed although the values were still within the required standard for infant diet. All the diets were suitable for infant food formulation with respect to their energy values.

Table 3: The proximate composition of flours and the experimental diets

Dietary sample	Protein (g/100g)	Fat (g/100g)	Crude fibre (g/100g)	Moisture (g/100g)	Ash (g/100g)	Carbohydrate (g/100g)	Energy (Kcal)
BF	6.69±0.50	2.60±0.10	0.80±0.40	7.82±0.20	3.00±0.50	79.09±0.40	366.36±0.30
BN	12.10±0.20	7.30±0.30	1.70±0.10	6.26±0.11	4.40±0.30	68.24±0.11	387.06±0.10
GN	23.58±0.10	40.20±0.30	1.80±0.20	5.68±0.50	8.90±0.4	19.84±0.20	535.48±0.20
BASAL	6.02±0.30	3.28±0.11	2.20±0.50	6.59±0.55	1.93±0.20	88.82±0.60	379.12± 0.50
BF-BN	14.79±0.40	8.20±0.20	3.05±0.10	5.23±0.50	4.20±0.20	63.60±0.30	387.36±0.20
BF-GN	17.53±0.40	12.60±0.60	3.31±0.60	5.14±0.80	3.24±0.50	57.79±0.20	414.68±0.10
BF-BN-GN	18.29±0.50	10.20±0.50	3.58±0.80	5.20±0.50	4.60±0.40	57.43±0.60	394.68±0.50
Comm	16.00±0.40	9.00± 0.20	5.00±0.20	4.00±0.30	5.00±0.50	61.00±0.10	389.00± 0.30

BF- Breadfruit flour, BN- Breadnut flour, GN- Groundnut flour, BASAL- Basal diet, BF-BN- Breadfruit-breadnut diet, BR-GN -Breadfruit-groundnut diet, BF-BN-GN -Breadfruit-breadnut-groundnut diet, Comm-Commercial baby food

Animal Feeding Experiment

The animals fed with the basal diet were found to become leaner and weaker each passing day of the experiment. Changes were observed in their disposition and in their consumption rate. Loss of weight was dramatic from average weight of 33.28gm at day one to 28.06 gm on the twenty eighth days. This implies the basal diet does not support growth well in rats, perhaps due to its low protein value (Adepeju *et al.*, 2016). On the other hand, the animals fed with other diets increased in weight especially in the Commercial diet group followed by the breadfruit-breadnut-groundnut, then by the breadfruit-groundnut and lastly by breadfruit-breadnut as shown in Figure 4. There was a great vitality exhibited by all

the animals on the experimental diets (except basal group) and the commercial diet- throughout the experiment. The breadfruit-breadnut-groundnut group followed the control (Commercial) group closely in all respects in the latter days of the experiment.

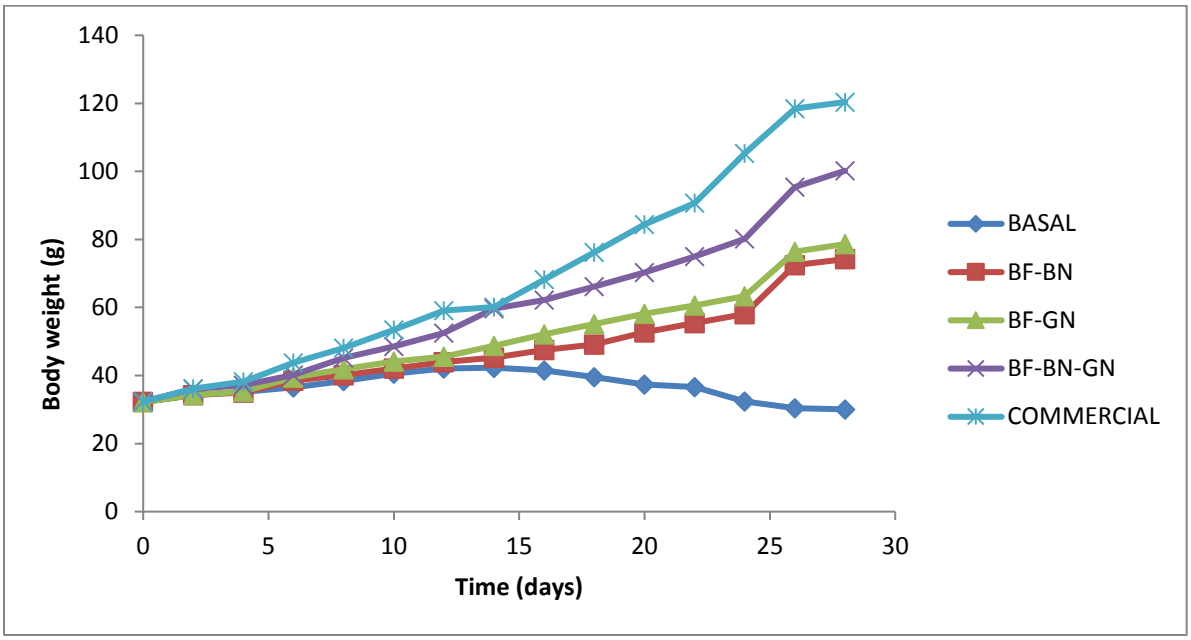

Fig. 4: Changes in mean weight of the experimental animals

The tissues of animal.fed with basal diets were found to be very small and indeed much smaller than those of animals from other experimental groups. There was large fat deposition in and around the adipose tissues of the animals fed Commercial diet.

The efficacy of the mixture of vegetable protein over the use of individual protein was confirmed by the weight of various tissues as given in Table 4.

Table 4. Weight of various tissues of the experimental animals.

Dietary sample	Liver	Kidney	Muscle
BF-BN	2.92±0.02	0.64±0.02	0.90±0.02
BF-GN	3.48±0.01	0.66±0.01	0.95±0.01
BF-BN-GN	4.87±0.03	0.84±0.05	1.63±0.02
Commercial	5.23±0.02	0.86±0.01	1.70±0.02
Basal	1.52±0.30	0.24±0.10	0.38±0.10
Control (zero-day animal.)	1.74±0.02	0.26±0.02	0.47±0.02

* The zero day animals (control) are the animal sacrificed on the first day of the experiment. The tissues collected from these animals served as the initial level for the other animal's tissues at the end of the experiment. BF-BN -Breadfruit-breadnut, BF-BN- Breadfruit-groundnut, BF-BN-GN-Breadfruit-breadnut-groundnut)

The livers of animals fed the breadfruit-breadnut-groundnut diet had the highest weights when compared with that of the breadfruit-breadnut diet and breadfruit-

groundnut diet groups. There was no significant difference (P>O.01) in weight of tissues of breadfruit-breadnut-groundnut group animal and Commercial diet group. This can be because both breadnut and groundnut used in the diet formulation were high in protein and may complement each other in the diet. The weight of kidney of breadfruit-breadnut diet group was almost same as that of breadfruit-groundnut group but higher than that of basal diet group.

The calculated Protein Efficiency Ratio (PER) values (calculated with the formula, weight gain per amount of protein consumed) and the Net Protein Retention (NPR) are indicated on Table 5.

Table 5: Protein efficiency ratio (PER) and Net protein ratio (NPR) of dietary sample (Mean ± SEM)

DIETARY SAMPLE	PER	NPR
BF-BN	3.20±0.10	3.31±0.02
BF-GN	3.42±0.01	3.90±0.05
BF-BN-GN	3.96±0.02	4.65±0.02
Commercial	4.36±0.01	4.90±0.03

BF-BN – Breadfruit-breadnut diet, BF-GN – Breadfruit-groundnut diet, BF-BN-GN – Breadfruit-breadnut-groundnut diet

The mean PER values are 4.36, 3.96, 3.42 and 3.20 for Commercial, Breadfruit-breadnut-groundnut, Breadfruit-groundnut and Breadfruit-breadnut respectively. The most favourable values were apparent in the control and those fed breadfruit-breadnut-groundnut diet whereas PER and NPR were inferior in groups receiving individual protein sources. The Breadfruit-breadnut-groundnut diet compared fairly well with Commercial which further confirmed its efficacy. The values of PER obtained in these diets exceeded the recommended values which is 2.1 (Ijarotimi, 2016). This shows that all the diets were far above the recommended requirement. This further ascertained that breadfruit and breadnut are good ingredients in complementary food formulation since the nutritional quality of food indicates their suitability for feeding young children (Livingstone et al., 1993). The NPR values followed the same pattern with commercial taking the lead, followed by the mixed diet and then Breadfruit-groundnut and finally by Breadfruit-breadnut groups. The NPR values followed the same pattern with commercial diet taking the lead, followed by the mixed diet and then Breadfruit-groundnut and finally by Breadfruit-breadnut diet group.

Table 6: Total protein level (mg/N) in various tissues of experimental animals

Dietary sample	Liver	Kidney	Muscle
BF-BN	81.40±0.20	53.85±0.80	46.62±0.20
BF-GN	86.50±0.30	56.80±0.20	48.78±0.50
BF-BN-GN	97.30±0.50	68.42±0.20	67.32±0.20
Basal	61.30±0.40	33.52±0.50	32.84±0.40
Commercial	130.72±0.10	74.70±0.20	78.06±0.10
Control(zero day animal)	62.40±0.50	32.43±0.20	35.66±0.30

Breadfruit-groundnut diet compared well so far with Breadfruit-breadnut-groundnut due to the effect groundnut (legume) which tends to improve the nutritional quality of the diet (protein).

The total protein level in the tissues is shown in Table 5. The control animals showed a clear lead over all other groups followed by the animal fed with the Breadfruit-breadnut-groundnut diet.

CONCLUSION

The study showed that the formulated diets promote growth better than the basal diet. The tissue weight measurement of the rats fed the formulated diets were better than that of the basal diet and compared favorably well with that of rats fed with control (commercial) diet. The study indicated that breadfruit-breadnut-groundnut, Breadfruit-breadnut and Breadfruit-groundnut diets may support growth in infants. The implications of these findings are not far reaching since all the components used in the formulation were obtained from local market. This would indicate that the diet formulated would be cheaper and more accessible to average mothers.

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Synthesis, Characterization and Antimicrobial Activity of Silver Nanoparticles from Aqueous extract of Elephant grass (*Pennisetum purpureum*)

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ABSTRACT

This paper assessed the use of aqueous extract of Elephant grass (*P. Purpureum)* in the synthesis of silver nanoparticles (AgNPs). The synthesis of AgNPs was confirmed by the measurement of the Surface Plasmon Resonance (SPR) at 480 nm using UV-visible spectrophotometry. Fourier Transform Infrared (FTIR) spectroscopic analysis of the nanoparticles indicated the presence of biomolecules as capping agents for the synthesized AgNPs which increased the stability of the nanoparticles thus synthesized. In addition, the antibacterial activity of the synthesized nanoparticles was tested as potential inhibitory activity against two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*).The results showed that the AgNPs have appreciable inhibition and antimicrobial effects on the two test organisms.

Keywords: Silver nanoparticles; antibacterial activity; green synthesis, elephant grass; *S. aureus; B. Subtilis*.

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INTRODUCTION

Nanotechnology, a field of research which centres on synthesis of particle with structures ranging from 1-100 nm has grown in the last decade to include the synthesis of metal nanoparticles (MNPs) with various chemical compositions, sizes and morphologies (Iravani *et al.*, 2014). MNPs have found wide applications in differs spheres of life including bimolecular detection, catalysis and plasmonic and antimicrobials (Elechiguerra *et al.*, 2005; Khlebtsov & Dykman, 2010; Saxena *et al.*, 2012; Veerasamy *et al.*, 2011). Nanoparticles of various metals including that of silver, gold, copper, zinc, titanium, cadmium and iron have been synthesized (Oberdörster *et al.*, 2005; Schabes-Retchkiman *et al.*, 2006; Vankar & Shukla, 2012). Among these metals, silver has been widely used in the synthesis of MNPs. AgNPs possessing less than 100 nm particle size and high- area- to volume ratio have been reported to possess increased reactivity relative to the bulk silver material (Oberdörster *et al.*, 2005). AgNPs been widely reported to possess antimicrobial sensitivity against disease causing organisms such as bacteria (Morones *et al.*, 2005).

AgNPs have been traditionally synthesized via physical or chemical route(Oliveira *et al.*, 2005); this routes involved the use of toxic and/ or expensive chemicals and specialised equipment. These limitations and increasing awareness towards green chemistry and other biological processes have led to the development of cheap and ecofriendly approach to synthesis of AgNPs involving use of either microorganisms such as bacteria, fungi, yeast or plant extracts (Hussain *et al.*, 2011; Udayasoorian *et al.*, 2011). However, the use of plant extracts in the synthesis of AgNPs is on the rise due to a wide range of metabolites/phytochemicals present in plants which hasten the synthesis and stabilize the AgNPs in solution (Franke *et al.*, 2010).

Pennisetum purpureum (Elephant grass), a common weed found in Nigeria is

known to be rich in metabolites such as tannins, alkaloids, flavonoids, saponins, cyanogenic glycosides and oxalates, which are known to have **antimicrobial properties (Okaraonye & Ikewuchi, 2009)**. Use of plant extracts in the synthesis and antimicrobial activities of AgNPs have been extensively studied (Azizi *et al.*, 2013) however, studies on synthesis of AgNPs from *Pennisetum purpureum* extract and antimicrobial activities of such AgNPs are limited. Therefore, this study focussed on the synthesis of AgNPs from *Pennisetum purpureum plant* extract and the antimicrobial activity of the Nanoparticles against bacteria.

MATERIALS AND METHODS

Collection of Elephant grass and preparation of extract

Fresh Elephant grass (*Pennisetum purpureum*) was collected from Joseph Ayo Babalola University campus, Ikeji-Arakeji, Osun State. The Elephant grass samples was washed several times with distilled water to remove dust particles and subsequently dried at room temperature. The elephant grass extract used in this study was obtained by adding 10 g of the dried elephant grass into an Erlenmeyer flask containing 150 ml of distilled water and boiled for 10 minutes at 60^{o}C before decanting it. The extract was allowed to cool and filtered using Whatman No.1 filter paper and was then stored in the refrigerator at 4^{o}C and used for further experiments.

Synthesis of silver nanoparticles

Silver nanoparticle was prepared by the adding 5 ml of 1 mM silver nitrate solution to 5ml of the elephant grass leaf extract and incubated at 60^{o}C in a thermostatted water temperature. The bio reduction of silver ion in solution was monitored at different time intervals by sampling aliquots of the reaction mixture for measurements of UV-Visible spectra of the solution. Reduction of the silver ions was observed by the change in colour of the reaction mixture to dark brown. The silver nanoparticle was purified by centrifuging the dark brown solution obtained several times at 2500 rpm for 20 minutes and the residues obtained were re-dispersed in water.

Characterization of silver nanoparticles

The absorption spectrum of the coloured reaction solution was recorded on a UV-Visible spectrophotometer by diluting small aliquots with distilled water in the range 200- 1200 nm. FTIR measurements were recorded on a Thermo Scientific Nicolet IF5 spectrophotometer (resolution 1000 – 4000 cm^{-1}). The analysis was carried out by pipetting a small aliquot of the sample into a potassium bromate plate and allowed to dry.

Screening of antibacterial activity of the synthesized nanoparticles

Antibacterial activities of the synthesized silver nanoparticles were assessed against two Gram positive bacteria: *Bacillus subtilis* and *Staphylococcus aureus* bacteria using agar well diffusion method with Muller Hinton Agar. Chloramphenicol (50 µl) was used as control. The experimental setup was replicated thrice. The plates were then incubated at 37°C for 24 hours and then examined for zones of inhibition.

RESULTS AND DISCUSSION

The UV–Visible spectrum of the silver nanoparticles synthesized from leave extract of *Pennisetum Purpureum* is as shown in Figure 1. When the extract was added to the boiling $AgNO_3$solution, the initial yellow colour of the solution turned dark brown colour, indicating formation of silver nanoparticles. The colour change was due to the reduction of silver ions. Metallic nanoparticles scatter and absorb light at certain wavelengths due to the resonant collective excitations of charge density at the interface between a conductor and an insulator known as surface plasmon resonance.

Metal nanoparticles have electrons which give surface Plasmon resonance absorption band due to the combined vibration of free

electrons on its surface in resonance with light wave. In this study, the absorption band of silver nanoparticles was observed at 430 nm (Fig. 1). The colour of the mixture of extract and silver ions changed from yellow to dark brown (Figure 2) on completion of reaction. This characteristic colour change is due to the excitation of the surface plasmon resonance of the silver nanoparticles silver nanoparticles (Mallikarjuna *et al.*, 2011) which gives rise to the observed colour change from yellow to brown in this study.

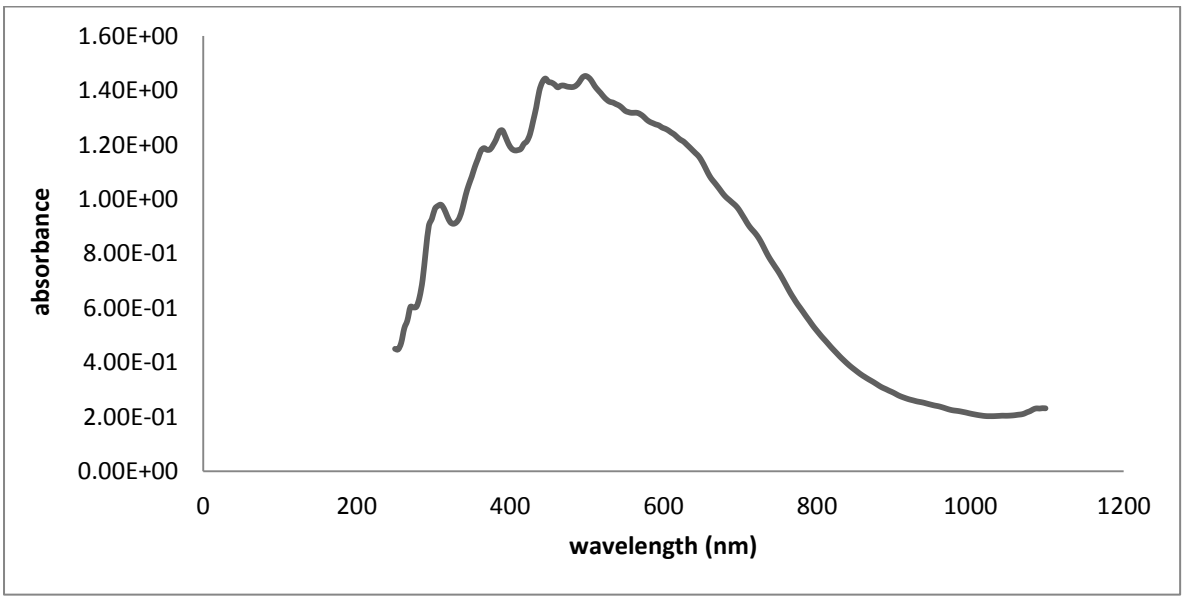

Figure 1: UV-Vis absorption spectrum of silver nanoparticles (SNP) synthesized from Pennisetum purpureum leaf extract

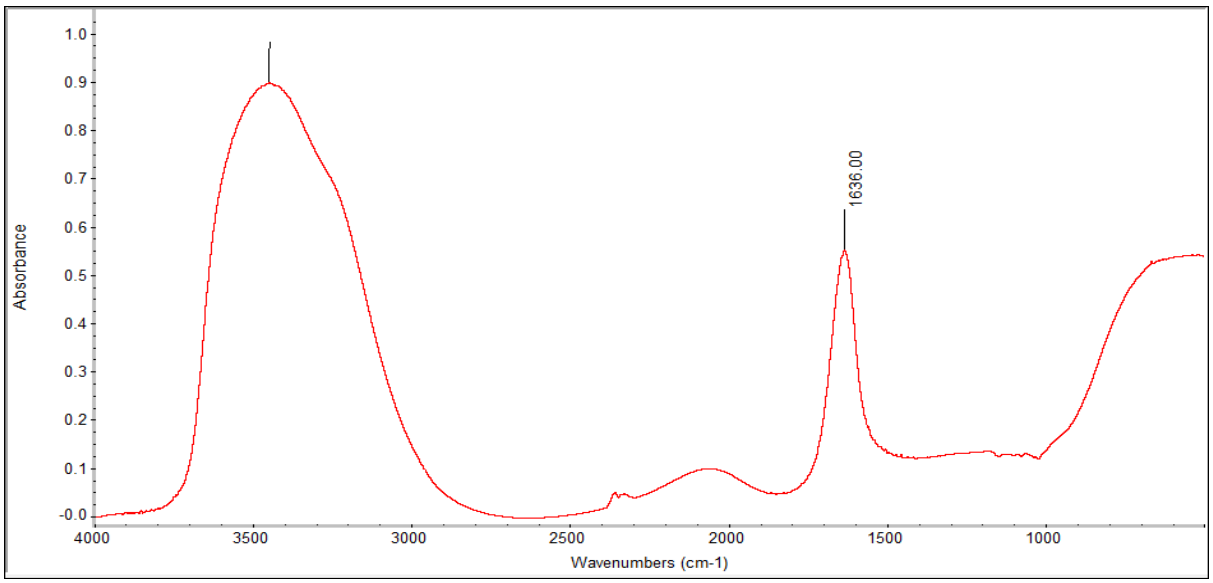

Figure 2: FTIR spectra of silver nanoparticles synthesized from *Pennisetum purpureum* extract.

FTIR measurements were carried out to identify the biomolecules responsible for capping and stabilization of nanoparticles synthesized from elephant grass leaf extract. The FTIR spectrum (Fig. 3) shows absorption bands at 3470 and 2050 cm^{-1} representing O-H and C-H stretching vibration of polyols. The absorption peaks located at 1634 and 650 cm^{-1} represented C=O and N-H vibration stretching of carboxylic acid and amines respectively. These peaks indicate that polyols (phenols and flavonoids), terpenoids and protein compounds were responsible for the reduction of silver ions to nanosilver and were further incorporated onto the silver colloids as capping agent preventing the agglomeration of the colloids thus stabilizing it (Kouvaris *et al.*, 2012).

The mean zones of inhibition exhibited by the elephant grass leaf extract synthesized silver nanoparticles; elephant grass leaf extract and bare silver nitrate solution against *S. aureus* and *B. subtilis* are shown in Table 1. The highest antibacterial activity/inhibition of 8.10±0.15 was recorded against *B.* subtilis while 4.2 ±0.21 was recorded against *S. aureus*

.

