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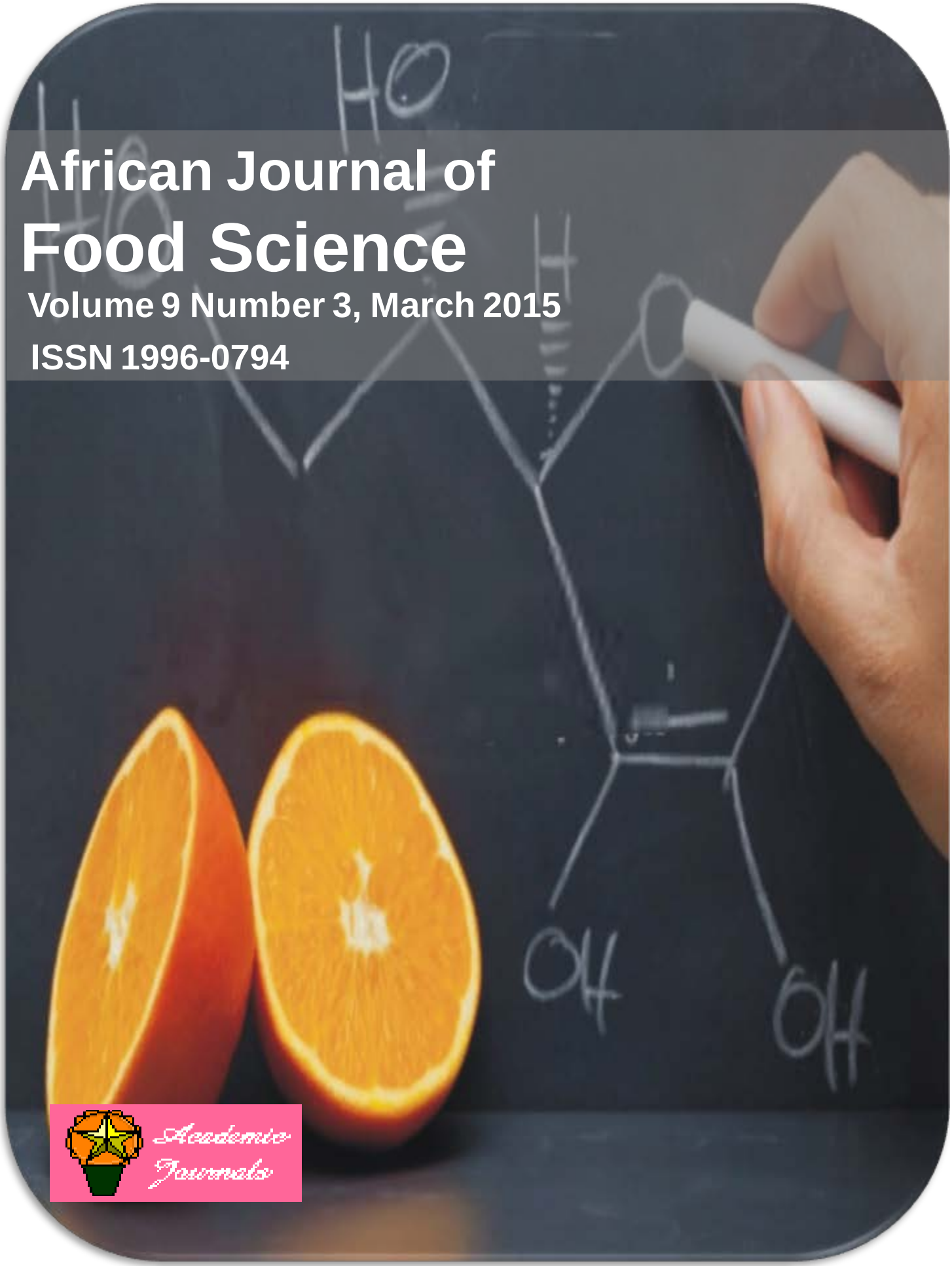

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: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

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Full Length Research Paper

Collection and evaluation of Roselle (*Hibiscus sabdariffa* L.) germplasm in Nigeria

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In order to assess the genetic diversity of Roselle (*Hibiscus sabdariffa* L.) in Nigeria, a survey was undertaken to collect the germplasm of the crop. The survey cut across 56 towns and 20 villages in 17 states including the Federal Capital Territory (FCT). Sixty three (63) farmers were interviewed and 60 accessions of Roselle were collected from them. Results showed that 41.7% of these accessions were having green calyx, while 31.7% were with red calyx. On the other hand, 20.0% of the accessions possessed deep red calyx while only 6.7% have light red and pink calyx. Collections from the North Central, North Eastern, North Western and South Western parts were replicated over states, towns and villages. The highest number of Roselle accessions was collected from Kaduna State (8 accessions) followed by Niger State (6 accessions); Jigawa State (6 accessions) while FCT and Bauchi State have 4 accessions each. This is an indication that these areas have the greatest diversity of the crop genetic resources in Nigeria and the states might be secondary centre of origin of the crop. However, morphological as well as molecular characterizations are required to arrange the Roselle accessions collected into a suitable group; this will eventually provide the raw materials needed for the Roselle improvement programme in Nigeria.

Key words: Genetic diversity, germplasm, Roselle accessions, improvement programme.

INTRODUCTION

Roselle (*Hibiscus sabdariffa* Linn.) is a shrub belonging to the Family Malvaceae (Mahadevan et al., 2009; Anjah et al., 2012). It is thought to have originated from Asia (India to Malaysia) or Tropical Africa. The plant is widely grown in the Tropics including Caribbean, Central America, India, Africa, Brazil, Australia, Hawaii, Florida and Philippines, as a home garden crop (Mahadevan et al., 2009). In Sudan, it is a major crop of export, especially, in the western part where it ranks after pearl millet, and followed by *Sesamum* (Leung and Foster, 1996; Gautam, 2004). The genus consists of about 300 species some of which are widely distributed as tropical herbs and shrubs (Heywood, 1978) or as annual erect, bushy, herbaceous sub-shrub (Amin, 2008). Some of the species include: *Hibiscus canabinus* L., *Hibiscus asper* (Hook.) F., *Hibiscus tiliaceus* L., *Hibiscus acetosella* Weiw ex Hiern and *Hibiscus scotelli* Bak. F.

The plant is about 3.5 m tall and has a deep penetrating taproot system. It has a smooth or nearly smooth, cylindrical, typically dark-green to red stems (Amin, 2008;

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Mahadevan, et al., 2009). The leaves are alternate, 7.5-12.5 cm long, green with reddish veins and long or short petioles. Leaves of young seedlings and upper leaves are deeply 3 to 5 or even 7-lobed and the margins are toothed. Flowers are borne singly in the leaf axils and are up to 12.5 cm wide, yellow or buff with a rose or maroon eye, that turn pink as they wither at the end of the day. The typically red calyx, consist of 5 large sepals with a collar (epicalyx) of 8-12 slim, pointed bracts (or bractioles) around the base. The fruit is a velvety capsule, 2-5 cm long, which is green when immature, 5-valved, with each valve containing 3-4 seeds which usually contain high percentage of oil (Rice et al., 1993). The capsule turns brown and splits open when mature and dry. Seeds are kidney-shaped, light-brown, 3-5 mm long and covered with minute, stout and stellate hairs (Julia, 1987).