Table 1: Zones of inhibition (mm) of AgNPs against test bacteria strains

Bacteria strains	AgNPs	Extract	$AgNO_{3\ (aq)}$	Control
S. aureus	4.2±0.45	negative	3.10± 0.80	9.70±0.20
B. subtilis	8.5± 0.20	negative	5.40±0.15	10.30±0.43

Control- Chloramphenicol

In comparison with the antibacterial activity of plant extract alone, the synthesized nanoparticle is clearly an effective antibacterial agent. This is due to the fact that the leaf extract showed no antibacterial activity against any of the test strains. However, it is rich in phytochemicals that was sufficient for the bio-reduction of Ag^{+} to Ag^{o} (Okaraonye & Ikewuchi, 2009). Though the antibacterial activities of the synthesised silver nanoparticles were lower than that of the control (chloramphenicol) in this study, the percentage inhibition of the silver nanoparticles against *S. aureus* and *B. subtilis* was still above average; 64 and 82.5 % respectively.

CONCLUSION

In this study, we have described a simple and green method for the synthesis of silver nanoparticles using the leaf extract of elephant grass (*Pennisetum purpureum*). The formation of silver nanoparticles was confirmed by UV-Visible spectroscopy. The FTIR spectrum showed that the phytochemicals found in the extracts were incorporated onto the silver nanoparticles as capping and stabilizing agents. The synthesized nanoparticles showed considerable bacterial inhibition against two gram positive bacterial *S. aureus* and *B. subtilis* in comparison to chloramphenicol, a potent antimicrobial drug.

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An Analysis of the Efficiency of Pipelined Processing Model in the Design of Integrated Devices

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ABSTRACT

In the implementation of pipelined processing, the performance or efficiency is usually represented by two parameters, speedup and throughput. It is important to investigate how the speedup and throughput change as pipelining is implemented. Pipelining is implemented by defining a particular operation which can be executed in a number of stages and the performance is dependent on the number of stages. The main task in the execution of the operations is to identify the optimal number of pipeline stages that gives good throughput and speedup based on cost performance. The MULTIPLY operation is chosen in this work using a fixed point multiplier pipelining for the analysis of efficiency measurement. Pipelined multipliers are used in implementing pipelining arithmetic unit when performing multiplication of fixed point numbers. It is known that multiplication takes longer time, so pipelining improves the performance and speed of this operation. The work analysed pipelined processing and identified critical point of efficient hardware utilization by showing possible redundancy for the achievement of a particular threshold.

Keywords: pipelining, efficiency, integrated, devices, speedup

INTRODUCTION

In recent times a lot of attention is devoted to the design and use of pipelined processors for a wide range of highly productive computing devices. Increasing the efficiency of such computing devices can be achieved by proposing new design algorithms and methods oriented toward large-scale integration (LSI) and very large scale integration (VLSI) (Ajit Pal, 2014; Omondi, 1999). Such increment of efficiency brings about problems connected with optimization of pipelined information devices at the structural, functional and circuitry design stages (Agarwal et al, 2000). Pipelining is one of the most important and popular technique that is used to enhance the performance of a processor (Golub and Ortega, 2014). It is a variant of parallel implementation technique that exploits instruction level parallelism which is done in the hardware. The choice of pipeline structure in relation to the design of microprocessors was investigated in (Hartstein and Puzak, 2002). The work intensified the question of dependency that exists between pipeline depth and microprocessor performance.

Kunkel and Smith (1986) considered the dependency issue in the context of gate delay using scalar processors as the example. In this work we investigated the efficiency of pipelined processors in relation to the algorithm of multiplication as a means of unifying processor's operations. The selected multiply algorithm for this work is based on classic variant of receiving partial product by one bit at a time with a pipelined serial carry-save adder summing these partial products.

In analysing the circuits of pipelined devices, the followings were investigated multiplication algorithms and their register structures, basic pipelined device structures and the coefficient of hardware expenditure. These analyses are aimed at isolating the most effective algorithm for carrying out MULTIPLY operation shown in Fig.1, assuming that the efficiency of algorithms increases with an:

- increment in inserting control function into the structure of pipelined devices;
- increase in pipelining power;
- increase in ability to lengthen the parallelisation power.

In (Handler, 1977), a classification scheme was proposed for identifying the parallelism degree and pipelining degree built into the hardware of a computer system. Parallel-pipelined processing in this case can be considered at three subsystem levels (Hwang and Briggs, 1988):

- Processor control unit corresponding to one processor or central processing unit (CPU);
- Arithmetic logic unit corresponding to the processing element; and
- Bit-level circuits corresponding to the combinational logic circuitry needed to perform 1-bit operations in the arithmetic logical unit (ALU).

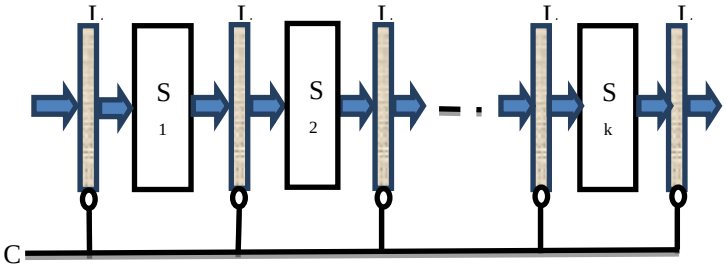
0. Start
1. Form Partial products for
   $a_i b_j \quad 0 \leq i, j \leq n-1$
2. $a_i b_j \ (i = 0, j = 0)$ are sent to the accumulator register.
3. Paired summing of odd terms is carried out
$\sum_{t=n-1}^{S} \sum_{j=S-1}^{n-1} a_i \, b_j + c_S$ and sent to the accumulator register
4. Paired summing of even terms is carried out
$\sum_{i=0}^{S} \sum_{j=S}^{n} a_i b_j + c_S$ and sent to the accumulator register
5. End.

Figure 1: MULTIPLY Algorithm

The number of pipe layer *N(S)* necessary for realising this algorithm is defined by formula (Hwang and Briggs, 1988):

$$N(S) = 3\left|\frac{N(S)-1}{2}\right| + N(S) Mod 2; \quad N(3) = 1 \qquad (1)$$

In designing pipelined integrated devices (PID), the choice of pipe stages and its optimal structure is very important. The choice of pipe layer to a large extent determines the internal structure of pipes and the system of connection amongst pipes (Weaver et al, 2002) Assuming that algorithms realised in PID allows arbitrary division into series of separate computing schemes then depending on the choice of algorithm for one or the other multiplication scheme, the efficiency of PID can change within a wide range. The choice and separation of algorithm for carrying out corresponding operations have to be done taking into consideration the complexity of each layer of PID and general number of layers in as Fig.2.



L – latch; C-control signal; $S_1 \ldots S_k$ – the i^{th} stage

Figure 2. Basic Structure of a Pipelined Processing Module

METHODS AND ANALYSIS

The analysis of how a pipelined processor's time is spent is important to determine the effective work done by the processor. If the total time is *T*, then it can be divided into the time that the processing unit is busy doing useful work - *Tb*, and the time that execution is not busy or held by any of a number of pipeline hazards, *Tnb*. This can also be regarded as the busy and not-busy times, which have been discussed in previous works (Macdougal, 1984; Emma et al, 1989). Typical pipeline holds include branch prediction errors and both decode and execution data dependencies as observed in (Emma and Davidson, 1987).

Generally, when estimating machine performance the estimated execution time, *T* is the most important measure. The impact of performance improvement can be measured in terms of the speedup *S*, expressed as the ratio of the execution time without the improvement (T_{wo}) to the execution time with the introduced improvement (T_w):

$$S = \frac{T_{wo}}{T_w} \qquad (2)$$

For example, if adding a 2MB cache module to a computer system results in lowering the execution time of some benchmark program from 24 seconds to 16 seconds, then the speedup would be 24/16, 1.5, or 50%. An equation to calculate speedup as a direct per cent can be represented as:

$$S = \frac{T_{wo} - T_W}{T_w}\ x\ 100 \qquad (3)$$

To develop a more fine-grained equation for estimating T it is necessary to have more information about the machine's clock period. In this case the total execution time for the program is given by:

$$T = IC\ x\ CPI\ x\ \tau \qquad (4)$$

where , τ …..is the machine clock period

CPI ….number of clock cycles per instruction.

IC … count of the number of instructions executed by the program during its execution

CPI and IC can be expressed either as an average over the instruction set and total count, respectively, or summed over each kind and number of instructions in the instruction set and program. Substituting the latter equation into the former we get:

$$S = \frac{IC_{wo}\ x\ CPI_{wo}\ x\ \tau_{wo} - IC_w\ x\ CPI_w\ x\ \tau_w}{IC_w\ x\ CPI_w\ x\ \tau_w}\ x\ 100 \qquad (5)$$

These equations and others derived from them are useful in computing and estimating the impact of changes in instructions and architecture upon performance. Using these equations we can also determine the coefficient of efficiency of the speedup. In the case of estimating the speedup obtained by replacing a CPU having an average CPI of 5 with another CPU having an average CPI of 3.5, with the clock period increased from 100 ns to 120ns, *S* will be 19%. Thus, without actually running a benchmark program we can estimate the impact of an architectural change upon performance. The choice of layers for PID is determined by the fact that at each time-sequence the next result bit is formed. We are applying this technique in the estimation of hardware efficiency in the design of pipelined integrated devices.

Therefore the number of pipelined integrated circuit (PIC) layer will be determined by the result bits necessary for obtaining the required computational precision. If for the purpose of separate computing step an action connected with the receipt of one next result digit is chosen then all computing processes will be the same. The realization of the same operations in every layer of PID allows the design of PIC with synchronized constant working

cycle which creates a simplified process for their production in the form of large scale integrated devices and very large scale integrated devices.

From the structural viewpoint, increasing the efficiency of PID is connected with adding some special extra registers called pipelined registers into the normal multiplying devices. The maximum number of pipelined registers that can be added is defined by formula

$$L_{max} = 3(Z - 1) \quad (6)$$

where Z = max(m, n);
m, n - bit size of the multiplicand and multiplier

The efficiency or utilisation rate of multiplication operation to a greater extent is defined by the following coefficient:

$$\propto = \frac{t_p}{t_y} \quad (7)$$

where tp – summed delay time on pipelined registers;
ty – pipeline stage execution time.

To determine the coefficient of acceleration of pipelined processing relatively to normal sequential processing firstly we determine total carry time, T_m for carrying out multiply operation in a multiplier and considering that in normal ALU multiplication the time is defined as:

$$T_m = Nt_y \quad (8)$$

where N is the length of the computed expression, but in a pipelined ALU with s-stages it is :

$$T_k = \left(\frac{t_y}{S+1} + t_p S\right)(N + S) \quad (9)$$

The coefficient of efficiency (acceleration) K_y of pipelined processing is then determined as:

$$K_y = \frac{T_k}{T_m} = \frac{\left(\frac{t_y}{S+1} + t_p S\right)(S+N)}{Nt_y} \quad (10)$$

Considering formula (2) then (10) can be expressed as:

$$K_y = \frac{T_k}{T_m} = \frac{\left(\frac{1}{S+1} + \propto S\right)(S+N)}{Nt_y} \quad (11)$$

and assuming that N is infinitely large then (11) can be rewritten as:

$$K_y = \left(\frac{1}{S+1} + \propto S\right) \quad (12)$$

We would like to compute the average number of cycles needed to execute an instruction, and the execution efficiency. If a branch is taken in a five-stage pipeline, then four cycles are needed to flush the pipeline and the branch penalty b is 4. The probability P_b that the branch is taken is .5. When the pipeline is filled and there are no branches, then the average number of cycles per instruction (CPINO_Branch) is 1. The average number of cycles per instruction when there are branches is then:

$$AvgCPI = (1 - P_b)(NoBrCPI) + P_b[P_t(1 + b) + (1 - P_t)(NoBrCPI)] = 1 + bP_bP_t \quad (13)$$

After making substitutions, we have 1.5 cycles. The execution efficiency is the ratio of the cycles per instruction when there are no branches to the cycles per instruction when there are branches. Thus we have execution efficiency = (NoBrCPI) / (AvgCPI) = 1/1.5 = 67%.

The processor runs at 67% of its potential speed as a result of branches, but this is still much better than the five cycles per instruction that might be needed without pipelining. There are techniques for improving the efficiency. As stated above, we know that loops are usually executed more than once, so we can guess that a branch out of a loop will not be taken and be right most of the time. We can also run simulations on the non-loop branches, and get a statistical sampling of which branches are likely to be taken, and then guess the branches accordingly. As explained above,

this approach works best when the pipeline is deep or the clock rate is slow.

No one doubts that there are significant differences between different types of operation run on processors in relation to the computational algorithms (Eludire, 2011). Maynard, et. al., (1984) have explored the pipelined operation differences between multi-user commercial workloads and technical workloads. The differences are associated with branch prediction accuracy, the degree of operating system calls, I/O content and dispatch characteristics (Gee et.al., 1991 and Charney et. al., 1997).

RESULTS AND DISCUSSIONS

We have simulated seven stages of register inclusion and obtained the K_y as a function of pipeline depth for each of them, and determined the optimum pipeline depth for each. In Fig. 2 we show the distribution of these optimum pipeline efficiency for different number of registers introduced into the processing module. The obtained result is shown in Table 1 and plotted in Fig. 3.

From Table 1 and the graphs shown in Figure 3, it can be deduced that when $N \geq 5$ multiply operation with dynamic introduced pipelined processing is effective. Pipelined processing is effective as far as the condition of $t_p \leq \alpha t_y$ is satisfied and from this we can determine α. The number of pipe layers necessary to ensure effective realization of pipelined processing by way of adding extra pipelined registers is determined from the following inequalities:

when $\alpha > 10^{-4}$ up to 8 layers of pipe;

$\alpha \leq 10^{-4}$ not more than 32 layers of pipe.

The following inequalities can be used in selecting an optimal number of pipe layers for achieving a given minimal efficiency T_k:

$0 < N \leq 4 \ldots\ldots 3\ to\ 4\ layers$;

$5 \leq N \leq 8 \ldots\ldots 5\ to\ 7\ layers$;

$N > 8 \ldots\ldots 7\ to\ 8\ layers$.

The choice of specific number of layers would depend on the needed α in the given range. Using a large number of layers in a pipe increases T_k and hardware expenditure which becomes comparable with realization of multiply operations without using pipelined registers shown in Fig.3.

Table 1: Coefficient of efficiency depending on number of pipeline registers introduced

S/N	1	2	4	8	16	32	64
0	4.498	8.996	17.992	35.984	71.968	143.936	287.872
1	4.673	7.010	11.683	21.029	39.722	77.108	151.879
2	5.024	6.698	10.047	16.745	30.142	56.934	110.519
3	5.549	6.937	9.711	15.260	26.359	48.556	92.949
4	6.250	7.500	10.000	15.000	25.000	45.000	85.000
5	7.126	8.314	10.689	15.440	24.941	43.944	81.949
6	8.177	9.345	11.682	16.354	25.700	44.391	81.772
7	9.404	10.579	12.930	17.632	27.035	45.843	83.457
8	10.805	12.006	14.407	19.209	28.814	48.023	86.442
9	12.382	13.620	16.097	21.049	30.955	50.766	90.389
10	14.134	15.419	17.989	23.128	33.408	53.966	95.083
11	16.061	17.400	20.077	25.430	36.138	57.553	100.383
12	18.164	19.561	22.355	27.944	39.122	61.477	106.187
13	20.441	21.901	24.821	30.662	42.342	65.704	112.427
14	22.894	24.420	27.473	33.578	45.788	70.208	119.049
15	25.522	27.117	30.307	36.688	49.449	74.971	126.015
16	28.325	29.991	33.324	39.989	53.318	79.977	133.295
17	31.304	33.043	36.521	43.477	57.390	85.215	140.866
18	34.457	36.271	39.898	47.152	61.660	90.677	148.710
19	37.786	39.675	43.454	51.011	66.126	96.354	156.812
20	41.290	43.256	47.189	55.053	70.783	102.242	165.160
21	44.969	47.013	51.101	59.278	75.630	108.335	173.745
22	48.824	50.946	55.192	63.683	80.665	114.629	182.558
23	52.853	55.055	59.460	68.269	85.886	121.122	191.593
24	57.058	59.340	63.905	73.034	91.293	127.810	200.844
25	61.438	63.801	68.527	77.979	96.883	134.691	210.307
26	65.993	68.437	73.326	83.103	102.656	141.763	219.977
27	70.724	73.249	78.301	88.405	108.611	149.025	229.852
28	75.629	78.237	83.453	93.885	114.748	156.474	239.927
29	80.710	83.400	88.781	99.542	121.065	164.110	250.201
30	85.966	88.739	94.285	105.378	127.562	171.932	260.671
31	91.397	94.253	99.966	111.390	134.240	179.938	271.335
32	97.004	99.943	105.822	117.580	141.096	188.128	282.192

S – number of pipeline stages (layers)

N1..N7 – number of registers introduced at the stages

T_K – quantified coefficient of efficiency

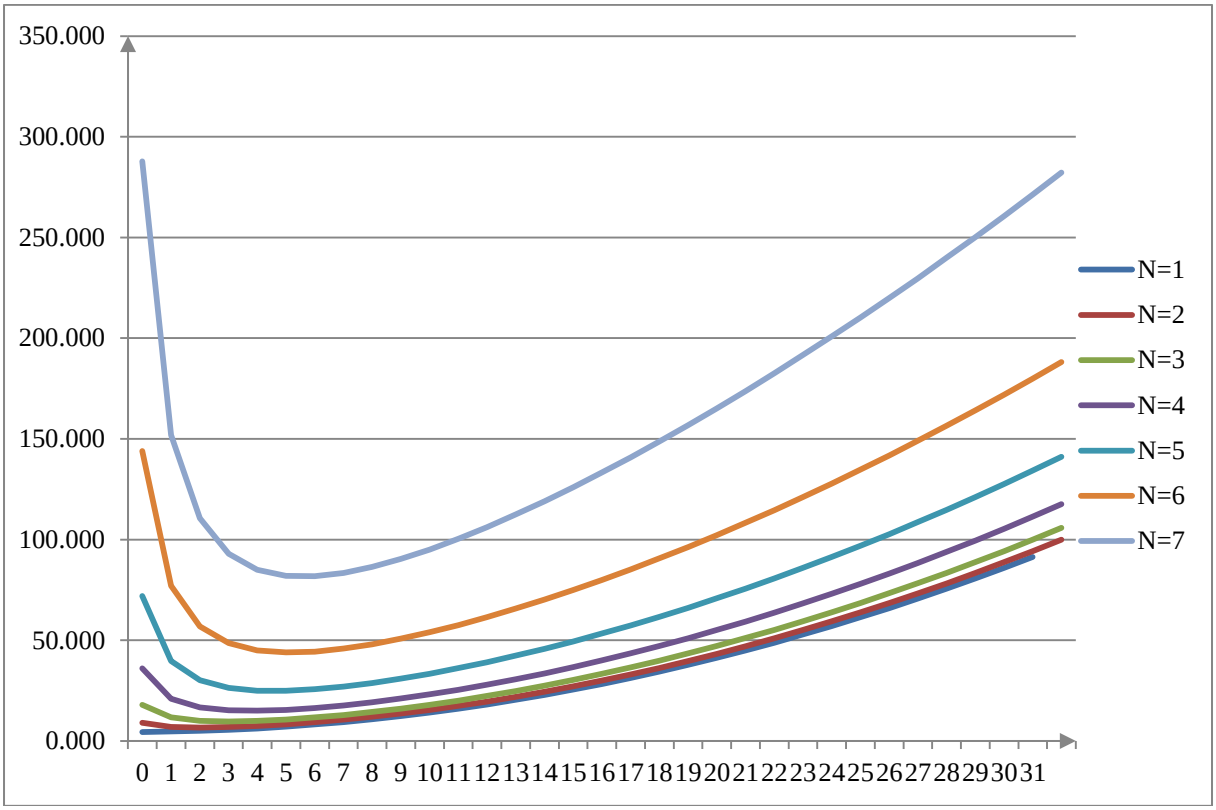


Figure 2: Dependency of Efficiency on the Number of Injected Registers and Pipeline Stages

CONCLUSION

An analysis has been presented on the choice of optimum pipeline registers for a microprocessor in the design of integrated devices. The theoretical position has been tested by simulating a variable depth pipeline model based on a pipelined multiplier, and has been found to be agreeable. It is found that the relationship between increasing the number of pipelined registers in a deeper pipeline to increase throughput, and limiting the number of pipeline stages, results in an optimum pipeline efficiency. That efficiency depends in a way on the detailed microarchitecture of the processor, details of the underlying technology used to build the processor, the algorithm and certain characteristics of the operations run on the processor.

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Effect of Fermentation on the Proximate and Anti - Nutritional Properties of African Bush Mango (*Irvinigia garbonensis)*

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ABSTRACT

This paper studies the effect of fermentation on the proximate and antinutritional properties of African bush mango (*Irvinigia garbonensis).* The sample of African bush mango (*Irvinigia garbonensis)* was divided into two portions of sample A (fermented) and sample B (unfermented). The two samples were subjected to analyses using standard methods. A had moisture content of 10.72% crude fat 41.50%, protein 13.42%, crude fibre 3.70%, ash content of 6.80%, carbohydrate 22.80% and the dry organic matter of 89.28% while Sample B had 7.26%, 60.34%, 8.0%, 4.30%, 3.0%, 17.10% and 92.74% for moisture, fat, protein crude fibre, ash, carbohydrate and dry organic matter respectively. The anti nutritional properties revealed that sample A had 0.056mg/100g phytate, 0.038mg/100g oxalate, tannin (0.011mg/100mg), saponin (0.113mg/100g) and 0.015mg/100g flavonoid while sample B showed that phytate had 0.124mg/100g, oxalate 0.26mg/100g, 0.041/100g (tannin), saponin 0.266mg/100g while flavonoid had 0.058mg/100g. The results of the study showed that both fermented and unfermented African bush mango (*Irvinigia garbonensis)* kernel has good nutrient values which can be consumed for body growth and maintenance.