The importance of this crop cannot be over emphasized; it is used for many different purposes, the most common of which are as a fibre crop, the young leaves are eaten as cooked vegetables especially with soup (Fasoyiro et al., 2005). The seeds are pounded into meal which is used as oily soup or sauce after roasting. Oil extracted from the seed is a substitute for castor oil while the residue is used in a fermented form as soup or cake (Aliyu, 2000).

The crop is used fresh for making wine, juice, jam, jelly, syrup, gelatin, pudding, cakes, ice cream and also dried and brewed into tea as well as flavours and carbonated soft drinks, other acidic foods, spice and used for butter, pies, sauces, tarts and other desserts (Walford, 1984; Qi et al., 2005). The grinded leaves and seeds are added to curries as seasoning. Roselle contains an acid, rhubarb-like flavour. The red calyces contain anti-oxidants including flavonoids, gossypetine, hibiscetine and sabdaretine (Qi et al., 2005). The fresh calyces are also rich in riboflavin, ascorbic acid, niacin, carotene, calcium, and iron that are nutritionally important (Mahadevan et al., 2009), as well as, amino acids and mineral salts (Cisse et al., 2009).They are also known for their unique flavour, characteristics that make them appealing to taste. Roselle drink had been improved nutritionally by producing fruit-flavoured Roselle drinks, which are richer in vitamins and minerals by addition of different fruits with higher consumption acceptability (Fasoyiro et al., 2004).

The crop is mainly grown as a vegetable from the savannah and semi-arid areas in Africa, while its use as a fibre crop is mostly in southern Asia. Formerly, it was traditionally cultivated in Nigeria for its leaves, seeds and stems; but is now being grown commercially for its calyces (Babatunde, 2003). Roselle is widely grown in northern parts of Nigeria, where the dried calyx is used for making a popular ‘zobo’ drinks (Falusi, 2007). Udom et al. (2001) reported that there are three common varieties of Roselle grown in Nigeria. Two of these varieties have red calyces while one has green calyces.

The green variety is more predominant in the Southern arts of Nigeria but the green variety is scarce while the other two red varieties are predominant in the Northern parts of this country; however, the green variety is also common in the Northern part of the country. The calyces from these varieties have a number of uses and promising prospects for industrial purposes (Alegbejo, 2000). These popular uses to which Roselle have been assigned had fuelled increasing demand for the crop thus, necessitating corresponding increased supply of the products. Though attempts have been made to achieve this increased supply through increased cultivation of the different varieties; the successes of such attempts have been limited by challenges ranging from unfavourable environmental conditions, as well as dwindling man-power and inadequate farming conditions. As the crop continues to play important horticultural roles in Nigeria, its improvement will surely enhance agricultural productivity, alleviate poverty and facilitate food security. But unfortunately, very little research attention has been given to the improvement of the crop. This background has made it necessary to collect and evaluate the germplasm of the crop, as a basis for research into its development and promotion as a major crop in Nigeria.

MATERIALS AND METHODS

Exploration and collection of Roselle germplasm in Nigeria

A survey of *H. sabdariffa* (Roselle) growers was conducted in south-western, north-central, north-western and north-eastern parts of Nigeria, representing the major Roselle producing areas of the country. The survey was conducted between October 2012 and January 2013, when the farmers were expected to be harvesting the crops. The states visited were Niger, Kogi, Nasarawa, Kwara, Ekiti, Ondo, Osun, FCT, Benue, Taraba, Plateau, Kebbi, Gombe, Bauchi, Kaduna, Katsina, Jigawa, and Sokoto. Questionnaires were administered through an interpreter in some cases and samples of available Roselle accessions under husbandry were collected. The questions asked included local name of accessions, source of seed supply, yield, Roselle seed preferences, constraints to cultivation and economic importance (Table 1).

The seeds were collected packed and sealed in thick paper envelopes each of which was given an accession code, local name and locality before they were finally stored in dry containers.

Measurement of the seed diameter

Ten seeds at random were selected from each of the accessions for the seed diameter. The seed diameters were measured using meter rule and the mean value was recorded as the average diameter.

RESULTS AND DISCUSSION

The survey covered 56 towns and 20 villages in 17 states including the Federal Capital Territory (FCT), Nigeria. Sixty three (63) farmers were interviewed and 60 accessions of Roselle were collected (Table 1). It was observed that most of the accessions were duplicated in most of the

Table 1. Sources and description of Roselle germplasm in Nigeria.

S/N	Accession number	Local name	Local Government	State	Calyx colour	*seed Diameter (mm)
1.	NGR-OD-001	Ishapa Otatupe	Ikare	Ondo	Green	3.55
2.	NGR-OD-002	Ishapa	Ogbese/ Akure	Ondo	Green	3.55
3.	NGR-OD-003	Ishapa Oloho	Ondo/Irele	Ondo	Green	3.65
4.	NGR-EK-004	Ishapa	Ijero	Ekiti	Green	3.30
5.	NGR-EK-005	Ishapa Toromoyan	Ado/ Ijan	Ekiti	Green	3.65
6.	NGR-EK-006	Ishapa Toromoyan	Omuo/ Ilasha	Ekiti	Green	3.55
7.	NGR-OS-007	Sapa	Iwo/ Ibode Osi	Osun	Green	3.35
8.	NGR-OS.008	Isapa	Ikoyi/ Ikire	Osun	Green	3.35
9.	NGR-OS-009	Isapa	Ilaorangun	Osun	Green	3.50
10.	NGR-KW-010	Ishapa	Oro	Kwara	Green	3.10
11.	NGR-KW-011	Ishapa	Offa	Kwara	Green	3.35
12.	NGR-NG-012	Emagi	Bida	Niger	Deep red	3.60
13.	NGR-NG-013	Emagi	Dabban/ Lavun	Niger	Red	3.35
14.	NGR-NG-O14	Emagi	Mokwa	Niger	Light red	3.30
15.	NGR-NG-015	Ama	Beji/ Bosso	Niger	Green L.V	3.50
16.	NGR-NG-016	Ama	Paiko/Paikoro	Niger	Green	3.45
17.	NGR-NG-017	Yakuwa	Kontagora	Niger	Pinkish G.L	3.20
18.	NGR-KD-018	Yakuwa	Kubau	Kaduna	Red	3.33
19.	NGR-KD-019	Barkatata	Sanga	Kaduna	Red	3.25
20.	NGR-KD-020	Yakuwa	Jema'a	Kaduna	Green	3.32
21.	NGR-KD-021	Zoborodo	Zaria	Kaduna	Red	3.27
22.	NGR-KD-022	Zobo	Chukun	Kaduna	Red	3.48
23.	NGR-KD-023	Zobo	Kachia	Kaduna	Red	3.29
24.	NGR-KD-024	Tseng	Jaba	Kaduna	Green	3.36
25.	NGR-KD-025	Zobo	Kagarko	Kaduna	Red	3.31
26.	NGR-JG-026	Bakin zobo	Kazaure	Jigawa	Deep red	3.24
27.	NGR-JG-027	Jan zoborodo	Gumel	Jigawa	Red	3.21
28.	NGR-JG-028	Jan zobo	Kaugama	Jigawa	Red	3.29
29.	NGR-JG-029	Farin zobo	Kazaure	Jigawa	Green	3.16
30.	NGR-JG-030	Farin zoborodo	Hadejia	Jigawa	Green	3.48
31.	NGR-JG-031	Bakin zobo	Kaugama	Jigawa	Red	3.34
32.	NGR-GB-032	Bakin zobo	Yamaltu-Deba	Gombe	Deep red	3.31
33.	NGR-GB-033	Barkata/ Gwaten	Dadin Kowa	Gombe	Green	3.28
34.	NGR-GB-034	Jan zobo	Kwani	Gombe	Red	3.41
35.	NGR-FCT-035	Emagi zuru	Yaba	FCT	Deep red	3.80
36.	NGR-FCT-036	Megi	Kuchi Goro (Amac)	FCT	Red	4.20
37.	NGR-FCT-037	Ama	Dakwa (Bwari)	FCT	Green	2.90
38.	NGR-FCT-038	Echi	Zuba	FCT	Deep red	3.70
39.	NGR-NS-039	Ogbomwa zobo	Eddo (Doma)	Nasarawa	Red	4.00
40.	NGR-NS-040	Echi zobo	Kiyi (Akwanga)	Nasarawa	Red	3.00
41.	NGR-NS-041	Yakwan Miya	Keffi	Nasarawa	Green	3.00
42.	NGR-PL-042	Yakuwa	Jos	Plateau	Red	3.90
43.	NGR-PL-043	Yakuwa	Jos	Plateau	Green	3.10
44.	NGR-BA-044	Farin Zobo	Toro	Bauchi	Green	3.00
45.	NGR-BA-045	Yakuwa/Bakin Zobo	Bauchi	Bauchi	Deep red	3.48
46.	NGR-BA-046	Yakuwa/Janzobo	Toro	Bauchi	Red	3.34
47.	NGR-BA-047	Farin Zobo	Gamawa/Katagun	Bauchi	Green	3.31
48.	NGR-BE-048	ASHWE	Gboko	Benue	Deep red	3.28
49.	NGR-BE-049	ASHWE	Gboko	Benue	Red	4.00
50.	NGR-BE-050	ASHWE	Yandev	Benue	Green	4.00
51.	NGR-TR-051	Farin zobo	Ardo Kola	Jalingo	Green	3.00

Table 1 Contd.

52.	NGR-TR-052	Jan Zobo	Karim Lamido	Jalingo	Deep red	3.41
53.	NGR-TR-053	Yakwa/Bakin zobo	Ardo Kola	Jalingo	Deep red	3.46
54.	NGR-KG-054	AGOLO	Ankpa	Kogi	Light red	4.00
55.	NGR-KG-055	AGOLO	Ankpa	Kogi	Red	4.00
56.	NGR-SK-056	YAKUWA	Sokoto	Sokoto	Deep red	3.29
57.	NGR-SK-057	JAN ZOBO	Sokoto	Sokoto	Red	3.16
58.	NGR-KT-058	YAKUWA	Katsina	Katsina	Deep red	3.48
59.	NGR-KT-059	YAKUWA	Katsina	Katsina	Green	3.34
60.	NGR-KB-060	YAKUWA	Birnin Kebbi	Kebbi	Deep red	3.31

* Values are means of the seeds measured in millimetre. Green L.V: Green. FCT: Federal Capital Territory. GREEN (L.V): late variety Pinkish (G.L): Pinkish calyces with green leaves.

Table 2. Predominant calyx colours among the accessions collected.

	Calyx colours				Total
	Green	Red	Deep Red	Others	
No. of accession	25	19	12	4	60
Calyx colour (%)	41.7	31.7	20.0	6.7	100.1

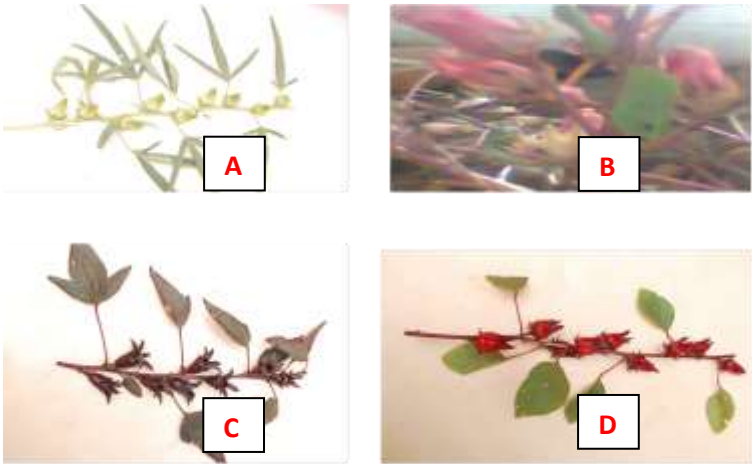

Plate 1. Roselle accessions with different calyx colour. A: Accession with green calyx; B: Accession with light red or pink calyx; C: Accession with deep red calyx; D: Roselle accession with red calyx.

towns and villages. The various calyx colours encountered were green, red, deep red, pink and light red (Plate 1). Results showed that 41.7% of these accessions were having green calyx, while 31.7% were with red calyx. On the other hand, 20.0% of the accessions possessed deep red calyx while only 6.7% had light red and pink calyx. Collections from the North Central, North Eastern, North Western and South Western parts were replicated over states, towns and villages in Nigeria (Table 2). The highest number of Roselle accessions was collected from Kaduna state (8 accessions) followed by Niger and Jigawa States (6 accession each); FCT and Bauchi State on the other hand have 4 accessions each (Table 1). This is an indication that these states had the greatest diversity of the crop genetic resource; it also showed that these regions might be the primary or secondary centre of origin of Roselle. This is in line with the report of Mohamed et al. (2012) that the genus *Hibiscus* has its centre of origin in Africa.

About 81.8% of the farmers preferred Roselle variety with dark red or red calyx because apart from having medicinal value, it is widely used in the preparations of

foods and drinks. This variety is grown in commercial quantities in Jigawa, Kaduna, Bauchi, Niger States and FCT. According to Stevels (1990), Roselle plants with anthocyanin pigmentation are able to withstand the harsh environment and more tolerant than the green variety. Hence, they are common in the dry zones of the areas of the production in Nigeria.

The farmers in the south-western part of Nigeria gave more priority to their starchy stable crops. 100% of them responded that they normally grow the green varieties of Roselle for vegetable. They also attend to this vegetable only when their main food crop has been established. The Roselle variety with green calyces is predominant in the south-western part of Nigeria. In view of the popularity of Roselle as a crop of considerable economic importance in Nigeria, there is a need to retain the diversity of the indigenous germplasm. A scientific morphological and molecular characterization of the materials collected is therefore necessary to ascertain the genetic diversity existing within the species in Nigeria.

Conflict of interest

The authors did not declare any conflict of interest.