Keywords: *African bush mango, proximate, anti nutritional properties, Irvinigia gabonensis, nutrients*

INTRODUCTION

African bush mango belongs to the family *Irvingiaceae.* It is known as wild mango or bush mango or Dika nut plant. It is an edible African Indigenous fruit tree that produces fruits and seeds (Atangara *et al*., 2002). The *Irvingia* species exist in two varieties, *Irvingia gabonensis* and *Irvingia wombolu*. Both species are reported to be gregarious and largely distributed in Africa. The fruit mesocarp of *Irvingia gabonensis*, is appreciated as snack or fresh fruit. Ground kernels are used to thicken and flavour soups. Bush Mango tree or dika tree (*Irviniacea spp*) is very valuable for its edible yellow mango like fruit, its kernel and the termite – resistant wood (Harris, 1996; Ayuk, *et al.,* 1999 and Adeosun, *et al.,* 2008). It grows naturally in the humid, lowland forests of tropic Africa but widely planted in central and western Africa (Ladipo *et al.,* 1995; Akubor, 1996). The fruit is a drupe with a thin outer skin, soft fleshy pulp when ripe and a hard stony nut encasing an extremely soft kernel (Okafor, 1978). There are two species of bush mango tree, the sweet edible pulp *(Irvinigia gabonesis*) and bitter inedible pulp (*Irvinigia wombolu*) (Ejiofor, 1994). The kernels of edible specie of *Irvinigia gabonensis* are called Ugiri in Igbo or Apon in Yoruba. Two species of the tree (*Irvinigia gabonensis*) which has a sweet edible pulp are common, however, kernels from both species exhibits similar valuable food properties. They are processed by grinding and crushing and then used to thicken soups and stews which a valuable local delicacy in Nigeria, Ghana, Gabon and Republic of Benin (Eka, 1980). Oil extracted from the kernel is raw materials for manufacturing pharmaceutical binders, confectioneries, edible fats, soaps and cosmetics (Okafor,

1978; Agor, 1994; Ayuk *et.al*., 1999; Okafor *et al*., 1999). The leaves, barks and root of *Irvinigia gabonesis* are also medicinal. Ethno- medicinal treatments utilize the bark kernels, leaves or roots as potential application in food and pharmaceutical industry. The dika tree (irvinigiacea spp) is very valuable for its edible yellow mango like fruit and the termite resistance wood (Ayuk *et al.,* 1999). The *Irvinigia gabonesis* kernel is very significant in the diet of rural women in West Africa (Ekpe *et al.,* 2007) and for controlling dietary lipids and weight gain (Leakey *et al.,* 2005 and Ogunsina *et al.,* 2008). *Irvinigia gabonensis* has been prized for its healing properties and now has become one of the most exciting discoveries in weight loss industry (Leakey *et al.,* 2005). *Irvinigia gabonensis* (Apon) is a tree found in West Central Africa, also known by the native as the wild mango or bush mango. The tree is valued for its dika nut in addition to producing a yellow edible fruits. *Irvinigia gabonensis* is high in fat, similar to other nuts and seed contain extraordinary fibre content (14%). Africa mango shows beneficial effects on diabetes patients and obesity as well as containing antimicrobial, antioxidant and GI activity. Africa mango constitutes an important part of the rural diet in West Africa for controlling dietary lipids and weight gain. Powdered dika kernel is commonly cooked with vegetables into "Ogbono" soup, a valuable local delicacy in Nigeria, Ghana and Gabon especially. *Irvinigia gabonesis* has played important role in the nutrition, economy and traditional medicine in western and south western to Tropical Africa from Nigerian to Angola through the help of the United Nations. A number of scientific contributions have been made available to enhance the production and commercial use of *Irvinigia* (Bajaj *et al.,* 1988).The kernels of Africa mango have classified use as oil seeds. The seeds are ground into paste, also known as dika bread, which is valued for its food thickening properties. The resulting product is used in soups, stews, the fat extracted from the kernels is similar to margarine or cooking oil. Flour may also be produced from the kernels. Numerous studies exist on the potential application of Africa mango in food, cosmetic and pharmaceutical products, and initiatives on phenotypic variation, amino acid profile, soil conditions and economic potential of the plant species document addition commercial interest (Burkill, 1994). The seeds are good source of nutrients, containing vitamins and minerals such as calcium, magnesium, sodium, phosphorous and iron. The pulp is also an excellent source of calcium (262mg per 100g) and vitamin C (66.7mg per 100ml). (Leakey *et al*., 2005). The tree grows naturally in the humid, low land forests of tropical Africa. It is widely planted in central and western Africa (Bajaj *et. al,* 1988). The kernel contains about 8.9% protein, 19.7% carbohydrate, 62.8% lipids, 5.3% dietary fibre and 3.2% ash by weight (Adeosun *et al.,* 2008). Fermentation is one of the oldest biotechnologies used in the enhancement of the nutrient content and preservation of food through the biosynthesis of vitamins, essential amino acids and proteins, by improving protein and fibre digestibility and by degrading antinutritional factors (FAO, 1983). Several food industries utilise microorganisms and the fermentation process in the preparation of foods (Ekundayo and Ojokoh, 2004). Fermentation process tends to reduce the toxicity of some foods (Namibisan and Sundaresan, 1985).The fermenting organisms include lactic acid bacteria, acetic acid producing bacteria and some alcohol producing yeast. Fermentation is a process of improving the organoleptic properties of the food by making the food more palatable and more edible. Fermentation process helps to remove toxins associated with food. These toxins are hazardous to health. This study investigated the effect of fermentation on the proximate and anti nutritional properties of African bush mango (*Irvinigia garbonensis*

MATERIALS AND METHODS

Sample Collection

Fruits of African bush mango (*Irvinigia garbonensis*) were purchased from market in Boripe Local Government of Osun State during the fruiting season on the onset of raining season. The mesocarp was peeled off to expose the endocarp. The seeds coats were broken to obtain the kernels. Other materials used are nylon, and jute bag.

Methods

The extracted kernels were divided in to two samples, sample A was fermented African bush mango kernels while sample B was unfermented one. Sample A was packed in to the jute bag and fermented for 48 hours. They were dried in the oven after fermentation. The two samples were blended separately in to air tight polythene bag and kept for analysis.

Determination of the proximate composition of African bush mango samples: Crude protein, either extract, ash, moisture content, crude fibre and total carbohydrate content of the sample were estimated by standard methods (AOAC, 1990).

Determination of anti-nutritional Composition

Determination of Tannic Acid (Tannin): Method of Makkar and Good child, (1995) was used. The tannin equivalent in the form of phenol was calculated from a standard curve.

Determination of Trypsin inhibitors: Trypsin inhibitor was determined by the method of as modified by Clarke and Oloso (1992).

Determination of Phytic Acid (Phytate): Phyatate content was determination by the anion exchange method as described by Harlnd and Oberleas (1986).

Determination of Oxalate: Oxalate content was determined using the method of Ngodi *et al*. (2005)

Determination of Saponin: 2g of each was weight in to 250ml beaker and 100ml of is isobutyl alcohol was added and left for 5 hours on a UDY shaker for uniform mixing to obtain a uniform solution. The mixture will then be filtered through a No 1 what man filter paper. The filtrate is transferred to another 100 ml beaker and was saturate with magnesium carbonate solution. The mixture was then filtered to obtain a clear colorless solution to be read on a spectrophotometer at 380 nm. 0 ppm to 10 ppm of standard saponin solution was prepared from 1000 ppm saponin stock standard solution and was saturated with magnesium carbonate as above and also filtered. The absorbance of the saponin standard solution (0-10ppm) was also read in 380 nm to obtain the gradient of plotted curve.

RESULTS AND DISCUSSION

Table 1. The proximate composition of both fermented and unfermented irvingia gabonensis kernel

PARAMETER	FERMENTED *Irvinigia garbonenses*	UNFERMENTED *Irvinigia garbonenses*
Moisture Content	10.72±0.02	7.26±0.01
Crude Protein	13.43±0.03	8.0±0.02
Crude Fat	41.5±0.01	60.34±0.01
Crude Fibre	3.70±0.02	4.3±0.01
Ash Content	6.8±0.03	3.0±0.02
Carbohydrate	22.80±0.02	17.10±0.01
Dry Organic Matter	89.28±0.01	92.74±0.02

Mean ± SD of triplicate determinations

Table 1 depicts the results of proximate composition of both fermented and unfermented irvinigia gabonensis [edible bush mango kernels]. The moisture content of fermented edible bush mango kernel was 10.72% while unfermented was 7.26%. The difference in percentage between the two was as a result of fermentation which could have increase the moisture content of fermented one. Through, the two moisture content compared favourably with the moisture content of legumes which range between 7.0-11.0% (Akroyed and Doughty, 1964). The value obtained is an indication that it can store for a long period without spoilage and will not be susceptible to microbial infection. The crude fat of fermented kernel was 41.5% while unfermented kernel of *irvinigia gabonensis* was 60.34%. The fat content of the two kernels were high and this is an indication that it is rich in oil which could be used as base materials in the manufacture of pharmaceutical binders, soups, cosmetics, confectioneries and edible fats (Ayuk *et al,* 1999). Also fat is important in the diet because it aids the absorption of fat soluble vitamins (Bogert *et al.,* 1994). The protein content of fermented to unfermented *irvinigia gabonensis* was 13.42% and 8.0% respectively. The protein values were low when compared with protein rich food like soybeans, cowpeas and melon ranging between 23.1-33.0% (Olaofe *et al.,* 1994). The recommended daily allowance for children range from 23.0-36.0g and for adults 44.56g. The crude fibre of fermented kernel of *irvinigia gabonensis* was 3.70% while unfermented was 4.30%. The reduction in fibre content was as a result of fermentation. The World Health Organization (WHO) has recommended an intake of 22.0-23.0g of fibre for every 1000 Kcal of diet (Fadare and Ajaiyeoba, 2008). Though it does not contribute to the nutritive value of foods, the presence of fibre (roughage) in the diet is necessary for digestion and for elimination of wastes, (Vadivel and Janardhana, 2005). The contraction of muscular walls of digestion tract is stimulated by fibre, thus contacting constipation (Narasinga *et al.,* (1989). The ash content of fermented kernel was 6.80% while unfermented one was 3.0%. The ash content of unfermented kernel compared well with the value (1.5- 2.5%) obtained for legumes nut seeds and tuber of legumes. The value of ash obtained in this study showed that the sample is a good source of minerals, therefore it can be recommended

for animal feeds (Pomeranz and Clifton, 1981). The values obtained for carbohydrate (by difference) were 22.80% for fermented kernel while 17.10% for unfermented kernel. This also indicated that fermented *irvinigia gabonensis* is a good source of energy and is capable of supplying the daily energy need of the body. The dry matter of fermented kernel was 89.28% while unfermented was 92.74%. This dry matter content was high. It could be attributed to experimental procedure as well as the cultivars of *irvinigia gabonensis*.

Table 2. Anti-nutrients properties of both fermented and unfermented irvinigia (Concentration mg/100g).

Anti-nutrient	Fermented *Irvinigia garbonenses*	Raw *Irvinigia garbonenses*
Phytate	0.056 ±0.01	0.124±0.01
Oxalate	0.038 ±0.03	0.26±0.01
Tannin	0.011 ±0.01	0.041±0.02
Saponin	0.113 ±0.01	0.266±0.03
Flavonoid	0.015 ±0.02	0.058±0.01

Mean ± SD of triplicate determinations

The table also depicts the anti nutrients properties of both fermented and unfermented *irvinigia gabonensis,* phytate of fermented to unfermented kernel is between 0.056mg/100g to 0.124mg/100, oxalate 0.038mg/100 to 0.260mg/100. Tannin was 0.011mg/100g to 0.041mg/100g, saponin 0.113mg/100g to 0.266mg/100. Flavonoid was 0.015mg/100 to 0.058mg/100 respectively. Comparing the phytate contents of fermented with unfermented kernel; it was low in value reported for peanut (1.36%), Ndjouenkeu *et al.,* (1996) and the value reported for dehulled and whole soybeans (1.07-65%) (Okafor and Ujor 1994); Pomeranz and Clifton (1981) but it is lower than the (0.18%) value reported for raw locust beans (Eka, 1980). Considering the processing effect on the phytate content of *irvinigia gabonensis*, fermentation has reduced it. This is in line with the work of Idowu *et al.,* (2013) who reported 31.1% reduction in phytic acid content of kenkey (fermented maize) and 45.5% reduction reported by Sadasivam and Manickam (1996) in fermentation of common beans to Tempe. It is also in agreement with the result of Ngodi *et al*., (2005). Fermentation has been reported of playing significant role in reducing phytic acid content of cereals, legumes and tubers as a result of the activities of endogenous phytase from both raw material and inherent microorganism which hydrolyse phytic acid in many fermented food preparation into inositol and orthophosphaste (Fadare and Ajaiyeoba, 2008). The oxalate is capable of chelating divalent cationic minerals like calcium, magnesium, iron and zinc thereby reducing biovailability of such minerals. The tannin content of *irvinigia gabonensis* for both fermented and unfermented is lower than the reported values of some undehulled common beans 0.95%. Though fermentation reduced the value, reduction in tannin due to fermentation might have been caused by the activity of Phenol oxidase or fermented

microflora anti tannins (Fadare and Ajaiyeoba, 2008; Fagbemi *et al*, 2005). The negative nutritional effects of tannins are diverse and incompletely understood, but it cause growth depression by decreasing the digestibility of proteins and carbohydrate. This is not likely the consequence of interaction of tannins with either proteins or starch to form enzyme resistant substances (Burkill, 1994). The saponin value of fermented kernel is reduced when compared with that of fermented one, this reduction will improved the organoleptic properties of *irvinigia gabonensis* since sapons are a factors that contribute to undesirable organoleptic properties of some legume product. There was reduction in the value of flavonoid of both fermented and unfermented *irvinigia gabonensis* kernel. The positive nutritional effect of flavonoids that they are bioactive compounds producing different antihypertensive action mechanism (Ndjouenkeu *et al.*, 1996)

In conclusion, the results of the study showed that both fermented and unfermented *irvinigia gabonensis* kernel has good nutrient values and the two varieties can be crushed, grinded, used in preparation of stew called Ogbono or Apon. The stew is good for consumption for both old and young ones as it will combine effectively with other food components in providing the required elements to the body. Based on the results obtained for both fermented and unfermented *Irivinigia garbonensis*, it can therefore be recommended in preparation of stew.

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Effects of Municipal Solid Waste on Soil Bacterial From Oke-Ijebu in Akure Metropolis

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ABSTRACT

Soil from dumpsites waste with no proper waste handling method are sources of pathogens to the soil and stream. This in turn may contribute to the emergence of community acquired infections. This study was conducted to obtain an insight into bacteria from dumpsite soil and the nearest stream in Oke Ijebu along Oja Oba road, Akure, Nigeria. Bacteria from the soil of the dumpsites was isolated in a nutrient agar medium plate, representative colonies of the isolates were subjected to further analysis. The bacteria recovered from the sample include: Bacillus anthracis, *Enterobacter* spp, *Staphylococcus* spp, *Pseudomonas aeruginosa, Paenibacillus lantus*, *Shigella sonnei, Salmonella* spp and *Citrobacter* spp were identified. The highest mean bacteria count was from the dump site soil which was 1.013×10^{8} cfu/g while the lowest mean bacteria count was 1.85×10^{5} cfu/g. There is a need for environmental agencies and governments to take appropriate preventive measures to avert potential problems due to dumping of domestic waste near a water body.

Keywords: *Soil, Dumpsites, Domestic waste.*

INTRODUCTION

The soil contains many types of microorganisms such as bacteria, Actinomycetes, fungi and algae which are important because they affect the physical, chemical and biological properties of the soil (Tortora *et a.l*, 2007). Soil is a mixture of broken rocks and minerals, living organisms and decaying organic matter called humus this also includes water and air (Yakowitz, 2008). Organisms in the soil needs air and water to survive, having these essential materials; air, water, and organic matter makes it possible for plants, bacteria, fungi and small animals like earthworm and insects to live in the soil (Sangodoyin, 1993). Microorganisms play an important role on nutritional chains that are important part of the biological balance in life. Where bacteria are essential for the closing of nutrient and geochemical cycles such as the carbon, Nitrogen, Sulphur and phosphorus cycle (Rao and Subba, 1999). Without bacteria and some fungi, soil would not be fertile and organic matter such as straw and leaves would accumulate within a short time. Soil contains varieties of microorganism including bacteria that can be established in any natural environment (Khupe, 2006). Bacteria are the most important and abundant microorganism which is present in surrounding environment. These are very small, unicellular, primitive and non-chlorophyll containing microorganism (Costerton, *et al*., 2015). Soil contains varieties of microorganism including bacteria that can be established in any natural environment. Bacteria are the most important and abundant microorganism which is present in surrounding environment. These are very small, unicellular, primitive and non-chlorophyll containing microorganism (Adegoke,

2001).

The Waste products in dump-site with no paper waste handling method are sources of pathogens to the soil, which in turn contribute to the emergence of community-acquired infections (Tortora *et al*., 2007). Solid waste generation is a growing global issue due to the large increase in solid waste production. This increase in waste quality requires improving and expanding the solid waste management options (Yakowitz, 2008). Landfill disposal is the most commonly used waste management method worldwide (Rabah *et al.*, 2008), physically, chemically, and biological process occur within a conventional landfill to promote the anaerobic degradation of solid waste and result in the production of leachate and landfill gas for a very long time. Waste can be loosely defined as any material that is considered to be of no further use to the owner and is, hence, discarded (Yusuf and Sonibare, 2004). However, most discarded waste can be reused or recycled. Waste can be loosely defined as any material that is considered to be of no further use to the owner and is, hence, discarded (Onuorah *et al*., 2015), one of the principles of most waste management philosophies. What may be of no further use to one person and regarded as waste to be dumped, may be of use to the next person, and is the basis of the rag picking trade, the sifting through of refuse at landfills for recovery and re-sale, a very fundamental historical waste management practice still functioning in many countries, often conducted on a highly organized commercial basis (Azam *et al*., 2003). The developing world is experiencing rapid population growth and a massive shift towards urban population. Human activities create vast amount of various wastes and pollutants (Mark *et al.*, 2000). The release of these materials into the environment sometimes causes serious health problems (Rabah *et al.*, 2008). The level of wastes produced by dense human and domestic animal population often exceeds the local ecosystem's biodegradative capacity, resulting in serious environmental pollution and epidemic outbreaks of disease (Ronald, 1988).

The colifom counts of bacterial of some natural water supplies in Nigeria far exceed the level recommended by the World Health Organization. Surface associated bacterial population in rivers, play an important part in the biodegradation of allochthonous substances such as pollutants derived from human activities (*Costerton et al.*,2005). Therefore, the objective of this study is to investigate the effects of dumpsite on soil microbiota.

MATERIALS AND METHOD

Sample Area

The soil and water samples were collected along Oke-Ijebu Akure, Ondo State Nigeria.

Sterilization of materials

Sampling bottles, beakers, petridishes, test tubes, and conical flasks, (glassware) were washed in water with detergent, rinsed and allowed to dry. They were after sterilized in hot air oven at 160°C for 60 minute (1hr). Media and distilled water were sterilized by autoclaving at 121°C for 15minutes. Workbench was disinfected using 70% ethanol, all the work was done aseptically (near the flame).

Collection of sample

The soil and water samples were collected along Oke-Ijebu Akure, Ondo State. Nigeria. 400 grams of soil sample from a refuse dump site was taken into a plastic container and 1 litre of water sample from stream was collected into a sterilize pre-washed container and appropriately labeled, and were immediately taken to the laboratory for microbiological analysis.

Isolation of bacteria from soil sample

The bacteria was isolated using pour plate method on Nutrient Agar. The petri dishes

was incubated at 37^0C for 24 hours.

Serial dilution method for Soil sample soil

1g of the soil sample was weighed and dispersed in a sterile beaker; 10ml of sterile distilled water was added to dilute the soil sample. 9 test tubes were labeled 10^{-1}–10^{-9} dilution factors 9ml of distilled water was measured into each and then sterilized at 121°C for 15 minute using the autoclave (Olutiola *et al*., 1999).

Pure culture

For reducing microbial population, 1g of soil was dissolved in 10ml of distilled water to make soil suspension. Serial dilution was carried out for getting isolated single colony. In this research, nutrient medium was used for bacterial growth. Nutrient agar was prepared according to the manufacturer's instruction, by dissolving 28g of the powder into 1 liter of distilled water in a conical flask. The conical flask was plugged with cotton wool and covered with aluminum foil. The medium was heated and sterilized in the autoclave at 121ºC for 15 minutes.

Pour plate technique

1ml of the diluted samples were taken from the test tubes 10^{-2} and 10^{-5} and were dispensed into the petri dishes labelled with the same diluted factor and the NA agar was poured into each at 45°C, the plate were rocked and allowed to set(solidify) and incubated for 24 hours (Olutiola *et al*., 1991).

Isolation of different organisms

Isolation was carried out from the 2nd and 5th plate, sterile loop was used to streak the already prepared Salmonella shigella agar, (SSA), Mannitol salt agar (MSA), Eosin methylene blue (EMB) and then incubated for 24 hours at 37°C.

Identification of bacterial isolates

The identification of bacteria was based on biochemical characterization such as sugar fermentation tests, citrate, catalase, indole, methyl red, voges-prauskauer, starch hydrolyses, oxidase etc. was based on colonial appearances, wet mount preparation and the use of staining technique such as lactophenol cotton-blue, ((Ronald, 1988).

RESULTS

Table 1 show the colonial morphology of the bacterial isolates, which is based on form, size, surface, colour, elevation, margin, texture and optical quality. These characteristics aid in identification of bacteria.

Table 1: Colonial morphology of bacteria isolates

SAMPLES	FORM	SIZE	SURFACE	COLOUR	ELEVATION	MARGIN	TEXTURE	OPTICAL QUALITY
Colony 1	Swarming	Large	Rough	Cream	Flat	Lobate	Dry	Translucent
Colony 2	Circular	Small	Dull	Cream	Raised	Entire	Smooth	Opaque
Colony 3	Circular	Large	Rough	Grey	Raised	Entire	Dry	Opaque
Colony 4	Irregular	Large	Dull	White	Raised	Undulate	Smooth	Opaque
Colony 5	Circular	Medium	Dull	Non pigmented	Flat	Entire	Mucoid	Translucent
Colony 6	Circular	Punctiform	Dull	Cream	Convex	Entire	Mucoid	Translucent
Colony 7	Irregular	Small	Glistering	Greenish	Umbonate	Undulate	Dry	Opaque

Table 2 shows the biochemical characteristics of bacteria, these characteristics is base on gram staining reaction, motility, catalase, oxidase, methyl red, Voges Proskauer, citrate, urease, sugar fermentation test (such as Glucose and Lactose) and spore staining. The bacteria identify are *Bacillus anthracis Enterobacter* spp, *Staphylococcus* spp, *Pseudomonas aeruginosa, Paenibacillus lantus Shigella sonnei, Salmonella species and Citrobacter* spp.

Table 2: Biochemical characteristics of isolated bacteria

Probable Organism	Gram Stains	Mo	Cat	Ox	MR	VP	Glu	Lac	Growth on EMB	Spore Staining	Ci	Ur	In
Bacillus anthracis	+R	-	+	+	-	+	NR	NR	+	+C	-	-	+
Paenibacillus lantus	+R	+	+	-	N	+	A	A	+	+T	+	N	-
Shigella sonnei	-R	N	+	N	+	-	NR	NR	+	+C	-	N	-
Pseudomonas spp.	-C	-	+	N	-	+	NR	NR	+	+T	-	-	-
Citrobacter sp.	-R	+	+	-	+	-	+	+	-	_	+	-	+
Pseudomonas spp	-R	+	+	-	+	-	-	-	-	_	-	+	-
Enterobacter sp.	-R	+	+	-	-	+	+	-	-	_	+	-	-
Salmonella sp.	-R	+	+	-	+	-	+	-	-	+	-	-	-
Staphylococcus sp.	+C	-	+	N	-	+	+	-	N	_	-	-	-

KEY: Mo: motility; Cat: catalase; Ox: Oxidase; MR: Methyl Red; VP: VogesProskaeur; Glu: Glucose; Lac: Lactose; EMB: Eosin Methylene Blue; Ci: Citrate; Ur: Urea; In: Indole; –R: Gram negative rod; +R: Gram Positive rod; -C: Gram negative Cocci; +C: Gram Positive cocci; -: Negative reaction; + : Positive reaction; N: Not done.

Table 1.2 shows total bacteria count of the dump sites soil the second replicate had the highest count 1.88×10^5 cfu/g, the mean for the three replicate is 1.85×10^5 cfu/g while the total bacterial count for 10^5 is the third replicate is 1.06×10^7 cfu/g, the mean replicate is 1.013×10^8 cfu/g.

Table 3 : Shows bacterial count from the dump site soil

Sample Code	Total Bacterial Count 10^3	Total Bacterial Count 10^5
R1	184	96
R2	188	102
R3	183	106
MEAN	185	101.333333
SD	7	25.3333333
CV(%)	3.78	25
VAR	7	25.3333333

Keys; VAR : Variance, CV: Coefficient of variation, SD : Standard deviation, R : Replicate

DISCUSSION

In this study, the high counts of both bacteria obtained indicated that the contaminated soil had a high population density than the control soil whose counts showed values that could be easily utilized by the organisms. Also, it may be attributable to the destabilization of the soil ecological balance as a result of the contaminant discharged of refuse and waste products. This result of the mean bacterial count 1.013×10^8 cfu/g from soil sample was in conformity with that of Pal and Lalwani (2011) who reported. All the microbial isolates identified from the soil samples (Table 1.1), have been reported to be associated with wastes and waste biodegradation (Obire *et al.,* 2002). The presence and abundance of species of *Bacillus anthracis* observed in the contaminated soil may not be surprising as these organisms are indigenous to soil environment and are known to persist in such environment (Atlas and Bartha, 2007). However, the presence of *Salmonella* spp and S*higella sonnei Enterobacter , Staphylococcus* sp, *Salmonella* sp and S*higella sonnei* in the contaminated soil may be attributable to feacal contamination. Similar findings were reported Bala (2006) reported the isolation of similar organisms from water sources in Jimeta-Yola that were faecally contaminated. The presence of these organisms is a pointer to possible pollution and may have an effect on the soil ecological balance. These findings were in conformity to that of Johannessen and Boyer (2015).

CONCLUSION

The bacterial analyses of soil samples are important for detecting the presence of microorganisms that might constitute health hazards. This can serve as a guide to monitor and protect our environment in relation to soil within our vicinity. The presence of bacterial species including *Bacillus anthracis Enterobacter sp, Staphylococcus sp, Pseudomonas aeruginosa, Paenibacillus lantus Shigella sonnei, Salmonella sp Citrobacter sp.* May cause severe health hazards like stomach cramps, diarrhea, vomiting, fever, urinary tract infection, pneumonia, hepatic infections, bacteremia, skin and soft tissue and opportunistic infections on burns, wounds and also blood related infections.. Effective and frequent monitoring of refuse is suggested to safeguard the health of the people.

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Chemical Composition of Some Defatted Gourd Melon (Egusi) Seed Flours

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ABSTRACT

Increase consumption of fatty foods may promote hypertension and obesity, which are well known risk factors of stroke. *Citrullus colocynthis, Citrullus vulgaris*, (*Lagenaria siceraria* I African Wine Kettle gourd (AWK), *Lagenaria siceraria* II Basket Ball Gourd (BBG) and *Lagenaria siceraria* III Bushel Giant Gourd (BGG) seeds flour samples were defatted using n-hexane. Proximate, energy and sugar compositions of the samples were determined on dry matter weight using standard methods. Oil extraction significantly increased the percentage protein from (24.37-34.64) % to (56.17-67.95) % and percentage carbohydrate from (3.23-10.88) % to (13.29-27.85) %. Gross energy of the full-fat samples (FFS) and defatted samples (DFS) ranged from (2,440.74 to 2,693.82) KJ/100g and (1,331.60 to 1,452.45) KJ/100g respectively. There were increase in the values of the sugar contents of the gourd seeds with oil extraction. Predominant sugars in the FFS was Lactose, D-Ribose and Maltose sugar; ranged from (65.90 to144.40) mg/100g, (48.93 to106.00) mg/100g and (60.71 to 93.69) mg/100g respectively. These three sugars were also predominant in DFS, ranging in the same order (82.81 to 168.89) mg/100g, (55.13 to 98.46) mg/100g and (49 to 85.88) mg/100g. High value of maltose will enhance the use of these gourd melon seeds in drinks and beverages. The relatively low values of glucose ranging from (31.71 to 49.13) mg/100g in the FFS and (36.67 to 57.18) mg/100g in the DFS makes them suitable for the consumption of people especially for those with diabetes and the low fat contents make them more heart friendly for patients with hypertension.

Keywords: *Melon (egusi) seeds, Oil extraction, Sugar content, Energy content*

INTRODUCTION

Plants are primary sources of medicines and food used by humans every day. Their roots, stems, leaves, flowers, fruits and seeds provide food for humans (Amaechi, 2009; Hemingsway, 2004). *Citrullus collocynthis, Citrullus vulgaris* and *Lagenaria siceraria* species are gourd melon (egusi) seeds grown in most parts of Nigeria. Gourd melon seeds belong to the family Cucurbitaceae. They are versatile and include hundreds of species of vine bearing coiled climbing tendrils and some of the most unusual fruits in the world. This plant family is known for its remarkable genetic diversity and prevalent adaptation that includes tropical and subtropical regions, arid deserts and temperate locations (Oluba et al., 2008). Seeds of cucurbits are sources of oils and protein with about 50% oil and up to 35% proteins (Oluba et al., 2008; Achu, et al., 2005). Egusi (*Colocynthis citrullus L*.) belongs to the species of the genus *Citrullus* of cucurbitaceae family, which usually consists of outsized amount of varieties that are commonly known as melons (Mabaleha, et al., 2007). *Colocynthis citrullus L*. is among the 300 species of melon found in tropical Africa and it is cultivated for its seeds, which are rich in oil (53%) and protein (28%) (Ntui, et

al., 2009). The regions of its cultivation include: Middle East, Nigeria, Ghana, Togo, Benin, Cameroon and some other countries in Africa for the foods in the seeds and as a crop inter-planted with maize, cassava and yam (Uruakpa and Aluko, 2004). Some *Lagenaria siceraria* gourds melon (egusi) seeds are grown in Yoruba land, Nigeria mostly for utility purposes. *Lagenaria siceraria* I (AWK), otherwise called Akeregbe in Yoruba land, *Lagenaria siceraria* II (BBG) is called Igbaademu and *Lagenaria siceraria* III (BGG) is known as Igba-je. However, some indigenous rural dwellers eat the seeds of these gourd plants as soup thickners and are called Melon or Egusi in Yoruba. Like some common egusi that have been worked on, they are very good sources of fats and protein, with fat content of about 50% (Ogundele and Oshodi, 2010; Ogundele et al., 2012). However, increased consumption of fatty foods may promote hypertension and obesity, which are well known risk factors of stroke (Nguemeni, et al., 2014). Myristic and palmitic acids have been established as the most important of the dietary risk factors in cholesterol high density (CHD) (Bender, 1992). High level of blood cholesterol is associated with the incidence of CHD which increases the LDL (low density lipoprotein in which 46% of the molecule is cholesterol) (Bender, 1992). Hence, it is important to see the effect of removal of the oil content of these melon seeds on the nutritional value of the seeds.

MATERIALS AND METHOD

The melon seeds used for this research work are *Citrullus colocynthis*, *Citrullus vulgaris*, *Lagenaria siceraria* I (African wine kettle), *Lageneria siceraria* II (Basketball gourd) and *Lagenaria siceraria* III (Bushel giant gourd). The seeds were bought from Ilora in Oyo State and in Akure. The seeds were identified at Federal Research Institute, Ibadan, Oyo State, Nigeria. They were dehusked, dried, picked and milled in a blender into flour.

Defatting of melon (egusi) flour samples

Defatted samples of the five varieties of gourd melon seeds were prepared by putting some quantities of the flour sample in soxhlet apparatus and refluxing continuously under heat with n-hexane (b.p. 40-60 ^{o}C) for nine hours.

Determination of Proximate Composition

Proximate analysis of the samples were carried out on dry matter and expressed in percentages, using standard procedures recommended by Association of Official Analytical Chemists (AOAC, 1990). The fat content (FC) was determined using solvent extraction method with n-hexane (b.p. 40-60 ^{o}C) in a soxhlet extractor. The moisture content (MC) was determined using air oven as weight difference after oven-drying for 4-5 hours at 105 ^{o}C. Crude Protein (CP) was determined by Kjeldahal method to determine percentage nitrogen content and converted to protein content as percentage Nitrogen x 6.25. Total ash Content (TAC) was determined by weight difference after incinerating a known weight to ash in a muffle furnace. The Crude Fibre (CF) was determined according to Pearson, 1981. Carbohydrate was determined by difference 100 % - ∑(FC, MC, CP, TAC, and CF)). The Proximate analysis was carried out in triplicates and the results are in % dry matter weight of samples (Ogundele and Oshodi, 2010).

Determination of Defatting Efficiency (DE)

The defatting efficiency (DE) was carried out on the defatted samples as follows:

$$DE = \frac{FCRM - FCDM}{FCRM}$$

Where:

FCRM = Fat content of raw melon

FCDM = Fat content of defatted melon

2.4 Determination of Total Sugar

The sugar content was determined by the method of Shaffer Somogyi sugar-

thiosulphate (AOAC, 1990).

Preparation of the reagent solution used in sugar analysis

(a) Shaffer Somogyi Carbonate 50 reagent 5.0 g KI, 25 g each of anhydrous Na_2CO_3 and KNa tartrate. $4H_2O$ (Rochelle salt) was dissolved each in 500 mL H_2O in large beaker. Solution of $CuSO_4.5H_2O$ (75 mL) with concentration of 100 g/L was added through a funnel with tip under surface, with stirring occasionally and 20 g $NaHCO_3$ and 5 g KI were dissolved in the large beaker. The whole mixture was then transferred to 1 litre volumetric flask and 250 mL 0.10 M KIO_3 was added and later made up to the mark in another one litre volumetric flask. The mixture was covered and left over night before use.

(b) Iodine-oxalate solution: -2.5 g KI and 2.5 g $K_2C_2O_4$ were dissolved in distilled water and diluted to 100 mL. The solution was prepared fresh.

(c) Thiosulphate standard solution:- 0.005 M $Na_2S_2O_3$ solution was prepared daily from standardized stock of 0.1 M $Na_2S_2O_3$ solution.

(d) Starch indicator: 2.5 g starch and 10 g HgI_2 in little water and then dissolved in 500 mL boiling water.

Determination of individual sugars

Sample of the flour (2.5 g) was dissolved in 20 mL distilled water and hydrolysed by using 20 mL 0.1 M H_2SO_4. 5 mL of the resulting solution was pipetted into 25x200 mm test tube and then 5 mL reagent (a) was added and the mixed well by swirling. The test tube was placed in boiling water and heated for between 15 and 35 minutes. The test tube was thereafter removed carefully without agitation to a running water and allowed to cool for about 4 minutes. The cap on the tube was removed and 2 mL KI-$K_2C_2O_4$ was gently added. The mixture was mixed thoroughly to ensure that Cu_2O was dissolved and then allowed to stand in cool water bath for 5 minutes with mixing done twice during the period. The remaining mixture was later titrated against 0.005 M $Na_2S_2O_3$ using starch indicator. The blank was equally run as described above and then the test solution titre value subtracted from the blank. The titration was repeated until two concordant results were obtained. The amount of sugar present was calculated based on the equation of Somogyi sugar-thiosulphate equivalents given in Table 1.

Table 1: Shaffer-Somogyi sugar- thiosulphate equivalent for sugar calculation

Sugar	Heating Time (minutes)	Equation
L-Arabinose	30.00	y = 0.1234x +0.060
Fructose	15.00	y = 0.113x + 0.079
D-Galactose	30.00	y = 0.1332x + 0.033
Glucose	15.00	y = 0.1099x + 0.048
Lactose	25.00	y = 0.2031x + 0.030
Maltose	30.00	y = 0.2199x + 0.072
D- mannose	35.00	y = 0.1148 + 0.084
D-Ribose	25.00	y = 0.1381x + 0.098
L-sorbose	15.00	y = 0.1244x + 0.116
D-xylose	30.00	y = 0.1130x + 0.044

AOAC, (1990); (y = mg sugar in 5 mL; x = mL of 0.005 M $Na_2S_2O_3$)

Determination of Calorific energy values using bomb calorimeter

Ballistic bomb calorimeter (Gallenkamp CBB-330-030F) was used to ignite about 5 g of each sample electrically and burned in excess oxygen (with recommended oxygen pressure of 25 atmospheres) in the bomb. The maximum temperature rise of the bomb calorimeter was measured with the thermocouple and galvanometer system. The rise in temperature obtained was compared with that of benzoic acid to determine the calorific/energy values of the sample materials. Energy was also calculated using Atwater factor (FAO, 2002).

Statistical Analysis

One way analysis of variance (ANOVA) and least significance difference (LSD) were carried out on the replicate data generated using SPSS 18. The results are expressed as mean ± standard deviation. Duncan was also used to determine values that are significantly different with $p \leq 0.05$ (Ogundele and Oshodi, 2010).

RESULTS AND DISCUSSION

Proximate composition of full-fat seeds FFS and defatted flours samples DFS

The proximate composition values of the full-fat seed (FFS) flour samples and defatted flour samples (DFS) are relative to dry matter (mg/100 g dw) and are presented in Tables 2 and 3 respectively. The proximate composition of the five varieties of gourd melon (egusi) seeds in Table 2 (Ogundele and Oshodi, 2010; Ogundele et al., 2012) shows that the seeds are very high in crude fat content, ranging from 46.03±1.14 to 56.61±0.10 % with *Citrullus colocynthis* having the highest value and *Lagenaria siceraria* I (AWK) having the least value. On the average, the crude fat content of the five varieties of gourd seeds is 51.30 %. These values are consistent with the fat contents of some other melons in the same family like *Citrullus lanatus, C. mannii and C. melo* which are (56.67±4.90, 45.89±4.73, 42.67±3.43) respectively as reported by Loukou et al., (2007).

The fat contents of these varieties of melon seeds are however higher than the fat contents of seeds like chick pea (1.5 %) (Sanche-Vioquez et.al., 1998) and African yam bean (0.58 to 1.79 %) (Oshodi et al., 1995). Hence, *Citrullus colocynthis, Citrullus vulgaris, Lagenaria siceraria I (AWK), Lagenaria siceraria II (BBG) and Lagenaria siceraria III (BGG)* seeds have high vegetable oil content for human consumption and for industrial applications such as in the cosmetics and food industries.

Table 2: Proximate composition (%) of full-fat gourd seed flours

Parameter	Sample				
	Ogundele et al., 2012		Ogundele and Oshodi, 2010		
	C.colocynthis	C.vulgaris	LSI(AWK)	LSII(BBG)	LSIII(BGG)
Protein	24.37^{a}±2.13	32.96^{c}±2.53	34.64^{c}±0.08	27.71^{b}±0.41	32.70^{c}±0.35
Fat	56.61^{d}±0.10	49.59^{b}±1.40	46.03^{a}±1.72	53.35^{c}±0.24	50.91^{b}±1.57
Moisture	3.08^{b}±0.80	2.75^{a}±0.27	5.67^{d}±0.06	5.13^{c}±0.04	5.67^{d}±0.11
Ash	3.15^{a}±0.30	3.53^{ab}±0.32	3.75^{bc}±0.17	4.07^{cd}±0.22	4.50^{d}±0.22
Fibre	1.91^{a}±1.00	2.00^{a} ±1.00	1.62^{a}±0.25	0.75^{a}±0.15	2.99^{a}±0.50
Carbohydrate	10.88^{b}±3.03	9.17^{b}±2.88	8.75^{b}±1594	8.99^{b}±0.76	3.23^{a}±1.90

Values with different superscriptions on the same row are significantly different at $p \leq 0.05$ (Ogundele and Oshodi, 2010; Ogundele et al., 2012)

Defatting these oil seeds flour samples with n-hexane was very effective as presented in Table 3.

The defatting efficiency ranges from 95.56 to 99.17 %, showing that virtually all the seed oil was effectively removed with only a negligible fraction left during oil extraction. The fat content of the defatted seeds ranges from 0.47±0.35 % *(C. colocynthis*) to 2.20±0.79 % for *C. vulgaris*. These results are similar to the report given for the fat content of some defatted samples like defatted *Cassia fisula* seed (0.39) % (Akinyede and Amoo, 2009).

Table 3: Proximate composition (%) of some de-fatted gourd melon seed flours

Parameter	Sample				
	C.colocynthis	C.vulgaris	LSI(AWK)	LSII(BBG)	LSIII(BGG)
Protein	56.17^{a}±4.91	59.76^{ab}±0.13	67.95^{b}±0.28	62.04^{ab}±2.17	58.78^{ab}±4.26
Fat	0.47^{a} ±0.35	2.20^{c}±0.79	0.77^{ab}±0.73	0.96^{ab}±0.36	1.64^{bc}±0.69
Moisture	4.64^{ab}±0.76	5.73^{ab}±0.13	3.72^{a}±0.76	9.13^{c}±2.17	7.07^{bc}±1.48
Total Ash	6.46^{a}±1.19	6.42^{a}±1.28	8.75^{b}±0.72	8.31^{ab}±1.38	11.96^{c}±0.25
Fibre	4.39^{bc}±2.32	5.00^{bc}±1.00	5.52^{c}±0.77	3.42^{abc}±0.85	4.59^{bc}±0.91
Carbohydrate	27.85^{b}±5.16	20.89^{ab}±9.25	13.29^{a}±1.41	16.12^{a}±2.80	15.98^{a}±6.16
DE	99.17	95.56	98.33	98.20	96.78

Values with different superscriptions on the same row are significantly different at $p \leq 0.05$.
DE is the % Deffatning Effectiveness

The values of the percentage crude protein (dw) for *Citrullus colocynthis*, *C. vulgaris*, *Lagenaria siceraria I (AWK), L. siceraria II (BBG)* and *L. siceraria III (BGG*) seed flours in Table 2 are 24.37±2.13, 32.96±2.53, 34.64±0.08, 27.71±0.41 and 32.70±0.35 % respectively with an average of 30.48 %. These values are higher than the protein contents of five cultivated African yam bean ranging from (20.18 to 25.78) % (Adeyeye, 1997); whole *Adenopus breviflorus* seed (28.60 %) (Oshodi, 1992) and chickpea (24.70 %) (Sanchez-Vioque, 1998). These melon (egusi) seed flour varieties are good sources of protein and can compete favourably with some other good sources of protein like Conophor nut (29.09 %), Jatropha curcas seeds (29.40 %) and Cucumeropsis edulis which is another variety of "egusi", that was reported to contain 31.85 % protein (Akpabange et al., 2008). Hence *C. colocynthis, C. vulgaris, L. siceraria I (AWK), L. siceraria II (BBG) and L. siceraria III (BGG)* are essentially good sources of protein for human consumption and can be good substitutes for animal protein for the fast growing need of protein for children and the fast growing world population. In addition, they can also serve as good sources of protein concentrate for human and animal food formulation.

More importantly, the percentage protein content of the seeds is reasonably increased by defatting as presented in Table 3. The protein content of the defatted gourd seed flours ranges from (56.17±4.91) % for *(C.colocynthis*) to (67.95±0.28) % for *(L. siceraria I*). Defatting therefore increased the protein content of the seeds under study by 130.49, 81.31, 91.13, 123.89 and 79.76 % *for C. colocynthis, C. vulgaris, L. siceraria I (AWK), L. siceraria II (BBG) and L. siceraria III (BGG)* respectively.

The crude fibre contents of these seed flours are considerably low with values ranging from (0.75±0.15) % (*L. siceraria* II) to (2.99±0.50) % (*L. siceraria* III) as seen in Table 2. There is however no significant difference in these values at $p \leq 0.05$. These values are comparable with the crude fibre reported for several indigenous cucurbits like the varieties of African yam beans (ranging 1.61 to 2.38) % (Adeyeye, 1997), peal millet (1.8±0.30 %) (Oshodi et al., 1999), *Cucurbita lanatus* (1.33 %) and for raw *Jatropha catharica* (1.60 %) (Oladele, 2008). On the other hand, the crude fibres of the defatted samples are higher with values ranging from 3.42±0.85 % for *L. siceraria II* to 5.52±0.77 % for *L .siceraria I.* The increase in the fibre content of the defatted samples is possibly due to the relative availability of the fibre that was increased after removing almost 100 % fat from the samples. The high values of the fibre of the defatted gourd seed flours will aid the rate of digestion and absorption compared with the FFS. Hence, defatting makes the fibre in the gourd melon seed samples to be available more as roughages. Fibre consists of cellulose and hemicellulose, a heterogeneous group in which pentosan usually dominate over lignin and pectin substances. There is now evidence that dietary fibre has a number of beneficial effects related to its indigestibility in the small intestine (Asp, 1996). Due to physical properties, dietary fibre and polysaccharides also influence digestion and absorption processes in the small intestine (Cherbut et al., 1995).

Sugar content of FFS and DFS

The sugar contents of the FFS and DFS are seen in Figures 1 and 2. There is general increase in the values of the sugar contents of the gourd seeds with defatting Figure 3. This is consistent with the increase in carbohydrate values of the seeds with defatting as in Tables 2 and 3.

The predominant sugars in the FFS are Lactose, D-Ribose and Maltose sugar; ranging from (65.90 to144.40) mg/100g, (48.93 to106.00) mg/100 g and (60.71 to 93.69) mg/100 g respectively; while in the DFS, these three sugars are still predominating. The range of these sugars in DFS are: Maltose (82.81 to 168.89) mg/100g, Lactose (55.13 to 98.46) mg/100g and D-Galactose (49 to 85.88) mg/100g. Lactose and Maltose are both disaccharides, hence on the average, the gourd melon seeds have high disaccharide content. The high Lactose value is likely to account for the milky appearance of the FFS and DFS in solution, making the melon seeds of possible quality for infant mixes formulation. Maltose on the other hand will enhance the use of these gourd melon seeds in drinks and beverages. Xylose content in both the FFS and DFS arerelatedly low. Xylose is classified as a monosaccharide of the aldopentose type, which means that it contains five carbon atoms and includes an aldehyde functional group. It is the precursor to hemicellulose, one of the main constituents of biomass.