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African Journal of Food Science

Full Length Research Paper

Alphonso mango conservation through exposure to gamma radiation

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Dispensation of fresh produce especially fruits through radiation, involves exposure to short wave energy to achieve a specific purpose *viz.* reduce the weight loss and extend the ripening. An experiment was carried out to study the effect of irradiation and storage conditions in Alphonso mango on physiological weight loss and ripening. The experiment was laid out in completely randomized block design with factorial concept with three repetitions. The fruits were exposed to gamma radiation for different doses from the source of ^{60}Co at Board of Radiation and Isotope Technology, Bhabha Atomic Research Centre, Mumbai. There were sixteen treatment combinations of irradiation dose (I_1 -0.00, I_2 -0.20, I_3 -0.40 and I_4 -0.60 kGy) and storage temperature (S_1-Ambient, S_2-9°C, S_3-12°C and S_4-CA storage (12°C, O_2 2%, CO_2 3%). The data indicated that the fruits irradiated with 0.40 kGy gamma rays (I_3) recorded significantly minimum percent reduction in physiological loss in weight and extended the ripening. Similar pattern was noted when fruits were kept at 9°C storage temperature. Combined effect of 0.40 kGy gamma rays irradiation and 9°C storage temperature (I_3S_2) also recorded maximum reduction in the physiological loss in weight and ripening percent throughout the storage period.

Key words: Gamma rays, *Mangifera indica* L., postharvest, ^{60}Co, refrigeration.

INTRODUCTION

Mangoes (*Mangifera indica* L.; family Anacardiaceae) are known as luxuries and expensive fruit in the markets of many industrialized countries. Asia accounts for 77% of global mango production and the Americas and Africa account for 13 and 19%, respectively (Yadav and Parmar, 2014). India is the global leader in mango production (Yadav and Patel, 2013). The high cost of mangoes in importing countries is due primarily to air freight charges. Mature fruits may take from three to eight days to ripen and this short period certainly limits the commercialization at long distance. Sea

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transport is less expensive and enables transportation of larger volumes and it thus would aid in the expansion of mango export industries. At the present stage of development, however, sea shipment does not guarantee good quality fruit on arrival for successful marketing. Mangoes are classified as climacteric fruits and ripen rapidly after harvest. Mango is generally harvested when physiologically mature and ripen under suitable conditions of air temperature and humidity. Therefore, if freshly harvested fruit is allowed to ripen at normal ambient conditions (this can vary between 22-32°C and 40-65% RH), ripening processes increase rapidly within the week (Yadav et al., 2013a).

The ripe fruit may stay edible for a few days thereafter. It is because of this fact that fruits must be stored under specific storage conditions, not only to maintain weight loss but also to prolong ripening. Mango is susceptible to chilling injury and an optimum air temperature of 12-13°C is generally recommended (Gomez-Lim, 1993). Irradiation of fruits has been successfully shown to delay ripening (Pimentel and Walder, 2004). Irradiation is also a physical process for the treatment of foods akin to conventional process like heating or freezing. It prevents food poisoning, reduces wastage to contamination and at the same time preserves quality (Mahindru, 2009). Therefore, the new knowledge is critical because it is important to maintain a balance between the optimum doses required to achieve safety and the minimum change in the quality of the fruit. In view of the above fact, it becomes quite clear that investigation for mango fruit is very important not only to reduce the ripening but also control the weight loss. So, irradiation can be used in combination with low temperature to assess the effects of different doses of gamma irradiation and storage temperature on reduction of the physiological loss in weight and ripening phenomena of fruit. The loss in weight of fruits is likely to reduce the quality of fruit drastically.

Therefore, one of the main objectives of any post harvest treatment is to reduce the extent of physiological loss in weight. The Alphonso variety of mango is famous for its excellent table fruit quality. This is a leading commercial mango cultivar of India and Pakistan and is taking position in the export market. The ripen fruits have attractive colour, and excellent sugar : acid blend. To study the combined effect of irradiation dose and storage temperature on prevention of mango was the main objective of study.

MATERIALS AND METHODS

The research was conducted during the months of June-July, in the year 2010 at Cold Storage, Post-Harvest Technology Unit and the laboratory of N. M. College of Agriculture, Navsari Agricultural University, Navsari (Gujarat), India. Export grade mangoes of cv. Alphonso were harvested on 12th June 2010. The selected mangoes from class I as per the quality parameters were specified and described in “post harvest manual for mangoes” published by APEDA (Anon., 2007). There were sixteen treatment combinations of irradiation dose (I_1 -0.00, I_2 -0.20, I_3 -0.40 and I_4 -0.60 kGy) and storage temperature (S_1-Ambient, S_2-9°C, S_3-12°C and S_4-CA storage at 12°C). The fruits were exposed to gamma radiation for different doses from the radio isotope ^{60}Co at ISOMED plant, Board of Radiation and Isotope Technology, Bhabha Atomic Research Centre, Mumbai. The fruits were packed in CFB box and kept at various temperatures, that is, at ambient, 9°C (90% RH), 12°C (90% RH) and at control atmospheric storage (12°C, O_2 2%, CO_2 3% and RH 90%). The dimensions of CFB box were 370 x 275 x 90 mm and gross weight of box with fruits was 3.0 kg. One CFB box had nine fruits for each treatment, each treatment was replicated thrice. The individual fruit weight was from 250-350 g. During the irradiation process, the fruit boxes were kept in the irradiation chamber through a conveyor system. The source rack with its ^{60}Co pencils was raised from the storage pool by automated control system. The product packages are then moved around the radiation source in such a way that they are exposed to radiation for predefined time. Transportation of fruits for irradiation at BARC was carried out in an air conditioned vehicle. Four fruits from each treatment of each replication were earmarked during the investigation for measuring physiological loss in weight. Fruits were weighted on first day of treatment and subsequently their weight was recorded at six day interval up to the end of shelf life. The physiological loss in weight (PLW) was expressed in percentage and calculated as proposed by Yadav et al. (2013a).

$$\text{PLW percent} = W_1 - W_2 / W_1 \times 100$$

Where W_1 = initial weight and W_2 = final weight.

The ripening was measured by the number of fruits having change in colour from greenish to yellow and soft in texture which were counted at six day intervals up to eating ripeness and expressed in percentage over total number of fruits taken for study. After 12th day of storage, few treatments had 0.00 values for PLW and ripening due to completion of their shelf life and unripening, therefore, individual mean value of irradiation and storage temperature was different from the original. So, only interaction effect was interpreted. Statistical analysis of the two year data was done by following the Fisher’s analysis of variance technique (Panse and Sukhatme, 1967) at Information Technology Centre, Department of Agricultural Statistics, N. M. College of Agriculture, Navsari Agricultural University, Navsari. Values were expressed as the mean standard deviation. The statistical significance of the differences was examined using analysis of variance. Results at probability value of less than 0.05 were considered signififcant.