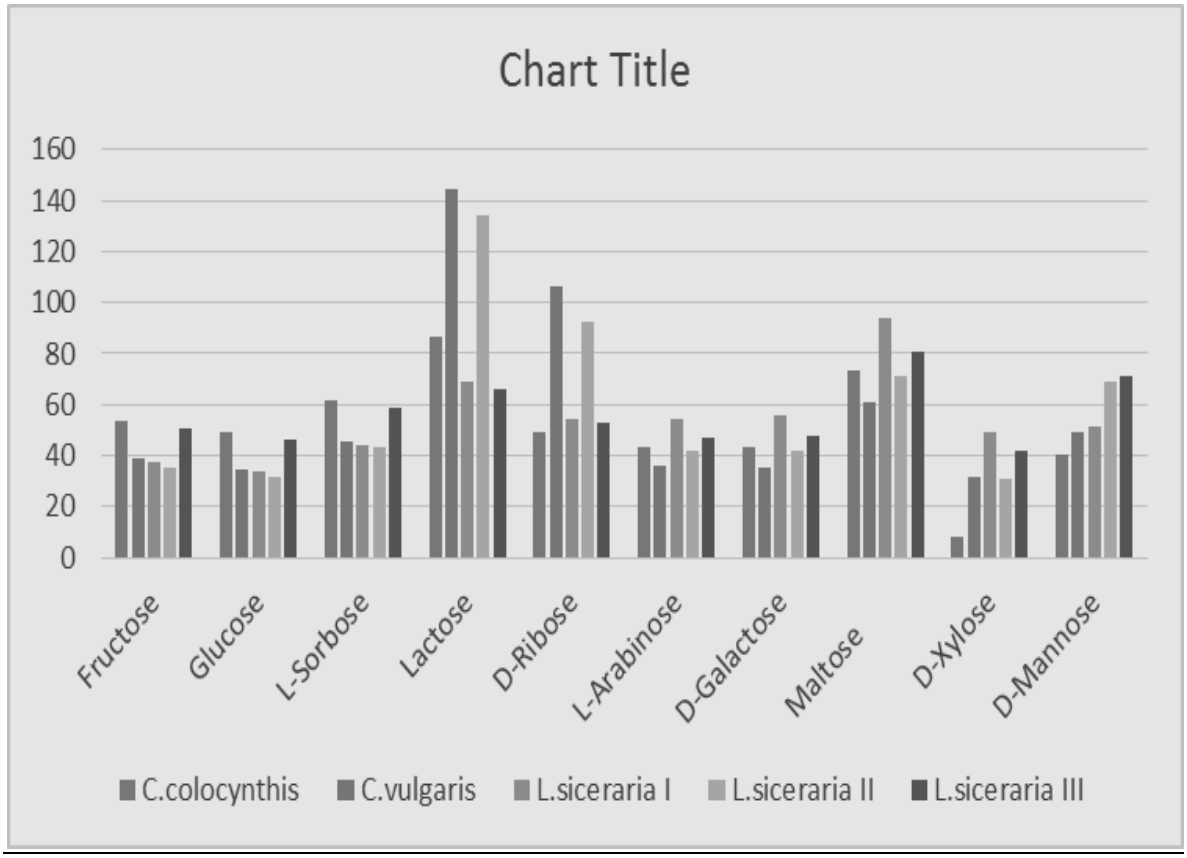

Figure 1: Varieties of sugar contents of full-fat gourd seed flour samples (mg/100g)

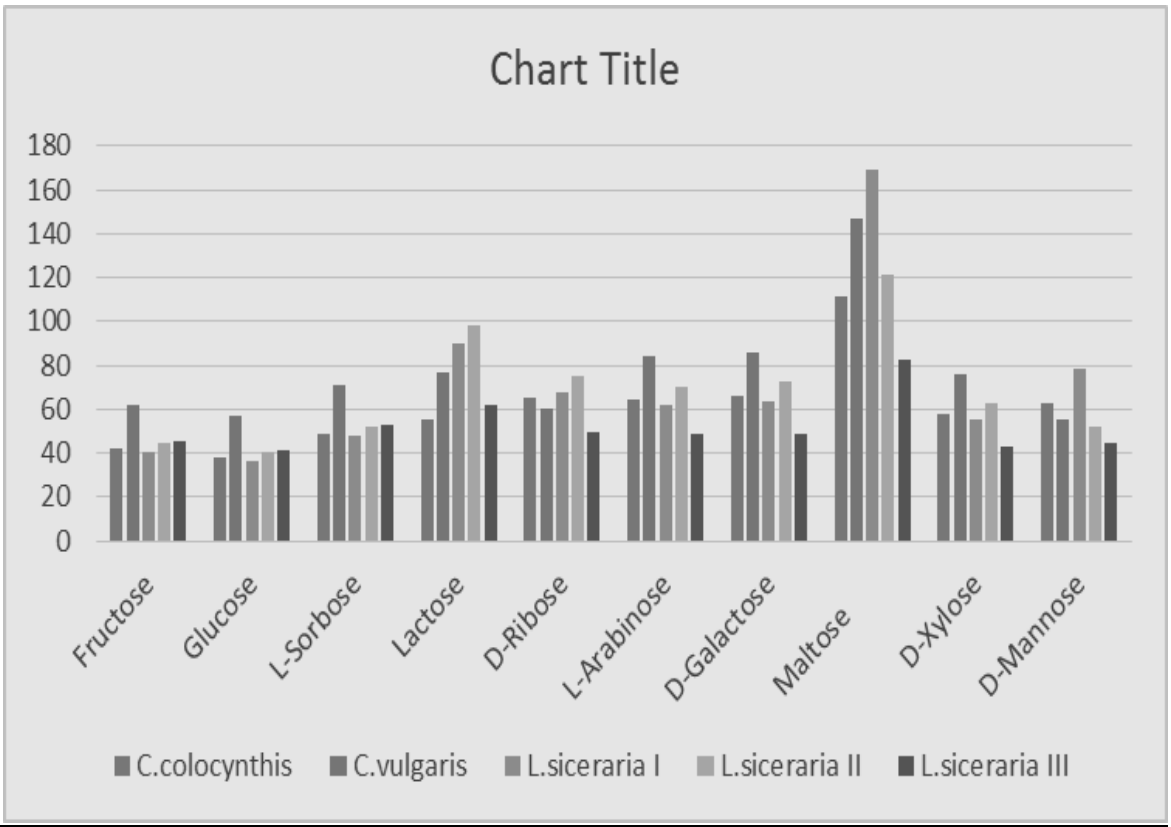

Figure 2: Varieties of sugar contents of de-fatted gourd seed flour samples (mg/100g)

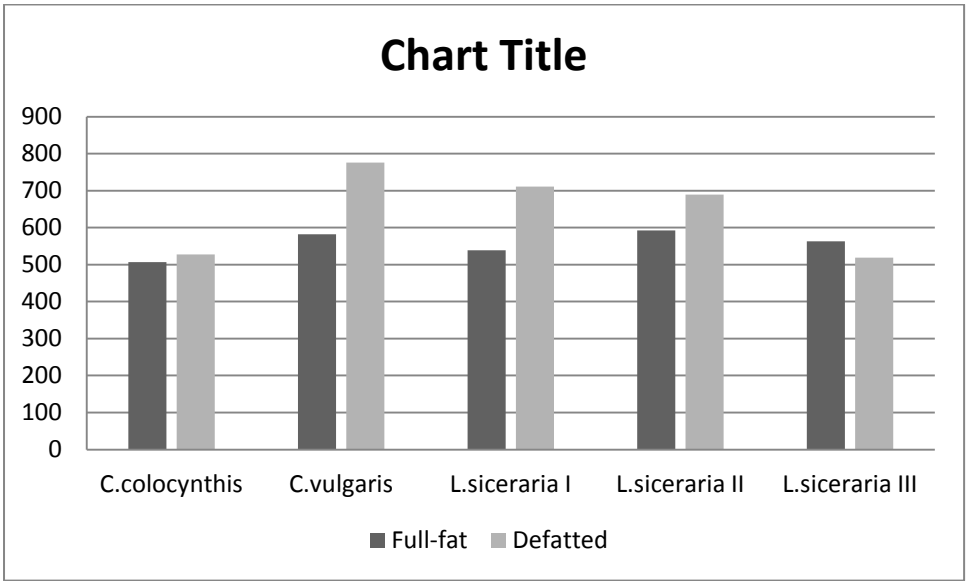

Figure 3: Comparison of the total sugar content of full fat and de-fatted gourd seed flour samples (mg/100g)

Xylose is also the first saccharide added to the serine or threonine in the proteoglycan type O-glycosylation, and, so, it is the first saccharide in biosynthetic pathways of most anionic polysaccharides such as heparin sulphate and chondroitin sulphate (Wikipedia, 2015). The values of the total sugar of the raw gourd melon seed flours range from 506.47 mg/100g (*C. colocynthis*) to 592.27 mg/100g (*L. siceraria* II BBG). There is corresponding increase noticed in the total sugar values of the defatted gourd seeds with values (mg/100g) ranging from 527.62 (*C. colocynthis*) to 775.96 (*C. vulgaris*). This is possibly as a result of corresponding increase in the Carbohydrate values of the defatted gourd melon seeds compared to the lower Carbohydrate values of the raw gourd melon seed. Fructose ranges from 35.40 mg/100g to 53.48 mg/100g in the raw samples and 40.67 mg/100g to 61.73 mg/100g in the defatted flour samples. Glucose, on the other hand ranges from 31.71 mg/100g to 49.13 mg/100g in the raw samples and 36.67 mg/100g to 57.18 mg/100g in the defatted samples. The relatively low values of the monosaccharide especially glucose and fructose is an importance factor. The importance of blood glucose response after a meal is often expressed as the glycaemic index (Asp, 1996). The low glucose content of these melon seeds is a factor that enhances their suitability for the consumption of adults especially the hypertensive ones.

Energy Content (EC)

Tables 4 shows the energy contents of the full-fat and defatted gourd melon (egusi) seeds. The gross energy of the raw melon samples determined using bomb calorimeter ranges from (2685.77 to 1275.08) KJ/100g for *L. siceraria (III)* and *L. siceraria (I)* respectively. The calculated gross energy for the raw melon samples ranges from (2440.74 to 2693.82) KJ/100g. The calculated gross energy values got from the result of the proximate analysis of protein, carbohydrate and fat, using Atwater factors are higher than the calorimeter determined energy for *L. siceraria* II *(BBG) and L. siceraria III (BGG)*

Table 4: Energy content of full-fat and defatted gourd seed flours (KJ/100g)

Energy of full fat melon (egusi) seed			Energy of defatted melon (egusi) seed flours
Sample	*Gross Energy1	**Gross Energy2	**Gross Energy2
C.colocynthis	2674.90	2693.82	1445.73
C.vulgaris	2685.45	2551.04	1452.45
L.siceraria I	2685.77	2440.74	1409.57
L.siceraria II	1751.08	2583.23	1331.24
L.siceraria III	1275.46	2507.23	1331.60

*Energy determined using Bomb Calorimeter; **Energy calculated using Atwater factor

The lower calorimeter determined energy for *L. siceraria* II *(BBG) and L. siceraria III (BGG)* may be due to energy lost to the environment and calorimeter during experiment. Defatting of the melon seeds however led to reduction in the calculated energy values, ranging from (1331.60 to 1452.45) KJ/100g for *L. siceraria II and C. vulgaries* respectively. The percentage energy due to fat in the raw sample ranges from (61.11 to 77.75) % and this was reduced to (2.02-5.06) % in the defatted melon flour samples. Obviously, the fat extracted is most likely responsible for this reduction since fat has the greatest energy production according to Atwater factors. These values are however close to the values of energy determined for some seeds like pumpkin seeds 27.0 to 27.20 KJ/g (Achinewhu and Isichei, 1990). The energy value of the seeds under study are higher than the total metabolisable energy for *C. africanum* fruit that was observed to be 420.42 KCal (Edem and Dosunmu, 2011), 448.83 KCal reported for *Gnetum africanum* seeds (Ekpo, 2007), 403.54 KCal reported for *Solanum nigrum* seeds (Edem et al., 2009) and 384.33 KCal reported for *B. coricea* seeds (Amaechi, 2009).

CONCLUSION

In conclusion, this research shows that oil extraction from these five varieties of gourd melon seeds significantly reduced the fat content of the seeds effectively by values ranging from 95.56 to 99.17 % (i.e. almost 100 % efficiency), increased the protein, ash, fibre, carbohydrate and eventually the sugar contents of the samples. The predominant sugars in the FFS are Lactose, D-Ribose and Maltose while glucose values are relatively low in both FFS and DFS. On the other hand, defatting led to increase in Maltose and decrease in Lactose level of the gourd melon seeds. The low glucose content of these melon seeds is a factor that enhances their suitability for the consumption of adults especially the hypertensive ones while reduction of the fat contents makes it more nutritious, elimination the risk for hypertension and obesity.

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Proximate composition and heavy metal content of bread: A case study of Ikeji Arakeji and Ipetu Ijesa, Osun State, Nigeria

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Abstract

Consumers' acceptability of bread should be dependent on both its nutritive and safety values. In assessing these values, nine composite samples of bread were widely sampled over three months, (Feb. - April 2011, in two towns in Ori-Ade Local Government Area of Osun State. Standard methods were used to determine the proximate composition (moisture, ash, protein, crude fibre, fat and carbohydrate contents) of the samples and trace metals (Pb, Cr, Co, Cu and Zn) were analysed using Atomic Absorption Spectrophotometer. Results of proximate composition, in %, as ordered above occurred in the ranges 1.96-4.32, 0.22-0.51, 1.21-7.91, 1.96-2.52 and 57.06-74.92 while those of trace metals, in mg/kg, were not detected (n.d)-0.30, 0.32-1.49, n.d-0.06, 0.03-0.21, n.d-0.41 and n.d-0.01. Apart from crude fibre content which in all the samples had values similar to those found in wheat flour used in making bread, values for other proximate components were low. Similarly, values for Co, Ni and Zn in all the bread samples were lower than results of Khaniki *et al*., 2005, Naghipour *et al*., 2014, Onianwa *et al*., 2001 and Al-Kamil, 2011 while Pb, Cu and Zn were not detected in any sample from Ikeji Arakeji. All the bread samples could therefore be said to be safe for consumption but some lack essential nutrients for acceptability by consumers.

Keyw*ords:* *Bread, Proximate composition, Heavy metal*

INTRODUCTION

Bread is a common food in Nigerian. Its consumption cuts across people of different age, religion and status. A number of types exists and with varying composition depending on the source of raw materials and recipe for formulation. Bread has been described by Dewettinck *et al*. (2008) as a fermented confectionery produced mainly from wheat flour, water, yeast and salt by a series of processes involving mixing, kneading, proofing, shaping and baking. Other constituents are improvers and additives. As food, bread quality and safety are important. While the former deals with characteristics that appeal to sight, taste and nutritional value thus determining its acceptability to consumers, the latter concerns absence or acceptable levels of contaminants or other substances that make it injurious to consumers' health.

Generally, food is important for life's sustenance but unsafe food could be detrimental to health and pose a threat to life. Report by Deman (1990) states that human cells need forty-five chemical components and other elements called essential nutrients to be present in adequate healthy food. Aside oxygen and water, the remaining forty-three are grouped into the five main classes of carbohydrate, protein, fat, minerals and vitamins. A nutritive and well-balanced diet should be made of foods which supply all these essential nutrients.

Apart from the nutrients, trace elements, including heavy metals, are also important in nutrition either for their essential nature or toxicity. Mahindru

(2004) and Alegria *et al*. (1990) reported that copper, manganese, selenium, chromium, iron, zinc and molybdenum are examples of trace elements that are important in human diet. Excessive intake of zinc has been linked with copper deficiency (Bhutta, 2000) while lead exposure causes brain damage and this is more severe in young children. This work was therefore carried out to determine the nutritive and the safety values of bread produced and consumed in two towns, Ikeji Arakeji and Ipetu Ijesa, in Osun State indwelt by an ample number of Staff of Joseph Ayo Babalola University. These will be based on the trace metal composition of bread samples which has been done in a few other places and its proximate composition, one that is seldom found in literature.

MATERIALS AND METHODS

Sample collection

Ipetu Ijesa lies on latitude 7.467°, longitude 4.883° and has a higher land area and population than Ikeji Arakeji, a town on latitude 7.430°, longitude 4.948°. Both towns are in Oriade Local Government Area, Osun State, South-West Nigeria. Bread samples (1 to 6) were obtained randomly from markets, bus-stops, bread vendors and bakeries to form six composite samples from Ipetu Ijesa and three (7 to 9) from Ikeji Arakeji. These were kept in nylons and taken to the laboratory.

Quality control

Chemicals used for the work were of analytical grade and the water was glass-distilled. The containers utilized were soaked in solution of dilute trioxonitrate (V) acid, washed and well rinsed. Blank determination was carried out and the results deducted from those of samples. Standard solutions of metals of interest were prepared with appropriate salts and employed in calibrating the AAS (PerkinElmer A Analyst 200 Atomic Absorption Spectrometer Version 3.0) used for analyzing trace metals.

Proximate analysis and trace metal determination

Determination of moisture content

A clean dry crucible was weighed and used to get 2 g sample to obtain a total weight (W1). This was placed in an oven, gradually heated to 105 °C and maintained at this temperature for 6 hours. It was thereafter cooled in a dessicator and weighed again. The process was repeated until a constant weight (W2) was obtained. Percentage moisture content was calculated using the formula: % moisture content = 100 (W1 – W2)/(W1) (Joslyn, 1970).

Determination of ash content

A crucible earlier washed and dried was weighed. Moisture-free sample (5 g) was then placed in it to obtain a total weight (W1). This was placed in a muffle furnace and heated at 550 °C for 3 hours to obtain the ash. The ashed sample was weighed (W2) after cooling in a dessicator. Percentage ash content was calculated as 100 (W1 – W2)/(W1) (Joslyn, 1970).

Determination of crude fibre

A sample (3 g) was weighed (W1) into an extraction apparatus. It was extracted three times using light petroleum ether by stirring, settling and decanting. The extracted sample was air-dried and transferred to a clean dry 100 cm^3 conical flask. A 0.1275 M sulphuric acid (80 cm^3) taken at room temperature and brought to its boiling point was added to the sample in the conical flask and heated for 30 minutes. The flask was rotated after every few minutes to remove particles from the side and mix the content. A plate was perforated and filter paper placed to cover the holes in it. The plate was fixed to a Buchner funnel and the mixture immediately poured into it. Adjustment was done to the funnel so that filtration was completed within 10 minutes. The insoluble matter was washed several times with boiling water until free from acid. It was transferred back to the conical flask and 0.313 M (80 cm^3) NaOH measured at

ordinary temperature and brought to boiling point was added and heated for 30 minutes. The mixture was was then allowed to stand for a minute and then filtered immediatelly. The insoluble material was transferred to the filter paper by means of boiling water and then washed with 1 % hydrochloric acid and again with boiling water until free from acid. It was thereafter washed twice with ethanol and thrice with ether. The insoluble material was then transferred to a dry and weighed crucible. It was repeatedly dried at 100 °C, cooled and weighed to a constant weight (W2). The crucible and its content were then placed on a heating mantle in a fume cupboard to burn off the organic matter. It was then transferred to a muffle furnace, heated at 550 °C for 3 hours, cooled and the ash content weighed as W3 (A.O.A.C., 1990).

Determination of crude fat

Sample (3 g) was weighed (W1) into a folded fat-free filter paper and a small cotton wool placed on it. This was properly tied with thread at both ends and weighed (W2). It was then placed in the extraction thimble and a small amount of cotton wool placed on top. The apparatus was connected after the addition of 300 cm^3 (60-80 °C) petroleum ether. Extraction was carried out for 3 hours using heating mantle with continuous flow of water in the condenser. The sample was thereafter removed, air-dried, placed in an oven and heated at 80 °C. Heating, cooling and weighing were carried out until a constant weight (W3) was obtained (A.O.A.C., 1990).

Determination of crude protein

Sample (0.15 g) was weighed and transferred into Kjeldahl digestion flask. Catalyst (0.8 g) and concentrated sulphuric acid (2 cm^3) were then added to the sample in the flask. The content of the flask was then heated on the heating mantle for 1 hour until the liquid became clear. The digest was cooled and made alkaline using 15 cm^3 of 40 % NaOH. This was then transferred to the distillation apparatus using minimum volume of water. The ammonia steam distilled into 2 % boric acid (10 cm^3) with 5 drops of methyl red indicator for 15 minutes. The distilled ammonia was then titrated with 0.02 M hydrochloric acid. The method was used to determine nitrogen and the value got was multiplied by 6.25 to obtain crude protein (Barenholz, 2002).

Determination of total carbohydrate

The crude carbohydrate content was determined by difference in 100 % and the sum of percentages of moisture, fat, crude fibre and protein.

Determination of trace metals

Sample (5 g) oven-dried at 60 °C was weighed into a dry clean crucible. This sample was ignited in a muffle furnace at 500 °C for 6-8 hours to obtain a greyish white ash. It was cooled on an asbestos sheet and 5 cm^3 of 1 N HNO_3 added to it. To obtain a more perfect greyish white ash, it was evaporated to dryness by heating on a hot plate at 400 °C for 15 minutes. This ash was cooled on an asbestos sheet, 10 cm^3 of 1 N HCl was added and the content was filtered into 50 cm^3 volumetric flask. A solution (10 cm^3 portion) of 0.1 N HCl was used to wash the crucible and the filter paper three times to make up to volume with 0.1 N HCl. The filtrate was stored for trace metals [lead (Pb), chromium (Cr), cobalt (Co), copper (Cu) and zinc (Zn)] determination using Atomic Absorption Spectrophotometer (A.O.A.C., 1990).

RESULTS AND DISCUSSION

Table 1 shows results of proximate composition of the bread samples. Percentage moisture content was generally low in all the bread samples as it ranged from 1.96 to 4.32 %. Moisture content of a food substance is an essential factor in the determination of its nutritive value and is an index of its stability and quality. It can also influence its packaging and shelf life. Bread sample 1 therefore had the lowest shelf life and greatest tendency to spoil while sample 9 had the highest shelf life and lowest

vulnerability to spoilage. The bread samples also had low ash content ranging from 0.22 to 0.51 %, with that of sample 9 being the least. It is important to note that ash content gives an idea of the amount of mineral elements present in a sample.

Table 1: Proximate composition of bread (%)

Bread	Moisture	Ash	Fat	Crude Fibre	Protein	Carbohydrate
1	4.32±1.23	0.34±0.26	10.91±1.08	7.91±4.23	2.40±0.52	74.13±2.06
2	2.77±1.24	0.26±0.16	14.94±0.55	7.75±0.62	2.52±0.82	71.76±1.18
3	3.36±1.54	0.42±0.16	16.02±1.89	3.20±1.16	2.08±0.17	74.92±2.19
4	3.19±1.22	0.24±0.11	15.45±1.73	5.23±1.33	1.97±0.04	73.93±1.64
5	2.64±0.41	0.39±0.16	16.11±1.63	4.79±0.62	2.26±0.46	73.81±2.38
6	2.26±0.44	0.26±0.10	19.76±0.90	4.11±0.93	2.13±0.50	71.49±0.82
7	2.17±1.34	0.51±0.21	37.61±0.55	1.21±0.41	2.27±0.64	57.06±0.91
8	2.49±1.16	0.51±0.20	31.49±4.78	4.62±1.44	2.45±0.61	58.44±4.64
9	1.96±2.50	0.22±0.10	31.21±2.04	2.63±0.47	1.96±0.35	62.02±0.95

Results are mean±standard deviation (n = 4)

Bread samples from Ikeji Arakeji (7, 8 and 9) had higher percentage fat contents and are therefore better sources of energy than those from Ipetu Ijesa (1 to 6). This is because fat is a high source of energy that is essential to growing animals, especially children. Dietary fibre is an important ingredient in food and has become popular in prevention and managemnt of diseases. Crude fibre consists chiefly of cellulose and other vegetable cell wall substances. Range of crude fibre obtained in the bread samples is 1.21 to 7.91 % and is within that (2 to 12 g/100 g) obtained by Rodriguez *et al*. (2006) for dietary fibre in wheat flour, a major ingredient in bread. Variation or modification of its composition and structure could be caused by food processing.

Similarly, in comparison with the range in wheat (8-15 %) obtained by Shewry (2009), protein content of all the bread samples are low and ranged from 1.96 to 2.52 %. The author also remarked that of the twenty amino acids commonly found in protein, ten of them are essential and can not be synthesized by animals. These must therefore be supplied through the diet and bread is an example of such. Furthermore, the same author reported that wheat (white flour) contains starch, a form of carbohydrate, in the range of 65 to 75 % and the range of values got in majority of the bread samples (57.06-74.92 %), except samples 7, 8 and 9 from Ikeji Arakeji, fall within this. Starch is a good source of energy and a fraction of it resists digestion in the small intestine, passing into the colon and gets fermented to short-chain fatty acids (notably butyrate). Topping (2007) has

observed that this may have health benefits including reduction of colo-rectal cancer.

The concentrations of trace metals in bread samples in the current work are shown in Table 2 and those for comparison in Table 3. Lead was not detected in any bread sample from Ikeji Arakeji as well as samples 5 and 6 from Ipetu Ijesa while the values found in other samples from Ipetu Ijesa were lower than the range reported by Khaniki *et al*. (2005) and Magomya *et al.* (2013) except sample 2 as well as much lower than maximum concentration

Table 2: Concentration of trace metals in bread (mg/kg)

Metal	Bread sample								
	1	2	3	4	5	6	7	8	9
Pb	0.07	0.30	0.11	0.22	n.d.	n.d.	n.d.	n.d.	n.d.
Cr	0.47	0.32	0.32	0.52	0.38	0.34	1.49	0.97	0.79
Co	0.05	0.06	0.002	0.05	0.02	0.01	n.d.	0.01	0.002
Ni	0.03	0.12	0.05	0.06	0.11	0.09	0.21	0.17	0.04
Cu	0.41	0.23	0.08	0.08	n.d.	0.01	n.d.	n.d.	n.d.
Zn	n.d.	n.d.	n.d.	0.01	n.d.	0.004	n.d.	n.d.	n.d.

n.d. = Not detected

Table 3: Concentration of trace metals for comparison (mg/kg)

Metal	Similar work on bread	Maximum permitted[f]	Maximum level[g]
Pb	0.27-0.52 (Iran)[a] 0.34-3.13 (Nigeria)[c] 0.004-0.006 (Basra City)[e]	6 (in any solid food)	0.2 (in cereal grains)
Cr	0.70-2.80[b]	1 (in cereals)	-
Co	0.20-1.80[b]	-	-
Ni	0.43-2.28[a]	-	-
Cu	0.13-0.66 (Nigeria)[c]	-	0.5
Zn	2.93 (Nigeria)[d] 2.96-4.61 (Basra City)[e] 7.2 (USA)[e], 8.2 (Egypt)[e] 13-93 (Romania)[e]	-	0.3-1

[a](Khaniki *et al*., 2005); [b](Naghipour *et al*., 2014); [c](Magomya *et al.*, 2013); [d] (Onianwa *et al*., 2001), [e](Al-Kamil, 2011); [f](Choi, 2011) and [g](FAO/WHO, 2011) permitted in any solid food (6 mg/kg) stipulated by Choii (2011). However, all the values detected were higher than those reported by Al-Kamin (2011).

Copper and Zinc were also not detected in any bread sample from Ikeji Arakeji as well as sample 5 from Ipetu Ijesa. Besides, samples 1, 2 and 3 did not reflect trace of Zinc. These two, Copper and Zinc, are trace elements essential in food and their non-detection in these sample is detrimental to the consumers and depreciates their nutritive value. All levels of Copper detected are lower than the maximum allowable but only those in samples 1 and 2 fall within the range reported by Magomya *et al.* (2013). As for Zinc, levels in the only two samples where detected (from Ipetu Ijesa) are lower to those from similar works.

Chromium and Nickel were detected in all the samples and Cobalt was also detected in all except sample 7, again from Ikeji Arakeji. Cobalt is an integral component of vitamin B 12, a co-factor for two enzymes [methionine synthase and methylmalonyl coenzyme A (CoA) mutase] and thus an essential nutrient for non-ruminant animals and humans. All levels of Cobalt and Nickel detected were lower than those reported for similar works and the same holds for Chromiun except those found in bread samples 7, 8 and 9 (also from Ikeji Arakeji) that exist in the range reported by Naghipour *et al*. (2014).

CONCLUSION

From the ongoing, all the bread samples where trace elements were detected had concentrations or levels within the maximum allowable limits with some below and others within the ranges reported in similar works. Their proximate analysis as regards some components were however low and some essential trace metals were not detected. The bread samples could therefore be said to be safe for human consumption but defective in nutritive standard.

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Phenotypic Variation in Semen Traits Among Four Breeds Of Rabbit in Humid Tropics

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ABSTRACT

The semen quality characteristics shows the potential of the male, reflecting the functional activity of the testicle. Semen traits of 48 rabbits which include New Zealand White, Californian White, Palomino Brown and Havana Black rabbits raised in the humid tropics were appraised. The experiment was carried out at the rabbitry unit of the Department of Environmental Biology and Fisheries, Adekunle Ajasin University Akungba-Akoko, Ondo State. Semen volume, semen pH, motility, sperm concentration, percentage live sperms and sperm morphology were examined. Data obtained was analyzed using SAS 2010. The analysis of variance showed that the breed of rabbit had significant effect ($P< 0.05$) on the traits. The New Zealand White rabbit had the highest volume of semen (1.07 ± 0.07ml) followed by California (0.90 ± 0.03ml) while the ejaculate volume of Palomino brown and Havana black were statistically similar. Semen from New Zealand White bucks was more alkaline than semen from other breeds studied. The percentage of viable sperms in the semen samples obtained from New Zealand White bucks (84.33±3.24%) was similar with that of California breed (82.02 ± 4.23%). The least sperm viability was recorded for Havana black bucks (69.67±1.12%). Semen from California breed had the highest sperm concentration of 37.45±3.01 (10^6/ml). New Zealand White bucks had higher percentage of normal head sperms (90.02 ± 6.89%) than the Californian breed (89.45± 5.98%). Havana black and Palomino breed had relatively lower percentage of normal head sperms (85.08±2.56 % and 82.05±4.64% respectively). The highest percentage of double head and tail sperms were found in Havana black bucks. New Zealand White and California rabbits had better semen traits and will have better breeding and reproductive success compared with Palomino brown and Havana black rabbit in a humid tropical environment.

Keywords*: semen traits, breed , rabbit, tropics*

INTRODUCTION

Male reproductive functions include the production of semen containing normal spermatozoa in adequate number together with ability to mate successfully (Oyeyemi *et. al*., 2008). The semen quality characteristic represents the potential of the male, reflecting the functional activity of the testicle. The quantity and quality of semen produced depend on a great variety of environmental and genetic factors including age, breed, feeding, health status, rearing condition, season of collection, number of ejaculates collected and interval between collections (Alvarino, 2000 ; Brun *et al.*, 2002). Semen traits such as concentration, mass motility and percentage of motile sperm per ejaculate play important role in reproductive success of rabbit. Brun *et al.* (2002) observed that mass motility and the total number of motile spermatozoa per insemination dose were highly correlated with kindling rate in rabbits. Litter size (total born) was significantly influenced by

semen concentration and the number of motile sperm per ejaculate influences the kindling rate. Semen pH acts as an indicator to the normal status of the accessory secretion and the livability of spermatozoa (Jean *et al*., 2002). Generally, semen pH of bucks should be slightly alkaline.

The most relevant parameters that correlate with fertility rate are the number of spermatozoa inseminated and their motility. The use of a single attribute is not sufficiently accurate to predict the fertilizing ability of the semen (Lavara *et al*., 2005). Sperm morphology is considered as a predictor of success in fertilizing oocytes during *in vitro* fertilization. The sperm head morphology has been used earlier to determine fertility potential in rabbits (Lavara *et al*., 2008). According to Al - Yahya (2014), there is a positive correlation between normal morphology of the sperms and fertility. The normal shape and size of the head, mid piece and tail of sperms increase the probability of ovulation and zygote formation. Spermatozoa with longer mid piece swim more slowly, while those with elongated heads and longer tails swim faster. The sperm swimming velocity is an important factor for male fertilization success. The sperm swimming capacity depends on sperm morphometric, which includes the head length, head width, mid piece length and tail length.

Rabbits are very sensitive to high environmental temperature, where the dense fur and lack of sweat glands make heat loss very difficult above the zone of thermal neutrality. Elevation of ambient temperature affects puberty deleteriously, leads to testicular degeneration and reduces percentages of normal and fertile spermatozoa in the ejaculate of males (Okab, 2011). According to Marai *et al.* (2002), high temperatures and humidity can result in behavioural and physical changes in bucks that can affect breeding and reproductive success. Changes after thermal stress that can be observed on a semen evaluation include alterations in the shape of the sperm cell head and tail piece. Elevated environmental temperatures can be dangerous and may produce advanced signs of heat stress. Kasa and Thwaites (1992) observed significant increases in the ratio of dead sperms to live sperms after an increase in the level of heat stress. Sperm concentration, percentages of dead sperm, and sperm with intact acrosome were decreased with elevated temperature during summer (Okab, 2011). The study of El-Maghawry and Soliman (2002) also showed detrimental effects of exposure to high ambient temperature on the concentration of spermatozoa of rabbits

Selection of suitable breed of rabbit for a particular environmental conditions is very much essential for successful rabbit production (Kumaresan et al., 2011). This study was therefore carried out to investigate the variation in the semen traits of temperate breeds of rabbits-Californian White, New Zealand White, Havana Black and Palomino Brown when raised in a humid tropical environment that is characterized with elevated ambient temperature and high relative humidity. This will help in the prediction of breeding and reproductive success these breeds in the humid tropics.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Rabbitry Unit of the Department of Animal and Environmental Biology , Adekunle Ajasin University, Akungba-Akoko, Ondo State. Akungba–Akoko is located in Akoko South West Local Government Area of Ondo state, Nigeria. The area lies in the South Western region of Nigeria (7º 28' and 5º43') with ambient temperature of 27^0C and relative humidity of 46mm Hg.

Experimental animals and management

Forty eight (48) temperate breeds of rabbits which include California White, Palomino Brown, New Zealand White and Havana

black were used for this study. Palomino brown rabbits are golden brown and lynx, they are large meaty rabbits. Californian White rabbits are rounded in body and have short smooth coat they are first bred in the 1920's with the intent of creating a better commercial meat rabbit, as a result of crosses between the Himalayan, and the standard Chinchilla. New Zealand White are multipurpose breed because they can be raised for meat, as pets and for laboratory purpose.

The rabbits were raised from weaning (4weeks) till maturity (12 weeks) under the same housing and feeding conditions. The experimental animals were kept in a wooden cage with each compartment of dimension of length × width× height: 80× 50 ×30 cm^3. The cages were constructed of wood and a wire mesh. The hutch was constructed in a way that it allow there waste to drop on the floor easily and has a single roof which covers all cages from rain or sunlight. They were fed with commercial pelleted diet; the diet used contained 15% Crude protein, 7% fat, 10% Crude fiber, 1.0% Calcium, together with available phosphorus of 0.35% and 2550Kcal/kg metabolisable energy. They were also supplied with forages. Clean water was also supplied to the rabbits ad- libitum.

Semen collection and analysis

A matured doe was used to tease buck and semen was collected into a clean, properly labeled calibrated container and was quickly transferred into laboratory for analysis. The following semen traits were measured:

Semen volume: The volume of the ejaculate was recorded from the calibrated container in milli- liters (ml),

Semen pH: Semen was liquefied with semen extender and the pH of semen was measured using pH meter.

Motility: The motility of sperms was determined with a drop of well-mixed liquefied semen on a slide covered with 20x 20mm cover glass and was observed under the microscope using the (X10 mag) and (X40 mag). The motility duration was 60 seconds during which the scoring was done.

Sperm concentration: The spermatozoa counts per one milliliter of ejaculate were also determined under the microscope.

Sperm viability percentage: The differential staining (one drop of semen was mixed with two drops of eosin) on a slide observed under the microscope aided the determination of the total live sperm cells. The unstained cells are the viable cells with intact cell membrane while the stained cells showed the dead/non-viable ones.

Sperm morphology: the percentages of sperms with normal head and tail as well as double head and tail were also determined.

Statistical analysis

Data obtained from the measurements was analysed using the General linear model of SAS (2010). The linear model is as specified below:

$Y_{ij} = \mu + A_i + e_{ij}$

Y_{ijk} = the parameter or interval

μ = overall mean for the parameter of interest

A_i = Fixed effect of ith breed (I=1-4)

e_{ijk} = random error associated with each record (Normally= Independently and identically distributed with zero mean and variance ($\eth^2 e$)

RESULTS AND DISCUSSION

The least square means of semen physical traits presented in Table 1 shows that the New Zealand White rabbit had the highest volume of semen (1.07±0.07ml) followed by California (0.90 ± 0.03ml) while the ejaculate volumes of Palomino Brown and Havana Black were statistically similar. Vicente (2000) reported higher average of semen volume (1.09ml) for New Zealand White bucks. According to Abd-El-Azim and El-Kamash (2011), the physical

characteristics such as ejaculate volume, semen density, and semen colour, mass and advanced motility were higher in New Zealand White rabbits compared with Californian and Baladi black rabbits. Abd-El-Hakeam *et a*l. (1992) reported a significantly higher semen volume for New Zealand White compared with California bucks. Moce *et al*. (2000) also found that ejaculate volume was higher in bucks of New Zealand White compared with other breeds studied. Castellini et al. (2006), however reported higher average semen volume for California bucks than New Zealand White bucks.

Measurements of semen pH is of great importance because it acts as an indication to the normal status of the accessory secretion and the livability of spermatozoa (Jean *et al.,* 2002). Generally, semen pH of bucks should be slightly alkaline. The effect of breed was significant ($P<0.05$) on semen pH in this study. Semen from New Zealand White bucks was more alkaline than semen from other breeds studied. The highest mean pH value was recorded for semen from New Zealand White bucks followed by California White and Palomino Brown while the least pH value was observed with Havana black rabbit. However, Abd-El-Azim and El-Kamash (2011) reported that semen pH was higher in Californian white rabbits.

Sperm motility illustrates the degree of sperm activity and it is important in the passage of sperms through the oviduct and fertilization (Jean *et al.*, 2002). Sperms of New Zealand White bucks had higher percentage motility (78.33 ±1.67%) than the Californian White breed (73.78 ± 1.42%). The least sperm motility was recorded for semen samples obtained from Havana Black rabbit (67.05±0.97%). The percentage of viable sperms in the semen samples obtained from the New Zealand White bucks (84.33±3.24%) was similar with that of California breed (82.02 ± 4.23%). The least sperm viability was also recorded for Havana black bucks (69.67±1.12%). Abd-El- Hakeam *et al.* (1992) reported a significant difference in semen pH (7.89) and (7.59), in the sperm motility (60.27 %) and (66.22 %) between New Zealand White and California. Brun *et al.* (2004) reported that California bucks gave significantly higher sperm motility percentage compared with the other breeds studied. New Zealand White bucks showed a significantly higher (by 6-10 %) ratio of intact spermatozoa (Bodnar *et al.* , 2000).

However, semen from California White breed had the highest sperm concentration of 37.45±3.01 (10^6/ml). This was followed by New Zealand White bucks with 34.27±1.79 (10^6/ml), Palomino Brown with 33.80±2.53 (10^6/ml) and Havana Black rabbit with 29.52±1.45 (10^6/ml). Abd-El-Hakeam *et al*. (1992) reported a non-significant difference in packed sperm volume (sperm concentration) between New Zealand White and California White bucks.

TABLE 1: Least square means of the semen physical traits as affected by breed

Parameters	New Zealand	California	Palomino	Havana black
Volume(ml)	1.07±0.07[a]	0.90±0.03[b]	0.78±0.04[c]	0.76±0.05[c]
pH	7.99±0.04[a]	7.45± 0.07[b]	7.16±0.08[c]	7.08±0.05[d]
Motility (%)	78.33±1.67[a]	73.78±1.42[b]	70.02. ±1.09[c]	67.05±0.97[d]
Viability (%)	84.33±3.24[a]	82.02 ± 4.23[a]	72.00±1.63[b]	69.67±1.12[c]
Concentration (10^6/ml)	34.27±1.79[b]	37.45 ±3.01[a]	33.80±2.53[c]	29.52±1.45[d]

[a b c d]Mean on the same row with different superscripts are significantly (P<0.05) different

Table 2 shows the percentage sperm morphology of the different breeds studied. New Zealand White bucks had higher percentage of normal head sperms (90.02 ± 6.89%) than the Californian breed (89.45± 5.98%). Havana Black and Palomino Brown breed had relatively lower percentage of normal head sperms (85.08±2.56 and 82.05±4.64%, respectively).

The highest percentage of double head sperms was found in Havana black followed by California White, while the least double head sperms was recorded for New Zealand White bucks. Bodnar *et al.* (2000) reported lower average abnormal spermatozoa content of the ejaculates for New Zealand White compared with Pannon White and Angora rabbit. In the case of New Zealand White a lower rate of tail abnormalities were found (Bodnar *et al* ., 2000).

However, California White breed had the highest percentage (85.78±2.19%) of normal tail sperms while the least percentage was observed in the semen samples from Palomino bucks. The least percentage of double tail was found in New Zealand White bucks while the highest percentage was recorded for Havana Black bucks.

The Pearson correlation of the semen traits presented on Table 3 shows a wide range of coefficients among the semen traits. There was a negative correlation between semen volume and semen pH. There was a positive correlation of 0.85 between semen volume and motility. The higher the semen volume, the better the motility of the sperms. The correlation between semen volume and concentration was insignificant. There was a positive correlation between sperm motility and the normal morphology of sperm tail. The correlation coefficient of normal tail and sperm motility was 0.94. There was a positive correlation between semen pH and the percentage of double head sperm as well as between semen pH and the percentage of double tail sperm. Semen pH correlated significantly with the percentage of primary and secondary sperm abnormalities (Bodnar *et al*., 2000).

Havana black bucks had relatively lower semen volume, sperm motility, viability and concentration with higher sperm abnormalities. This could be as a result of their inability to tolerate high ambient temperature and relative humidity which characterized the humid tropical environment. Fadare (2014) reported a high heat stress index for Havana Black rabbits raised in the humid tropics. Elevated temperature has a significant effect on semen pH and sperm abnormalities. Roca *et al*. (2005) observed that semen volume and motility indices changed with elevated temperature. The report of Okab (2011)

showed that sperm concentration, percentages of dead sperm, and sperm with intact acrosome decreased with elevated temperature.

TABLE 2: Least square means of the sperm morphology as affected by breed

Parameters	New Zealand	California	Palomino	Havana black
Normal head(%)	90.02± 6.89[a]	89.45±5.98[a]	82.05±4.64[b]	85.08±2.56[b]
Double head(%)	2.33 ±0.33[d]	7.89±5.44[b]	4.20±0.85[c]	9.78±3.89[a]
Normal tail (%)	81.33±3.24[b]	85.78±2.19[a]	73.00±4.63[d]	78.34±2.52[c]
Double tail(%)	12.27±5.79[c]	14.67±1.23[b]	14.80±3.53[b]	18.34±1.56[a]

[a b c d]Mean on the same row with different superscripts are significantly (P<0.05) different

TABLE 3: Pearson correlation of the semen traits

	Vol	pH	Motility	Viability	Conc.	Nhead	Ahead	Ntail	Atail
Vol	1.00								
pH	-0.42	1.00							
Motility	0.85	0.57	1.00						
Viability	0.49	0.34	0.85	1.00					
Conc.	0.12	0.27	0.53	0.46	1.00				
Nhead	0.29	0.35	0.02	0.45	0.36	1.00			
Ahead	0.31	0.72	0.08	0.31	0.39	-0.69	1.00		
Ntail	0.21	0.26	0.94	0.34	0.25	0.78	0.23	1.00	
Atail	0.15	0.74	-0.68	0.47	0.38	0.32	0.75	-0.87	1.00

Vol=Semen volume, Nhead=Normal head, Ahead=Abnormal head, Ntail=Normal tail, Atail= Abnormal tail

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CONCLUSION

New Zealand White bucks had higher values in some traits such as semen volume, semen pH and sperm motility. The percentage of viable sperms in the semen samples obtained from New Zealand White bucks was similar with that of California breed. Semen from California breed had the highest sperm concentration and highest percentage of normal- tail sperms among the breed studied. Havana black bucks had relatively lower semen volume, sperm motility, viability and concentration with higher sperm abnormalities. New Zealand White and California rabbit had better semen traits compared with Palomino brown and Havana black rabbit in a humid tropical environment. New Zealand White and Californian White will have better breeding and reproductive success in the humid tropics and should be considered by rabbit breeders in humid tropical environment.