RESULTS AND DISCUSSION

The data indicated that the physiological loss in weight of fruits increased with the advancement of storage period and significantly influenced by irradiation, storage temperature and their interaction. It is evident from Figure 1 that the physiological loss in weight was significantly influenced by various treatments of gamma rays. Consistently and significantly, minimum PLW was observed in treatment I_3 (0.40 kGy) at 6th, 12th, 18th, 24th, 30th and 36th days of storage, that is, 2.155, 3.856, 5.790, 3.810, 2.410 and 2.900 percent, respectively and the maximum PLW was observed in I_1 (0.00 kGy) at different storage conditions. Similarly, storage temperature affects the PLW and Figure 2 shows that the significantly minimum PLW was observed in treatment S2 (9°C) at 6th, 12th, 18th, 24th, 30th and 36th day of fruit storage (1.095, 2.209, 3.270, 4.670, 6.070 and 5.120%, respectively). Whereas, maximum PLW percent was recorded in fruits kept at ambient temperature (S¬1) at different storage conditions. Interaction between irradiation and

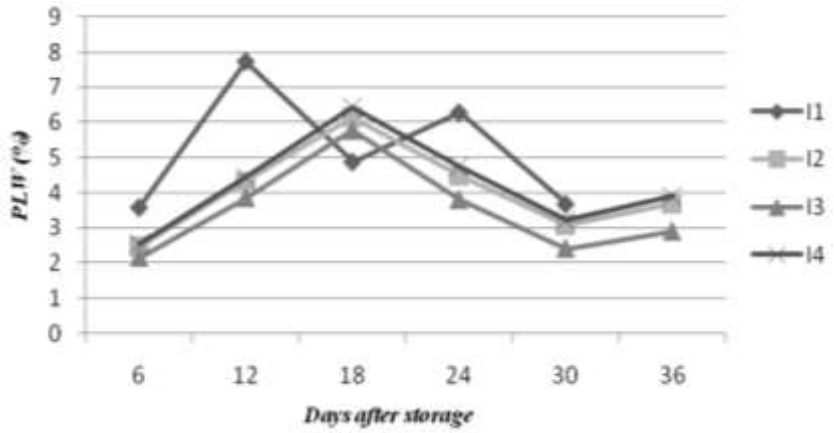

Figure 1. Exposure of gamma rays influenced PLW (%) of Alphonso mango at different days of storage.

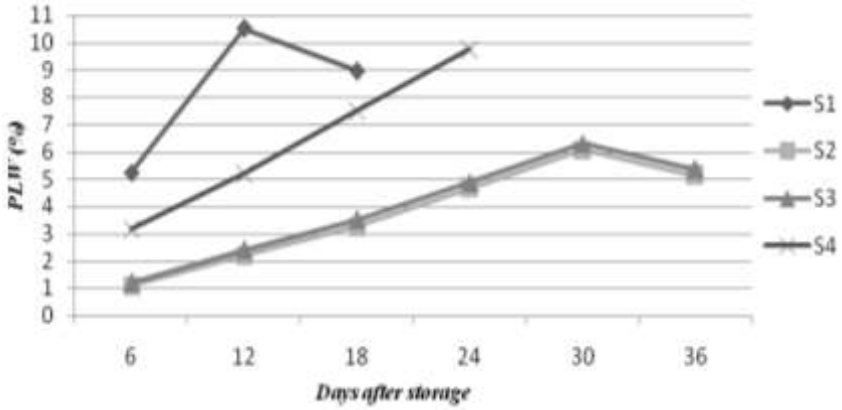

Figure 2. Storage condition influenced PLW (%) of Alphonso mango at different days of storage.

storage temperature was found significant and irradiated fruits significantly reduced the PLW over unirradiated fruits at all conditions of the storage. The shelf life extended up to 36 day, and on this day, only fruits exposed to 0.40 and 0.60 kGy irradiation and stored at 9°C has shelf life and the minimum reduction in PLW percent was recorded at six day interval in the fruits exposed with 0.40 kGy irradiation and stored at 9°C (I_3S_2), that is, 5.660% at 36th day, 4.720% at 30th day, 3.480% at 24th day, 2.750% at18th day, 1.777% at 12th day and 0.920 at 6th day of storage (Figure 2). The remaining treatments were discarded due to the lost of their shelf life at every stage of over ripening. The PLW was possibly on account of loss of moisture through transpiration and utilization of some reserve food materials in the process of respiration (Mayer et al., 1960). It is evident from the data that the physiological loss in weight of mango fruit was significantly influenced by the various exposed dose of gamma rays and different storage temperature (Figure 3). The irradiation significantly reduced PLW during storage period over control which might be attributed to reduction in utilization of reserve food material in the process of respiration (Purohit et al., 2009). The delay in respiration rate as a result of irradiation is also reported by Singh and Pal (2009) in guava. Similar findings were also observed by Yadav et al. (2013c), Prasadini et al. (2008) and El-Salhy et al. (2006) in mango. Similarly, in the different storage conditions, the highest physiological loss in weight was observed in fruits subjected to ambient temperature. Lower physiological loss in weight was noted in 9 and 12°C and in CA (12°C) storage temperature which might be due to lesser water vapour deficit as compared to ambient condition and the low temperature which had slowed down the metabolic activities like respiration and transpiration (Yadav et al., 2013b). The observations are in accordance with the results of Roy and Joshi (1989) and Waskar and Masalkar (1997) in mango; Nagaraju and Reddy (1995) and Aina (1999) in banana and Gutierrez et al. (2002) in guava.

The significant minimum reduction in PLW of mango fruits subjected to irradiation and stored at various temperatures, that is, at 9 and 12°C and in CA (12°C) might be due to the

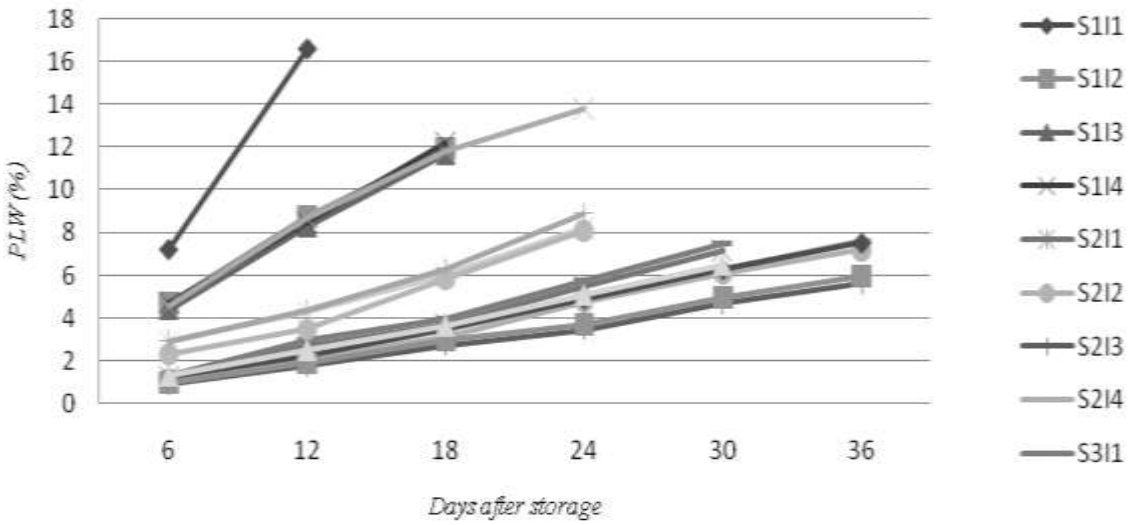

Figure 3. Combined effect of gamma rays and storage condition on Alphonso mango during storage.