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Biochemical and Histological Studies on the effects of Lonart Ds on *Plasmodium berghei* Infected Mice.

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ABSTRACT

Biochemical and histological studies on the effects of Lonart Ds on *Plasmodium berghei* infected mice was investigated. Fifty mice were divided into five study groups of ten mice each. The groups were positive control, negative control, plasmodium with half therapeutic dose, plasmodium with therapeutic dose and plasmodium with double therapeutic dose respectively. Serum ALT, AST and ALP activities were significantly increased in negative control mice when compared with other groups, while parasitized mice with half therapeutic dose and double therapeutic doses showed significant increase in serum enzyme level when compared with positive control at ($P < 0.05$). Parasitized mice with therapeutic dose showed a significant decrease in AST and ALT levels and no significant difference was observed in ALP level when compared with positive control. This result was also supported by histological examination of the liver of parasitized mice treated with therapeutic dose showing marked improvement in renal epithelium and reduction in hepatic macrophage.

Keywords: *Aspartate transaminase, Alanine transaminase, Alkaline phosphatase and Liver tissue.*

INTRODUCTION

Malaria is a disease of global public health importance. Its social and economic burden is a major obstacle to human development in many of the world's poorest countries. In heavily affected countries, malaria alone accounts for 40% of public health expenditure, 30% to 50% of hospital admissions, and up to 60% of outpatient visits (WHO, 2007). Malaria is transmitted from person to person by the bite of mosquitoes infected with the protozoan parasite *Plasmodium*. Four *Plasmodium* species are capable of causing malaria in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale*. Of these *Plasmodium falciparum* is responsible for over 90% of cases and almost all of the malaria deaths worldwide (WHO, 2008b).

Artemisinin derivatives have been shown to produce faster relief of clinical symptoms and faster clearance of parasites from the blood than other antimalarial drugs (McIntosh and Olliaro, 1999; Adjuik *et al*., 2004 and WHO, 2006). A mosquito infects a person with sporozoites in the process of taking a blood meal. The sporozoites then enter the bloodstream and migrate to the liver. In the liver, they multiply into merozoites which infect and rupture the liver cells in an attempt to escape back into the bloodstream where infection continues. The invasion of liver cells by the sporozoite form of the malarial parasites can cause organ congestion, sinusoidal blockage and cellular inflammation (Jarikre *et al*., 2002). These changes in hepatocytes can lead to the leakage of parenchymal (transaminases) and membranous (alkaline phosphatase) enzymes of the liver into the circulatory system (Burtis *et al*., 2001). Hence the increase in liver enzymes (AST, ALT and ALP) which have been observed among malarial infected patients. Maegraith, 1981, Onyesom and Onyemakonor, 2011 also demonstrated that the various liver enzyme

(AST, ALT and ALP) activities in serum increased with increase in malarial parasite density and confirmed that the hepatic (liver) stage of the parasite's life cycle in its human host is accompanied by significant perturbation in the hepatocyte's parenchyma and membrane, leading to leakage of the liver enzymes into the general circulation. Artemether and lumenfantrine combined drug is currently receiving global attention as the most potent therapy for malaria infection. The treatment protocol is simple and there is no incident of drug resistance. However, there is a paucity of data on the biochemical studies evaluating the safety or toxic risk potentials associated with this synergistic drug.

MATERIAL AND METHODS

Animal treatment

Animals were grouped for experimental studies and were treated with Lonart DS following plasmodium parasite inoculation. The mice were weighed at the start of the experiment and randomly assigned on the basis of their weight into five study groups of ten mice each. Group A (positive control) received normal diet, Group B (negative control inoculated with plasmodium) received normal diet while groups C, D and E were administered half therapeutic dose, therapeutic dose and double therapeutic dose of Lonart DS respectively.

Parasite inoculation

The malaria parasite used was a chloroquine-sensitive strain of *Plasmodium berghei* (NK-65), obtained from the National Institute for Medical Research (NIMR), Ibadan, Nigeria and kept at the Department of Biochemistry Joseph Ayo Babalola University, Ikeji-Arakeji, Nigeria. The parasites were maintained by serial blood passage in mice. Parasitized erythrocytes were obtained from a donor-infected mouse by cardiac puncture in heparin and made up to 20ml with normal saline. Animals were inoculated intraperitoneally with infected blood suspension (0.2 ml) containing 1x 10^7 of parasitized erythrocytes on day zero. Parasitaemia was assessed by thin blood film made by collecting blood from the cut tip of the tail and this was stained with Geimisia stain (WHO, 2000). Infected mice with parasitaemia of 5-7% were allocated into four study groups of ten mice each (Builders *et al*., 2011).

Curative (established infection or rane test)

The curative potential of the drug was done employing the method described by (Ryley and Peters, 1970). The mice were injected intraperitoneally with standard inoculum of 10^7 *P. berghei* NK 65 infected erythrocytes on the first day (day 0). After 72 hours and following confirmation of parasitemia, the mice were divided into 4 groups of ten mice each.

Sample collection and biochemical assays

24 hours after the 3 days experimental period all mice were sacrificed by suffocation in chloroform vapor and dissected. Blood was obtained by cardiac puncture using sterile syringe and needle, the blood sample was allowed to stand for 30minutes to clot at room temperature and further spun at 2000rpm for 15 minutes in an MSE table top centrifuge. The serum removed with sterile needle was then used for biochemical investigations.

Assay for alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferases (AST) activities.

Alkaline phosphatase (ALP) (E.C 3.1.3.1) activity was assayed according to the method described by (Bassey *et al*., 1946) as modified by (Wright and Plummer, 1974). The procedure as described by Pratt and Kaplan (2000) and Lee *et al* (2008) were employed for the assay of aspartate aminotransferase (AST) (E.C 2.6.1.1) and Alanine aminotransferase (ALT) (E.C 2.6.1.2) activities respectively. All measurements were done using campsec

spectrophotometer.

Histological study

For light microscopic examination, liver tissues from each group were fixed with 10% buffered formalin, embedded with paraffin. After routine processing, paraffin sections of each tissue were cut into 5m thickness and stained with haematoxylin and eosin (Drury et al., 1967). The photomicrographs of the relevant stained sections were taken with the aid of a light microscope.

Statistical analysis

All data collected were summarized as mean ± SEM. Significant differences were determined using a Student's t- test and the differences were considered significant if $p < 0.05$.

RESULTS

Effect of Plasmodium and lonart DS treatment on serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) of mice.

This study was aimed at assessing the extent to which plasmodium affected serum enzyme levels in mice and how treatment with varying doses of Lonart DS ameliorated the conditions of the plasmodium infected mice.

Table 1: Effect of plasmodium and Lonart DS treatment on serum enzyme activities

TREATMENT GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)
GROUP A Control	118.0 ± 0.27	56.9 ± 0.25	49.5 ± 0.28
GROUP B Plasmodium treated	136.9 ± 0.25	131.6 ± 0.23	180.4 ± 0.17
GROUP C Half therapeutic dose	a, b 130.8 ± 0.31	a, b 60.2 ± 0.21	a, b 61.9 ± 0.25
GROUP D Therapeutic dose	a, b 116.7 ± 0.35	a, b 48.8 ± 0.89	a, b 49.3 ± 0.22
GROUP E Double therapeutic dose	a, b 135.4 ± 0.23	a, b 65.8 ± 0.31	a, b 59.7 ± 0.39

Values are mean ± SEM, n = 9

a; indicate significant difference in results of the different therapeutic doses in mice compared with control at 0.05 level of significance.

b; indicate significant difference in results of the different therapeutic doses in mice compared with the plasmodium treated group at 0.05 level of significance.

Effects of plasmodium and Lonart DS on histopathology of liver

The histopathological effect of plasmodium and Lonart DS (at different doses) were done on the liver sections of the experimental mice. Microscopic observation indicated chronic infiltrations of polymorphs, marked degenerating features in hepatocytes of plasmodium treated mice when compared with control, but the mice treated with half therapeutic dose showed perivascular inflammatory cells and vascular congestion when compared with control while mice treated with normal therapeutic dose showed reduction in hepatic macrophage and the mice treated with double therapeutic dose showed the presence of mild perhepatic inflammatory cels

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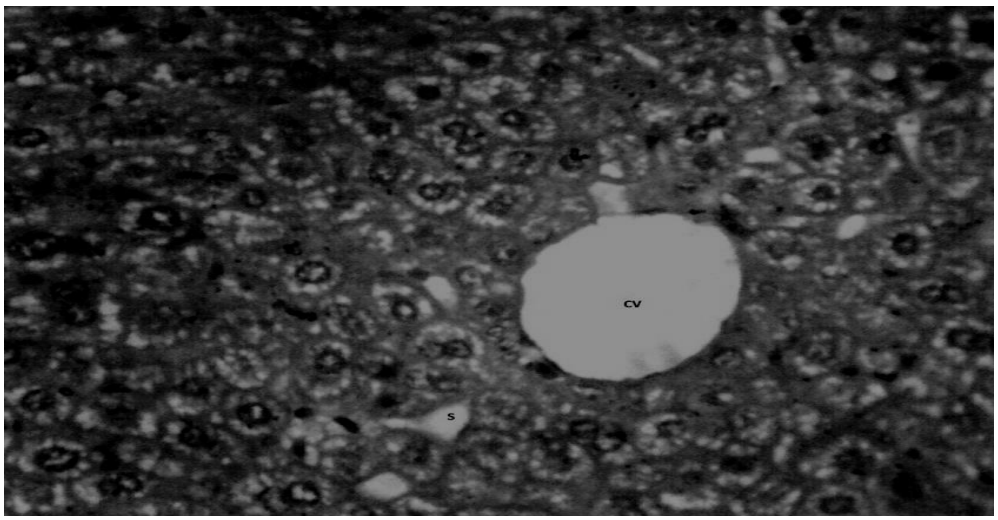

Plate 1.1: Photomicrograph of liver section of control mouse showing hepatic tissue composing of hepatocytes disposed in sheet, the central vein (CV) and sinusoids (s) are well outlined and free of congestion, inflammatory cells and interstitial collections. Cells appear essentially normal. (H&E ×400).

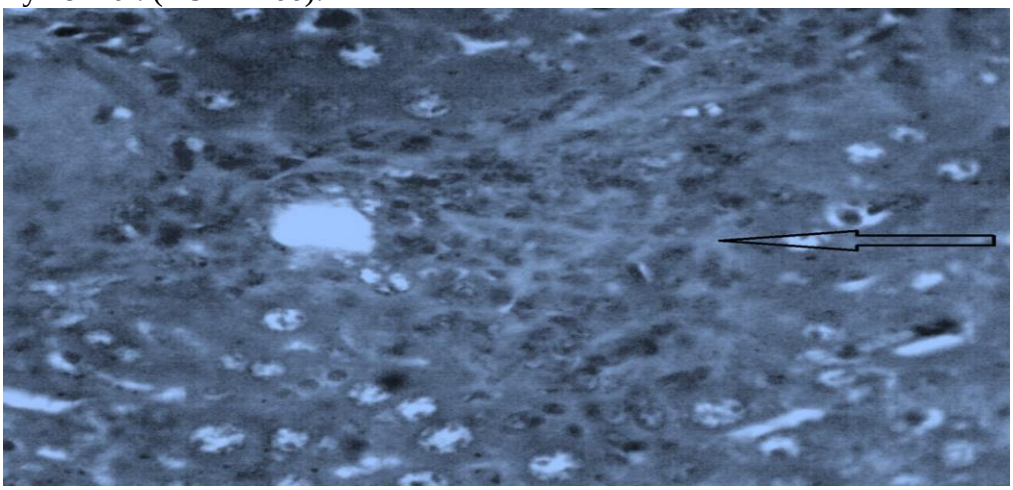

Plate 1.2: Photomicrograph of liver section of plasmodium treated mouse showing chronic infiltrations of polymorphs, marked degenerating features in hepatocytes. There is heavy presence of hepatic microphages. (H&E ×400).

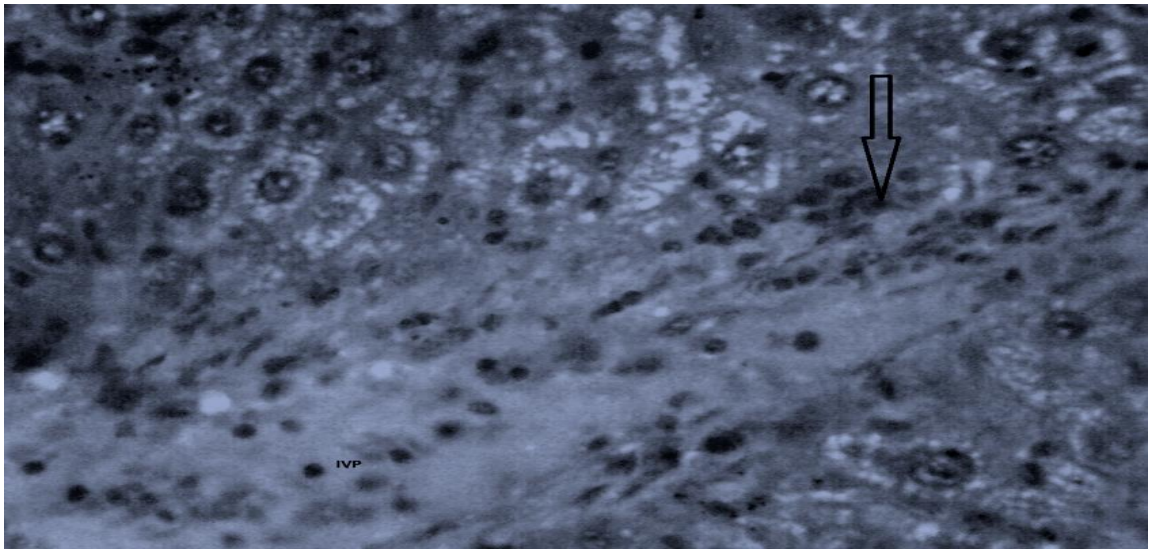

Plate 1.3: Photomicrograph of liver section of mouse treated with half therapeutic dose shows heavy presence of intravascular polymorphs (IVP), perivascular inflammatory cells (arrow), vascular congestion, the hepatocytes appear essentially unremarkable. (H&E ×400)

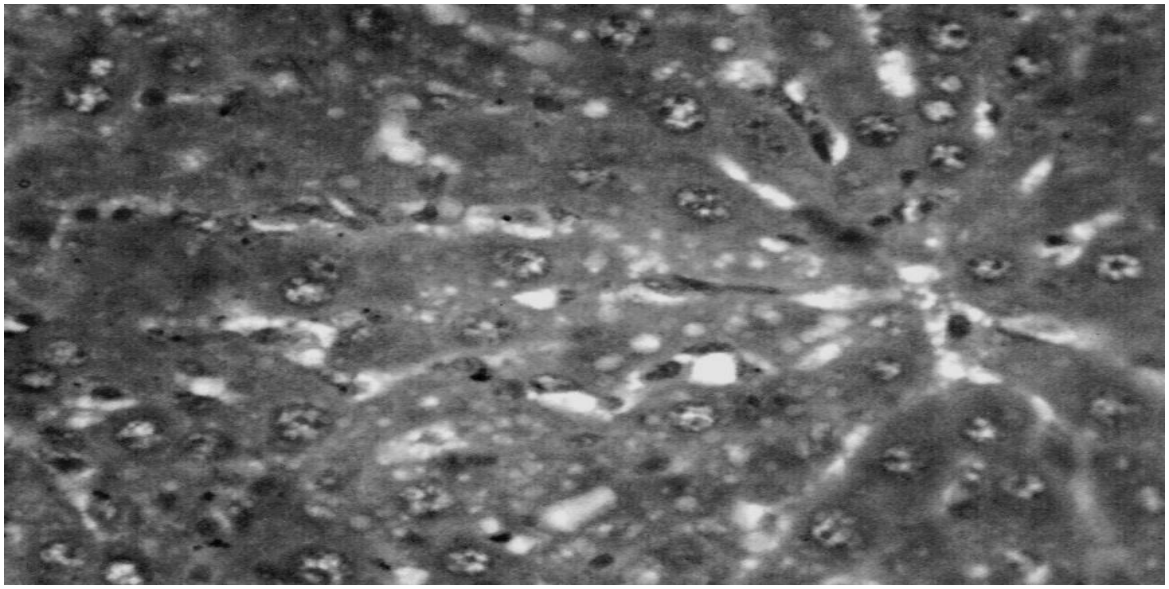

Plate 1.4: Photomicrograph of liver section of mouse treated with normal therapeutic dose showing mild dilation of the sinusoids, reduction in hepatic macrophage, the vascular channels are free of inflammatory cells and congestion (H&E ×400).

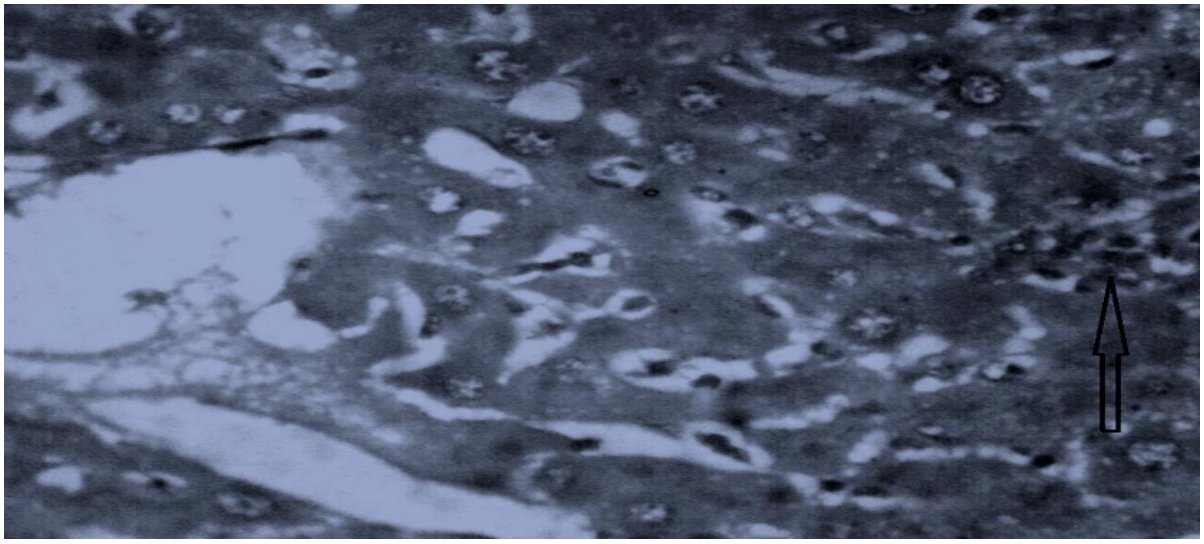

Plate 1.5: Photomicrograph of liver section of mouse treated with double therapeutic dose showing increased sinusoidal spaces, reduced microphage, presence of mild perihepatic inflammatory cells, portal vessels are free of congestion and inflammatory cells (H&E ×400).

DISCUSSION

Liver destruction can affect the metabolic processes in the body due to the role of liver in general metabolism of the organism. Enzymes are necessary for normal cellular metabolism including that of the liver (Rajamanickam and Muthuswamy, 2008). This study investigated the possible effect of Lonart DS on this cellular damage. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are considered indicators of hepatocellular health (Yang and Chen, 2003), (Vozarova *et al*., 2002).

A significant increase in the activities of ALT, AST and ALP in the blood and liver of control parasitized mice as compared with all the other groups was observed. This increase in enzyme activities may be as a result of liver injury caused by the *Plasmodium* infection and the consequent release of the enzymes into the blood stream. This is shown in the histopathological examination of the liver of the parasitized mouse which possesses marked chronic infiltrations of polymorphs, marked degenerating features in hepatocytes. The level of these enzymes in serum reflects pathological and physiological state of the liver and hence where Lonart DS exhibit its effects most. The distortion of tissues which occurs with subsequent passage of enzymes into the blood stream depends on the severity of cellular damage hence the need to monitor these enzymes (AST, ALT and ALP) to ascertain the physiological state of the tissues of mice administered half therapeutic, therapeutic and double therapeutic doses of Lonart DS.

The liver plays a central role in transforming and clearing chemicals and is susceptibility to toxicity from these agents. This is primarily because of its unique metabolic responsibility and close relationship with the GIT. Previous studies have reported that some antimalarial agents such as chloroquine (Pari and Amail, 2005) and amodiaquine (Farombi *et al*., 2000) can induce hepatic damage. Previous authors however, did not agree on the capacity of ACT to induce liver injuries, indicating a slight decrease in the activities of AST, ALT and ALP when compared to the normal control suggest that this drug does not disrupt or induce hepatic injury similar to those suggested by Georgewill and Ebong, 201). They reported normal hepatic cells in mice administered with ACT. Whereas Adaramoye *et al*., (2008) reported increased liver damage in rats administered

with ACT, in the present study it was observed that administration of Lonart DS caused a significant but dose dependent increase in activities of serum enzymes AST, ALT and ALP. This may suggest the ability of the drug to predispose to hepatic toxicity (Vahdati-Mashhadian and Rakhshandeh,(2005); (Ewaraiah and Satyanarayana, 2010) .

A significant decrease was observed in enzyme activities of groups treated with half therapeutic, therapeutic and double therapeutic doses of Lonart DS when compared with the plasmodium treated group indicating that the drug has reduced the sudden increase in enzyme activity.

The pharmacokinetics of the ACTs has also shown that their primary site of metabolism is the liver. Thus it would be expected that the liver would be susceptible to injury from these agents. However, results from this current study shows a mild presence of inflammatory cells in plasmodium treated mice, mice treated with half therapeutic and double therapeutic doses when compared with the control, but there was a marked improvement in the liver section of mice treated with normal therapeutic dose. Plasmodia infection caused marked congestion, marked hepatocytes necrosis and mild vascular congestion in the liver and kidney respectively. The presence of the parasite might have induced the cells of these organs causing the observed damages. Heavy parasitemia have been implicated in the occurrence of tubulointerstitial damages as well as glomerulonephritis and renal failure in the kidney of the infected patients Mahakur et al., (1983) Rajapurkar, (1994) and Saroj and Bhabani, (2008).

CONCLUSION

From this study it can be concluded that Lonart DS has anti- plasmodial effect on *Plasmodium berghei* infected mice in varying degrees and in a dose dependent manner, but may cause marked renal and hepatic toxicity when administered at half and double therapeutic doses as such caution should be taken in administering the drug beyond the therapeutic dose.