Table 1. Irradiation and storage conditions influencing the ripening of Alphonso mango during storage.

Source	Ripening (%) days after storage														
	6					12					18				
	I_1	I_2	I_3	I_4	Mean	I_1	I_2	I_3	I_4	Mean	I_1	I_2	I_3	I_4	Mean
S_1	33.530 (35.374)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	8.380 (10.084)	97.480 (80.840)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	24.370 (21.450)	0.000* (1.65)	83.810 (66.250)	83.730 (66.182)	85.750 (67.794)	63.320 (50.471)
S_2	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)
S_3	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)
S_4	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	34.950 (36.227)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)	8.740 (10.297)
Mean	8.380 (10.084)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)		24.370 (21.450)	0.000 (1.654)	0.000 (1.654)	0.000 (1.654)		8.740 (10.297)	20.950 (17.803)	20.930 (17.786)	21.440 (18.189)	
Source	I		S		I X S	I		S		I X S	I		S		I X S
S.Em ±	0.003		0.003		0.006	0.003		0.003		0.007	0.014		0.014		0.029
CD ($P \leq 0.05$)	0.009		0.009		0.019	0.010		0.010		0.022	0.043		0.043		0.086

Table 1. Contd.

Source	Ripening (%) days after storage									
	24					30				
	I_1	I_2	I_3	I_4	Mean	I_1	I_2	I_3	I_4	Mean
S_1	0.00* (1.65)	0.00* (1.65)	0.00* (1.65)	0.00* (1.65)	0.00 (1.65)	0.00* (1.65)	0.00* (1.65)	0.00* (1.65)	0.00* (1.65)	0.00 (1.65)
S_2	0.00 (1.65)	0.00 (1.65)	0.00 (1.65)	0.00 (1.65)	0.00 (1.65)	83.43 (65.95)	53.21 (46.82)	50.05 (46.82)	65.18 (45.01)	62.97 (52.90)
S_3	41.57 (40.13)	0.00 (1.65)	0.00 (1.65)	0.00 (1.65)	10.39 (11.27)	97.82 (81.47)	58.16 (49.68)	51.93 (49.68)	74.03 (46.08)	70.48 (59.14)
S_4	98.26 (82.39)	79.56 (63.09)	75.05 (60.01)	83.57 (66.06)	84.11 (67.89)	0.00* (1.65)	0.00* (1.65)	0.00* (1.65)	0.00* (1.65)	0.00 (1.65)
Mean	34.96 (31.46)	19.89 (17.01)	18.76 (16.24)	20.89 (17.76)		45.31 (37.68)	27.84 (24.95)	25.49 (23.60)	34.80 (29.12)	
Source	I		S		I X S	I		S		I X S
S. Em ±	0.02		0.02		0.03	0.01		0.01		0.02
CD ($P \leq 0.05$)	0.05		0.05		0.10	0.03		0.03		0.06

Figures in parenthesis indicate ARC SINE transformed value, *Indicate fruits completely discarded, I = irradiation, S = storage temperature.

mutual complementary effect of irradiation and low temperature (Yadav et al., 2013a). Table 1 Indicates that the ripening percentage of fruits were influenced by the advancement of storage period and significantly affected by irradiation, storage temperature and their interaction. Irradiated fruits significantly delayed the ripening process over unirradiated fruits at all conditions of the storage. Irradiated fruits still did not fully ripe up to 30th day of storage, stored at 9°C. On this day, fruits exposed to 0.40 kGy recorded minimum unripe fruits, similarly, fruits stored at 9°C showed only 63.14 ripening in fruits. The remaining treatments were discarded due to the lost of their shelf life. The fruits exposed to gamma rays (0.20 and 0.40 kGy) and stored at 9 and 12°C had more than 50% unripe (I_3S_2 and I_3S_3) on 30th day of storage, they showed 50.05 and 51.93% ripening, respectively. The remaining of the treatments had high ripening or discarded due to completion of their shelf life.

The fruits exposed to gamma rays and stored at 9 (S_2) and 12°C (S_3) storage temperature as well as unirradiated fruits kept at 9°C remained unripe (I_1S_2) at 24th day of storage (Figure 3). Unirradiated and irradiated fruits kept at ambient temperature were discarded due to the end of their shelf life and the remaining were under ripening. At 18th day of storage, unirradiated fruits were discarded due to full ripening at ambient conditions, remaining unripe or showed minute ripening. At 6th and 12th day of storage, ripened fruits (33.530 and 97.480%, respectively) were observed in unirradiated fruits stored at ambient temperature (I_1S_1). The remaining of the treatments was fully unripe. Ripening percentage is a physiolo-gical process which designates the period from harvest until the fruits attain the stage of maximum consumer acceptability. The unirradiated mangoes early showed ripeness whereas, gamma rays exposed mangoes had significantly delayed ripening. The possible mechanisms that have been postulated include: a) irradiations results in decreased sensitivity to ripening action of ethylene and b) alteration in carbohydrates metabolism by regulating certain key enzymes, which interfere with production of ATP which is required for various synthetic processes during ripening (Udipi and Ghugre, 2010). Same findings were noted by Yadav and Patel (2014) and Farzana and Panhwar (2005) in mango and Aina et al. (1999) in banana. The lower and delayed ripening was noted at 9 and 12°C and in CA (12°C) storage as compared to ambient temperature. The decrease in ripening percent and increase in days for ripening at low temperature may be due to

desirable inhibition of enzymatic activities leading to reduction in the respiration and ethylene production (Mane, 2009). These results are supported by Mann and Singh (1975) in mango and Deka et al. (2006) in banana. The minimum and delayed ripening in fruits is due to exposure to gamma rays and storage temperature at 9 and 12°C and in CA (12°C) storage as compared to fruits unirradiated and kept at ambient temperature in the present study which might be due to the joint balancing effect of irradiation and low temperature (Yadav and Patel, 2014).

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Multivariate analysis of nutritional diversity of selected macro and micro nutrients in pearl millet (*Pennisetum glaucum*) varieties

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Analysis on economically viable indigenous food cereals like pearl millet as alternative strategies to curb under nutrition and boost food security is of utmost importance to widen the essential nutrient sources for human beings. To contribute to this area, macro and micro nutrient analysis was carried out on 60 pearl millet genotypes. On each of the genotype, 7 biochemical parameters (starch, amylose, amylopectin, protein, K, Zn and P) were analyzed. Starch content of the genotypes ranged from 27 - 46.7% with a mean of 34.2%, while most of the genotypes had more amylopectin than amylose with exceptions of a few varieties with a ratio of 2:1. The protein content had a range of 4.6 - 9.9% with a mean of 7.1%. Zinc was among the highest level followed by phosphorous and finally potassium. The principal component analysis (PCA) showed that the first four PCA contributed to 79.8% of the variability among the pearl millet varieties. Cluster analysis grouped data into 6 clusters and a singleton with a genetic distance 0.37 – 8.73 showing great variability. Biochemical traits are useful tool for determining genetic variability in pearl millet and can contribute to breeding programs and enhance food security.