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Isolation of Microbial Contaminants From Vegetables

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ABSTRACT

Vegetables are the edible parts of plant. Occurrence of microbial spoilage of vegetables is recognized as a source of potential health hazard to man and animals. The research focuses on isolation of microbes particularly bacteria and fungi from marketed vegetables. Samples were collected and Standard microbiological analyses were used to isolate bacteria and fungi. Eight bacterial isolates that were isolated are *Brevibacillus brevi, Bacillus subtilis, Branmehamlla cattarhalis, Escherichia coli, Salmonella typhi, Pseudomonas aruginosa, Serratia marcscen and Staphylococcus sp.* Four fungal isolates were also isolated; *Aspergillus flavus, Aspergillus niger, Penicillium sp, Saccharomyces sp.* Pepper (*Capsicum annuum*) has the highest bacterial count (6.53×10^9 cfu/ml) while shoko (*Celosia argentia*) has the highest fungal count (5.45×10^9cfu/ml). In this study, the high prevalence of fungal and bacterial contamination of these vegetables depicts unhygienic handling of these food materials at the point of cultivation, harvesting, transportation or selling. Therefore, there is need to safeguard the health of final consumers by proper washing and disinfection of these products which are consumed in their raw forms.

Keywords: *Bacterial isolates, Microbial load, Vegetables*.

INTRODUCTION

The word vegetable was first recorded in English in the early 15th century. It comes from old French and was originally applied to all plants. The word is still used in this sense in biological contexts. It is derived from Medieval Latin ***vegetabilis*** meaning "growing", "flourishing" (Wharton, 1970). Vegetables are the edible component of plant. This typically means the leaves, stems, bulbs, seeds and root of a plant. However, the word vegetable is not scientific and its meaning is largely based on culinary and cultural tradition (ICMSF, 1986; Bankefa, 2013; Akinyele *et. al.*, 2013).

Vegetables are important protective component of food that is highly beneficial for the maintenance of good health and prevention of diseases. They contain varying proportions of vitamins such as vitamin A, K, B6, provitamins, dietary minerals and carbohydrates. Vegetable contain various medicinal agents and are valued mainly for their high vitamin and mineral content (Marie *et al.*, 2013).

Studies have evaluated the association of fruit and vegetables consumption with the reduction of risk of specific diseases ((Hu *et al.*, 2014). However, vegetables often also contain phytotoxins and antinutrients, which interfere with the absorption of nutrients (Marie *et al.*, 2013). These include *a*-solanine, *a*-chaconine, enzyme inhibitors of cholinesterase, protease, amylase, cyanide, cyanide precursors, oxalic acid and others. These toxins are natural used to wipe off the insects, predators and fungi that might attack the plant (Ayanyemi, 2013).

The number of outbreaks of food borne illnesses associated with consumption of fresh vegetables has increased due to contaminants (Sengun and Karapinar,

2004). The incidence of microorganisms in vegetables may be expected to reflect the sanitary quality of the processing steps and the aseptic condition of the raw product at the time of processing (Amoah *et.al., 2007*; Ayanyemi, 2013). However, pathogenic microorganisms of human origin may also be present in minimally processed vegetables as the minimal technological processing may be unable to remove the original contamination resulting from air, soil, water, Insects, animal, workers, harvesting and transportation equipment. Certain fungi such as *Aspergillus spp*, *Fusarium spp*, and *Penicillium spp*. which are commonly occurring filamentous fungi may grow on vegetable and their growth may result in production of toxins known as mycotoxins, which may cause a variety of illness in human having allergic responses to immune suppression and cancer (Doyle, 2015).

Vegetables are frequently consumed raw without being exposed to aseptic processes that reliably eliminates pathogens. Washing fruits and vegetables in chlorinated water can reduce microbial load but cannot be considererd as the ultimate method to eliminate pathogens (Joseph, 2013). Eating or drinking contaminated foods can cause food-borne diseases. Many different types of bacteria, viruses and parasites can contaminate food, so there is numerous different food borne infections (Richard, 2013). Microorganism can be introduced to the crop on the seed itself, during crop growth in the field, during harvesting and postharvest handling, or during storage and distribution (Akinyele, 2009). Therefore, early interventive measures during crop development and harvesting and good agricultural practices (GAP) will provide drastic reductions in yield loss due to spoilage at all subsequent steps in the food-to-fork continuum (Eckert and Ogawa, 1988; Barth *et al*., 2009).

Vegetables particularly the leafy ones have been implicated in nearly half the gastrointestinal infections caused by norovirus in the United States. These foods are commonly eaten raw and may become contaminated during preparation by an infected food handler. Hygiene is important when handling foods that are eaten raw. Such products need to be properly washed, handled and stored to limit contamination (Akinyele *et al.*, 2013).

Some microbial contaminants are capable of colonising and creating lesions on healthy plant tissue (Zhang, 2014). The occurrence of microbial spoilage of vegetable is recognized as a source of potential health hazard to man and animals. This is due to the production of toxins by the microoganisms. Therefore, bacteriological safe fruits and vegetables are essential to maximize the health benefits promised by adequate consumption of these produce. The research focuses on isolation of pathogenic microorganisms particularly bacteria and fungi marketed vegetables.

MATERIALS AND METHODS

Study Area

Akure is situated at 7.25^0 North latitude, 5.19^0 East longitude and 396 meters elevation above the sea level. Akure is a big town in Nigeria, having about 420,594 inhabitants. Owena which is located in the suburb of Owena town in Ifedore Local Government Area of Ondo-State, between latitude 7.15^0 N, longitude 5.05^0 E (Lasisi, 2002).

Collection of samples

During the period of the study, six vegetables samples; Jews mallow (Ewedu,

Corchorus sp), Lagos spinach (shoko – *Celosia argentia*), tomato (*Solanum lycopersicum*), Chili (shombo – *Capsicum annuum*) Cayenne pepper (Bawa – *Capsicum annuum*) and water leaf (*Tallinum triangulare*), were bought from local markets in different towns located in Ondo state and Osun state. These states are located in the Southwest part of Nigeria. The samples were not collected aseptically because the main purpose was to know the level of contamination associated with vegetables ranging from harvesting, handling and selling.

Sterilization of materials

The glass wares were thoroughly washed with detergent rinsed thoroughly with distilled water and then air dried. The glass wares were sterilized in the hot air oven at 160°c for one hour. The inoculating loop was also sterilized by flaming. The work bench was disinfected by swabbing with 95% ethanol. All work in the laboratory was done in a sterile environment.

Preparation of media

The media used in this research work were nutrient agar, potato dextrose agar, Skimmed milk agar, starch agar, tributyrin agar, peptone water and they were all prepared according to manufacturer's instructions. The media was dissolved in the adequate amount of distilled water. The media were all homogenized and autoclaved at 121°c for 15 minutes.

Isolation and identification of fungi

The samples were serially diluted using sterile distilled water and homogenized. The diluted samples (10^{-4} and 10^{-6}) were plated onto sterile potato dextrose agar and incubated at 25°C for 72 hours. Distinct colonies were picked and purified by streaking on the same agar. Mycelia of the isolated fungi were picked on a slide, two drops of lactophenol-cotton blue was added and covered at an angle of 60° with a cover slip. Fungi isolates were characterized and identified based on their colonial morphology and microscopic characteristics at magnification of ×40 objective lens. They were identified using different identification keys (Nelson *et al*., 1983).

Isolation of bacteria

The samples were serially diluted using sterile distilled water and homogenized. The diluted samples (10^{-4} and 10^{-6}) were plated on sterile nutrient agar and incubated at 37°C for 24 hours. Distinct colonies were picked and purified by streaking on the same agar. The pure cultures were preserved on agar slants for further studies (Bradshaw, 1979). Colony counting was done by means of a Gallenkamp colony counter.

Identification of bacterial isolates

Spore and gram staining for microscopic characterization of bacteria were carried out. Other Biochemical tests such as; sugar fermentation test, coagulase test, catalase test, Methyl red test, voges-proskeuer test, SIM test, citrate test were also used to identify bacteria isolates.

Gram staining

A loopful of young culture was placed on the slide. Smear was prepared by spreading the culture in distilled water; the smear was air dried and heat fixed. The heat fixed smear was first stained with crystal violet for 60 seconds. After washing the slide, it was stained with safranin for 60 seconds, rinsed with water and air dried. The cells were examined under the light microscope using x100 objectives lens for gram's reaction and cellular morphology (Cheesbrough, 2006).

Bacterial smear was prepared on slide. The smear was air dried and heat fixed. The primary stain malachite green was applied on the heat fixed slide and was allowed to steam in water bath for five minutes.

Malachite green stain was re-applied to avoid drying out. The slides were removed from the steam and rinsed with water until the slide was clear. The slide was flooded with counterstain safranin for 60 seconds and then rinsed with water. The slides were viewed under oil immersion lens with a light microscope.

After the staining procedure, the endospores appeared green having retained the primary stain (malachite green). Vegetative cell appeared pink having retained the counterstain safranin (Onyeagba, 2004).

Catalase Test

A drop of hydrogen peroxide was placed on a grease free clean slide, a sterile inoculating loop was used to pick a loop-full of the isolate and spread on the hydrogen peroxide. Formation of bubbles indicates the presence of the catalase enzyme (Bailey and Scott, 1974).

Voges-Proskauer Test

The test isolates were inoculated into glucose phosphate broth and incubated at 37°C for 72 hours. After incubation, 40% Potassium hydroxide (alpha-napthol) was added to the culture and observed for colour change (Macfaddin, 2000).

Methyl-red Test

The test isolates were inoculated into test tubes containing glucose phosphate broth and incubated at 37°C for 72 hours. After incubation, the methyl red indicator (0.02g in 50ml of 95% ethanol) was added to the culture and observed for change in colour. Red coloration indicates a positive result while yellow coloration indicates a negative result (Beck, 2000).

Sulphur, indole and mortility (SIM) Test

The test isolates were inoculated into test tubes containing SIM agar by stabbing to the bottom of the tube and streaked, then incubated at 37°C for 24hrs. After incubation, colour change was observed. Black coloration indicates H_2S is produced while turbidity of the organism from the stab mark indicates the organism is motile. Kovacs reagent was then added to the culture and observed for change in colour. Red coloration indicates indole positive (Janda, 2006).

Citrate Test

The test isolates were inoculated into test tubes containing Simmon's Citrate Agar by stabbing to the bottom of the tubes and streaked, then incubated at 37°C for 24hrs. After incubation, colour change was observed, blue coloration indicates the organism is citrate positive (koneman, 2006).

Fermentation of sugar

The sugars used for this experiment were glucose, lactose, sucrose, and malatose. One gram of each sugar was weighed into different conical flask. Phenol red (0.01g) was added as an indicator and 5ml each of the sugar solution were dispensed into different tubes with Durham's tube inserted into each test tube. The tubes were plugged with cotton wool and labelled appropriately; it was then sterilized in an autoclave at 121°C for 15 minutes after which the tubes were allowed to cool.

The bacterial isolates were inoculated aseptically into sugar solution in the tubes and incubated at 37°C for 72 hours. The change of colour from red to yellow indicates acid production which implies the utilization of sugar by the organism and appearance of bubble in the Durhams tubes indicates gas production. Un-inoculated tubes were used as control (Cheesbrough, 2006).

Coagulase Test

A loop-full of test isolates were picked from a young culture, emulsified with serum placed on a clean grease free slide and rocked for 1 minute. The presence of

agglutination indicates a positive reaction (Winn *et al*., 2006).

RESULTS

Table 1 shows the colonial morphology of bacteria based on the form, size, texture, colour, opacity, surface, elevation and margin. Table 2 shows the microscopy and biochemical reaction of bacteria isolated from vegetables based on gram reaction, spore formation, motility, methylred, voges proskauer, catalase, and coagulase. Table 3 and 4 shows the total bacterial and fungal count of the marketed vegetable samples calculated in colony forming units per ml. Table 5 shows the cultural characteristics and the microscopic examination of fungi isolates by using selective media, potato dextrose agar

TABLE 1: Colonial morphology of bacteria isolated from vegetables

I SOLATES ID	FORM	SIZE	COLOUR	OPACITY	SURFACE	TEXTURE	ELEVATION	MARGIN
TO 1	Circular	Small	Cream	Opaque	Glistering	Moist	Raised	Entire
TO2	Circular	Small	Yellow	Translucent	Wrinkled	Butyrous	Flat	Enitre
TO3	Filamentous	Medium	Cream	Tanslucent	Veined	Moist	Flat	Filiform
SB1	Circular	Small	Green	Translucent	Smooth	Moist	Flat	Entire
SB2	Rhizoid	Large	Cream	Cloudy	Wrinkled	Moist	Raised	Undulate
SB3	Circular	Large	Cream	Cloudy	Glistering	Moist	Raised	Lobate
SB4	Circular	Puntiform	Cream	Translucent	Glistering	Moist	Flat	Entire
EW 1	Circular	Medium	Cream	Translucent	Glistering	Moist	Flat	Entire
EW2	Circular	Medium	Cream	Translucent	Glistering	Moist	Flat	Entire
SH1	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
PE1	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
PE2	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
PE3	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire
WL1	Circular	Small	Cream	Translucent	Glistering	Moist	Flat	Entire

KEYS: EW (Ewedu); SH (shoko); TO (Tomato); PE (Pepper); SB (Shombo); WL (Water leaf)

TABLE 2: Microscopy and biochemical reaction of bacteria isolated from vegetables

ISOLATES ID	GR	SPORE FORMERS	MOTILITY	MR	VP	CATALASE	COAGULASE
TO 1	+ Rod	+	+	+	-	+	-
TO2	+ Rod	+	+	-	+	+	+
TO3	+ cocci	-	+	+	-	+	+
SB1	- Rod	-	+	-	+	+	-
SB2	+ Rod	+	+	+	-	+	-
SB3	- Rod	-	+	+	-	+	-
SB4	+ Rod	+	+	+	-	+	+
EW 1	- Rod	-	+	+	-	+	-
EW2	+ Rod	+	+	+	+	+	-
SH1	+Rod	+	+	+	-	+	-
PE1	+cocci clusters	-	+	+	-	+	-
PE2	- Cocci	-	+	-	+	+	+
PE3	- Rod	-	+	-	+	+	-
WL1	+ cocci	-	+	-	+	+	+

Keys: Negative : - positive: + GR : Grams reaction MR : Methyl Red, VP : Voges Proskauer

Table 3: Total bacterial count of marketed vegetables

Samples	Bacterial count (10^{-4})	Bacterial count (10^{-6})
EW	1.60×10^5	2.64×10^9
SH	2.13×10^5	6.43×10^9
TO	2.55×10^5	3.23×10^9
PE	2.65×15^6	6.53×10^9
SB	2.76×10^5	5.75×10^9
WL	5.20×10^5	7.25×10^9

KEYS: EW (Ewedu); SH (shoko); TO (Tomato); PE (Pepper); SB (Shombo); WL (Water leaf); CFU/ML (Colony forming units per ml)

Table 4: Total fungal count of marketed vegetables

Samples	Fungal count ($10^{-4)}$	Fungal count (10^{-6})
EW	2.43×10^5	5.00×10^9
SH	2.63×10^5	5.45×10^9
TO	$1.1\ 3 \times 10^5$	3.61×10^9
PE	1.09×10^5	3.82×10^9
SB	1.26×10^5	3.93×10^9
WL	1.06×10^5	3.23×10^9

KEYWORDS: EW : (Ewedu); SH : (shoko); TO : (Tomato); PE : (Pepper); SB : (Shombo); WL : (Water leaf); CFU/ML : (Colony forming units per ml).

TABLE 5: Characteristics of the fungi isolates on potato dextrose agar

Isolates ID	Cultural characteristics	Microscopic examination of slide culture	Organism
TO 1	Whitish colonies becoming brown black with age	Non septatesporangiosphore are directly opposite the branched rhizoids. Sporangia are subglobose. Sporangiospores are ovoid in shape and columellaaresubglobose.	*Rhizopus stolonifer*
TO2	Wrinkled gray colony becoming with brownish gray with age.	Non septate mycelia branching sporangiophores; columellapyriform, ellipsoidal, pointed conical	*Mucor spp*
TO3	White fluffy growth of colonies with elevated mycelia that turned black after 36 hours	Black with sulphur yellow area on the surface single celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bud of the septate hyphae.	*Aspergillus niger*
SB1	Colonies are granular, velvety or wooly and yellow brown	Conodiophores are long and rough just beneath the globose vesicle. Philades are circumferential and are biseratial. Conidia are round, smooth or slightly rough and form long chains.	*Aspergillus flavus*
SB2	Whitish pink mycelial growth with colonies. Colonies are whitish-pick with micro-conidia, ovoid to mycelia growth a purple tinge myce-liumellipsodal in shape are extensive and cottony in culture	Macro conidia culture are borne on phialides on branched conidiospores. Septate fusiform, slightly curved and pointed at both ends	*Fusarium spp*
SB3	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
SB4	Powdery olivaceous green with sterile margin. Orange to red, wrinkled, radially furrowed	Conidia head has a symmetric penicillin being tangled in chains of conidia	*Penicillium spp*
EW 1	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
EW2	Powdery olivaceous green with sterile margin. Orange to red, wrinkled, radially furrowed	Conidia head has a symmetric penicillin being tangled in chains of conidia	*Penicillium spp*
SH1	White fluffy growth of colonies with elevated mycelia that turned black after 36 hours	Black with sulphur yellow area on the surface single celled spores (conidia) in chains developing at the end of the sterigma arising from the terminal bud of the septate hyphae.	*Aspergillus niger*
PE1	Colonies are granular, velvety or wooly and yellow brown	Conodiophores are long and rough just beneath the globose vesicle. Philades are circumferential and are biseratial. Conidia are round, smooth or slightly rough and form long chains.	*Aspergillus flavus*

PE2	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
PE3	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*
WL1	Shiny, creamy, white colonies	Single celled structures	*Saccharomyces spp*

TABLE 6: SUGAR FERMENTATION PROFILE OF BACTERIA ISOLATED FROM MARKETED VEGETABLES

ISOLATES	GLUCOSE	SUCROSE	LACTOSE	FRUCTOSE	GAS	INDOLE	H_2S	PROBABLE ORGANISM
TO 1	-	-	-	+	-	+	-	*Brevibacillus brevi*
TO2	+	+	-	+	-	+	-	*Brevibacillus brevi*
TO3	+	+	-	+	+	-	-	*Staphylococcus sp*
SB1	+	-	-	+	-	-	-	*Pseudomonas aruginosa*
SB2	-	-	+	+	+	-	-	*Salmonella typhii*
SB3	+	-	+	+	-	-	-	*Salmonella typhii*
SB4	-	-	+	-	+	-	-	*Bacillus subtilis*
EW 1	+	-	+	-	-	-	-	*Escherichia coli*
EW2	-	-	+	+	+	+	-	*Escherichia coli*
SH1	-	+	-	+	-	+	-	*Paenibacillus validus*
PE1	-	+	-	-	+	-	+	*Branmehamlla cattarhalis*
PE2	+	+	-	+	+	-	-	*Branmehamlla cattarhalis*
PE3	+	+	+	+	+	+	-	*Serratia marcscens*
WL1	+	+	+	+	+	+	-	*Staphylococcus sp*

KEYS: Negative - ; Positive +

DISCUSSION

Fourteen bacteria isolates were isolated from freshly marketed vegetables which includes Jews mallow (Ewedu, *Corchorus* sp), Lagos spinach (shoko – *Celosia argentia*), tomato (*Solanum lycopersicum*), Chili (shombo, *Capsicum annuum*) Cayenne pepper (Bawa, *Capsicum annuum*), water leaf (*Tallinum triangulare*) based on colonial, morphological, sugar fermentation and biochemical tests. The presence of *Brevibacillus brevi, Paenibacillus validus, Bacillus subtilis* as also detected by Breidt (2009) revealed the unhygienic practices of the traders when handling vegetables or contamination from soil, air and dust.

The *E.coli* and *S. typhii* isolated in the study is in consonance with the study conducted by Baiyewu (1998). This may be linked to animal dung and manure used during the cultivation of vegetables as fertilizers. *S. typhii* has been implicated to be responsible for typhoid fever (Baiyewu, 1998). Most strains of *Staphylococcus spp* are known to be pathogenic due to the heat stable enterotoxin they produce. The presence of *Staphyloccus sp*, as supported by the study of Ayoola (2007) may lead to contamination of food and eventually affects the health of the consumers.

Branmehamella cattarhalis isolated from pepper (*Capsicum annuum*) may be due to air contamination. It is of high potential risk when consumed raw or under-cooked by the

consumers. It causes upper respiratory tract infection such as sinusitis and otitis (Barth, 2009).

Pseudosomonas aeruginosa is a prominent inhabitant of soil which is responsible for diseases of vegetables. It is associated with spoilage of vegetable. According to Amusa (2007) it is an important cause of infection and is a frequent cause of nosocomial infections such as pneumonia, urinary tract infection (UTIs), and bacteremia. It invades burns area, causes septic shock and responsible for cystic fibrosis in human. *Serratia marcescens*, an opportunistic human pathogen isolated in the study had also been implicated as one of the organisms causing nosocomial infection (Micheal, 2013).

According to Akinyele (2013), fungi isolate *Rhizopus stolonifer* and *Mucor mucedo* found in the study causes food spoilage. They grow on the surface of moist, carbohydrate-rich foods, such as breads, fruits and vegetables. The presence of *Penicillium sp* and *Aspergillus niger* agreed with study of (Akinyele, 2013), and could be due to the fact that these organisms are spore formers and are known as common environmental contaminants nevertheless, they have been implicated as food borne pathogens.

In this study, the high prevalence of fungi and bacteria was further enhanced by unhygienic mode of transportation of these consumable products. Also, local practice of using organic manure, such as human, animal and poultry dropping as fertilizer might have contributed immensely to the occurrence of the pathogen. The contamination of vegetables by pathogenic bacteria and fungi could also be as a result of poor handling practices in food supply chain, storage conditions, distributions, marketing practices and transportation (Effiuvwevwere, 2000).

Consequent upon all these findings it is therefore very necessary and important for regulatory authorities in conjunction with government are to formulate a aseptic techniques of producing, handling, processing, storing and retailing vegetables especially in developing countries such as Nigeria. Vegetables should be thoroughly washed with clean portable water before consuming raw and before cooking in order to reduce food borne infections. It is therefore necessary and important that both the farmer who harvests the vegetables for transportation, the marketers and consumers take necessary and appropriate precautions in preventing contamination and eating of contaminated vegetables. We therefore recommend that government at different levels should intensify the provision of portable water in our farm settlements to reduce the use of un-treated waters for farming. Animal dung used as manure should be well treated with disinfectants or autoclaved to reduce their level of contamination of the vegetable by pathogenic organisms. Likewise, standard stalls should be erected in our market places where the sellers can conveniently display their goods without been too close to the road to reduce air borne contamination of the consumables in the markets.

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