Key words: Nutritional contents, food security, breeding, principal component analysis, genetic distance, cluster analysis.

INTRODUCTION

Millet has been cultivated since the pre-historic ages in areas of North Africa and Central Asia. The whole grain is used in soups, stews or as a cooked cereal. Millet can also be popped; roasted or sprouted (Ronzio, 2004). Africa was the largest producer of millet in 2009 (20.6 million metric tonne), followed by Asia 12.4 million metric tons and India 10.5 million metric tons (FAO, 2009). Pearl millet is one of the most extensively cultivated cereals in the world, after rice, wheat and sorghum, and particularly in arid to semi-arid regions. Pearl millet is so important

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that it is estimated to be planted on around 14 million hectares in Africa and 14 million hectares in Asia (FAO, 2009).

Pearl millet (*Pennisetum typhoideum*) is the most widely grown type of millet because of its tolerance to difficult growing conditions such as drought, low soil fertility and high temperature, areas where other cereal crops, such as maize (*Zea mays*) or wheat (*Triticum aestivum*), would not survive (Maqbool et al., 2001). It is widely grown as a multi-purpose cereal grain crop principally for food, and also for feed, fodder, fuel and mulch on more than 26 million hectares, primarily in arid and semi-arid regions of India and Africa (FAO, 2000).

Indigenous foods like pearl millet are rich and inexpensive sources of protein, carbohydrates, dietary fibre, minerals and vitamins to millions of peoples in developed and developing countries, and are some of the basic foods of the indigenous populations of Africa (Luthria and Pastor-Corrales, 2006). Nutritionally, pearl millet is comparable and even superior to major cereals with respect to energy value, proteins, fat and minerals. It makes an important contribution to human diet due to high levels of calcium, iron, zinc, lipids and high quality proteins. Besides, it is also a rich source of dietary fiber and micro nutrients (Anu Sehgal and Kwatra, 2006; Malik et al., 2002). Carbohydrate components of pearl millet grains comprise of starch, dietary fiber and soluble sugars. Starch which consists of glucose in form of amylose and amylopectin is a predominant component of pearl millet endosperm. Pearl millet grains are all very high in calories- precisely the reason they do wonders for growing children and pregnant women (www.icrisat.org).

Micronutrient malnutrition can be defined as deficiency in one or more vitamins and minerals of importance for human health. It is an outcome of inappropriate dietary composition and disease (Nube and Voortman, 2006). Dietary micronutrient deficiencies affect a large part of the global population.

The World Health Organization estimates that globally some two billion people are affected by iron deficiency and that some 750 million people suffer from iodine deficiency (WHO, 2006; Unicef, 2006). Also, zinc deficiency is increasingly recognized as an important public health problem (Ramakrishnan, 2002; Black, 2003a, b). First, among poor populations, overall food intakes are often below minimum requirements and as a result not only the intake of macronutrients (carbohydrate, fat, protein), but also the consumption of micronutrients (minerals, trace elements and vitamins) can be inadequate (Nube and Voortman, 2006).

More than two billion people are reported to be iron deficient, which makes iron deficiency the most widespread human micronutrient deficiency in the world (Rengel et al., 1999). In recent years, interest in the occurrence of human zinc deficiency, in particular among children, has been growing strongly (Hotz and Brown, 2004).

Evaluating genetic diversity of germplasm can assist in differentiating varieties with the greatest novelty which as a result, is most desirable for the incorporation into crop improvement programs. Genetic diversity refers to the variation of heritable characteristics present among alleles of genes in different individuals of populations of species that serves as an important role in evolution by allowing a species to adapt to a new environment (Weir, 1996; Kremer et al., 1998).

The estimation of genetic distance using phenotypic and/or molecular markers can help determine suitable germplasm for incorporation into future plant breeding programs. Thus, assessment of genetic diversity in pearl millet germplasm and determination of their phenotypic and biochemical activities would help to know the breeding potential of a particular variety.

Quantitative assessment of genetic diversity is significantly important to determine the extent of genetic differences between and within crop species (Adugna, 2002). Genetic distances are measures of the average genetic divergence between two sequences, species or between populations within a species or taxa (Souza and Sorrells, 1991). Genetic similarity is the converse of genetic distances, that is, the extent of gene similarities among cultivars.

Genetically diverse parents produce high heterotic effects and yield desirable segregates. The pattern of genetic relationships between and within accessions can be shown by multivariate analyses. Cluster analysis on the other hand is a useful statistical tool for studying the relationships among closely related accessions. Therefore, the objective of this study was to evaluate and identify the quantities of particular macro and micro nutrients of pearl millet varieties and their suitability in food security and breeding.

MATERIALS AND METHODS

Sample preparation

The accessions evaluated were collection of open pollinated varieties (OPVs), commercially released varieties in East and Central Africa, local varieties and hybrids. These 60 pearl millet varieties sourced from ICRISAT, Kenya were grown in two sites Marigat (KARI –Perkerra) and Koibatek (Agricultural Training Centre, ATC-Koibatek) in Central Kenya region for two seasons. ATC- Koibatek lies at latitude 1° 35' S, and longitude 36° 66' E, altitude 1890 m a.s.l. in agro-ecological zone UM4, with low agricultural potential.

Average annual rainfall is 767 mm; mean annual minimum and maximum temperature are 10.9 and 28.8°C, respectively. KARI Perkerra-Marigat lies at a latitude of 1°45´ N and longitude 36°15´ E with an altitude 1067 m.a.s.l. The centre is situated in agro ecological zone 5 (LM5), soils are volcanic fluvisols of sandy/silty clay loam texture, slightly acidic to slightly alkaline. Annual rainfall mean is 654 mm. Mean annual minimum and maximum, temperatures are 32.4 and 16.8°C, respectively, and under field evaluation, the yield range from 3482 -1305 kg ha^{-1}. These varieties were powdered and analyzed in duplicate in their biochemical characteristics.

Determination of protein content

Finely milled pearl millet grain of 0.1 g were weighed and transferred into a digestion tube. Selenium catalyst mixture weighing 1 g was mixed with the samples and 5 ml of concentrated sulphuric acid (96%) was added into the tube. The tubes were then heated cautiously in the digestion apparatus, at the fume cupboard until the digest was clear. The sample was transferred to a 100 ml volumetric flask, and distilled water was added into 100 ml graduated flask upto the mark. Boric acid indicator solution of 5 ml was then transferred to 100 ml conical flask containing 5 drops of mixed indicator and was placed under the condenser of the distillation apparatus. 10 ml of the clear supernatant liquid of the digest was then transferred into the apparatus, and then 10 ml of 46% sodium hydroxide was added and rinsed again with distilled water. Distillation was then commenced. After the first distillation, drops reached the boric acid indicator solution, colour changed from pink to green. A total of 150 ml of the distillate was collected. The solution was titrated with 0.0174 N sulphuric acids until the colour changed from green to pink.

Determination of starch content

Powdered sample of 0.25 g was homogenized in hot 80% ethanol to remove sugars. The residue was then centrifuged and retained. The residue was dried well over a water bath. To the residue, 5.0 ml of distilled water and 6.5 ml of 52% perchloric acid was added, and then extracted at 0°C for 20 min. The supernatants were centrifuged, pooled and made up to 100 ml. A quantity of 0.1 ml of the supernatant was pipetted out and made up to the volume of 1 ml with distilled water. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and the volume made up to 1 ml in each tube with water. An amount of 4 ml of anthrone reagent was then added to each tube and sample heated for 8 min in a boiling water bath. Sample was cooled rapidly and the intensity of green to dark green colour was read using a spectrophotometer at 630 nm. The glucose content in the sample was determined using the standard calibration graph, and then the value was multiplied by a factor of 0.9 to arrive at the starch content.

Determination of amylose content

Powdered sample of 0.1 g was weighed, and 1 ml of distilled ethanol added followed by 10 ml of 1 N NaOH. The sample was heated for 10 min in a boiling water bath. The volume was made up to 100 ml. The extract taken was 2.5 ml and 20 ml of distilled water was added followed by three drops of 0.1% phenolphthalein. Drop wise HCl 0.1 N was then added until the pink colour just disappeared. 1 ml iodine reagent was added till the volume was 50 ml and the colour read at 590 nm using a spectrophotometer. Standard amylose solution 0.2, 0.4, 0.6, 0.8 and 1 ml was taken and the colour developed as in the case of the test samples. The amount of amylose present in the sample was calculated using the standard graph.

Determination of mineral content

A powdered sample of 0.1g was weighed and put into a dry, clean and labeled digestion tube. 5 ml of digestion mixture was added to each tube and also to 2 reagent blanks for each batch of samples. The sample was then digested at 360°C for 2 h after which the solution was clear. It was then allowed to cool. After cooling, 25 ml of distilled water was added and mixed well and left to cool again. The solution was then made up to 100 ml with distilled water and allowed to settle. Then, potassium and zinc was determined as follows, 4 ml of the wet digested sample solution was pipetted into a 100 ml volumetric flask, made to mark with distilled water and mixed well. The sample was then aspirated and directed into the atomic absorption spectrophotometer starting with the standards and blank solutions. The readings of the amount of the selected elements using their respective cathode lambs were recorded and the concentrations of the elements were determined by plotting a calibration curves.

Phosphorous was determined by taking 10 ml of the wet-ashed digestion solution into a 50 ml volumetric flask. 0.2 ml of 0.5% p-nitrophenol indicator solution was the added. The solution was made alkaline (yellow colour) by the addition of 6 N NH_3 solutions dropwise with gentle shaking followed by dilute 1 N HNO_3 dropwise until just colourless. Next, 5 ml of a mixture of ammonium molybdate/ammonium vanadate reagent was added. Finally, the solution was made to mark with distilled water and mixed well. It was then left for 30 min and the absorption measured using a U.V spectrophotometer and the concentration determined using a calibration curve.

Statistical analysis

All analyses were performed in duplicate (n = 2), the data was presented as means standard error of deviation (± SEM) and analysis of variance was determined at $p \leq 0.05$ level of significance. Correlations between different parameters were established using Pearson correlation coefficient. Multivariate analysis was undertaken using JMP statistical software, version 10. Principal component analysis (PCA) was used as a tool of data reduction to summarize the standardized data from biochemical composition analysis. The determination of genetic dissimilarity was Euclidean distance and the hierarchical agglomerative clustering method. Euclidean measure of distance was used for the estimation of genetic distance (GD) among varieties.

RESULTS AND DISCUSSION

Healthy diets contain an adequate and balanced combination of macronutrients (carbohydrates, fats and protein) and essential micronutrients (vitamins and minerals). Pearl millet is the major source of energy and protein for millions of people in Africa and has been reported that millet has many nutritious and medical functions (Obilana and Manyasa, 2002; Yang et al., 2012). In this study, starch content of the genotypes ranged from 27 - 46.7% with a mean of 34.2%. The first level of genotypes *Tsholotsho bearded*, *CIAKAUNGE-Vil3*, *ICMV 93771*, *KIRAKAORIGINAL, IP8772* and IP8773 had high amounts of starch contents that were not significantly different from 41.6 - 46.7%, *ICMA 00111 X SUDAN 11, ICMA 00888 X MIXTURE (KIRAKA/GACALIVIL2)*, *ICMA 00888 X HSD 2163* genotypes had the lowest starch of 27.6, 27.2, and 27.0 respectively. Foods with a low glycemic index like pear millet starch are useful to manage maturity onset diabetes, by improving metabolic control of blood pressure and plasma low density lipoprotein cholesterol levels due to less pronounced insulin response (Asp, 1996).

Most of the genotypes had less amylose than amylopectin with exceptions of a few varieties with a ratio of 1: 2. The amylase content ranged from 5.3 - 21.6% with a

mean of 11.5%. Genotypes *IP8766*, *ICMA 00111 X SUDAN 11*, *IP 10470*, *IP 8783*, *DEMBI YELLOW*, *SDMV 90031*, *SDMV 94014*, and *ICMV 221-1* had amylose levels of 16% and above, while *ICMA 93222 X HSD 2163*, *ICMA 00888 X SIUKU Vil 4b*, and *CIAKAUNGE – Vil 3* had the lowest amounts (Table 1). The first five genotypes above had more amylose than its amylopectin contents and these are favorable for baking, and preparing snacks. ANOVA showed that the first level of amylopectin *CIAKAUNGE-Vil3*, *ICMV 93771*, *KIRAKA ORIGINAL* and *Tsholotsho bearded* had amounts of 38.7, 36.2, 33.5 and 32.8% that were not significantly different. *ICMA 00111 X SUDAN 11* had the lowest content of 8.0% (Table 1).

The protein content had a range of 4.6 - 9.9% with a mean of 7.1% which is similar to studies done by Chethan and Malleshi (2007) and Singh and Raghuvanshi, (2012) who reported an average of 5 – 8 and 7%, respectively. *SDEA 4L-17 X HSD 7193*, *SDEA 4L-17 X CIAKAUNGE Vil3*, *MIXTURE (KIRAKA/GACALI Vil 2), IP 10471*, *ICMV 221-1*, *ICMA 00888 X HSD 7193*, *DEMBI YELLOW*, *ICMV 221*, *CIAKAUNGE-Vil 3* and *ICMV 91450* had amounts of 8% and above. Adeola et al. (1995) showed that the essential amino acid profile of pearl millet protein had lysine, threonine, methionine, tryptophan and cystine more than in proteins of sorghum and corn. These varieties have good protein and thus are good for human and livestock feeds.

Pearl millet contains various essential micro nutrients needed by the body. There are wide fluctuations in the total mineral and trace elements contained in pearl millet, the biggest factor determining this is the nature of the soil it is grown in. These minerals are required in the human body for numerous functions in the body. The genotypes *ICMV 221-1*, *GACAATIVIL-6 (ICRISAT), DEMBI YELLOW*, *SDMV 90031*, *KIRAKA ORIGINAL* and *SOSATC 88* exhibited the highest levels of phosphorous that were not significantly different. The phosphorous content ranged from 28.3 -1593.0 ppm with a mean of 362.1 ppm. This is slightly higher than those found by Singh and Srivastava (2006) who reported that the finger millet phosphorus content ranged from 130 to 295 mg% with a mean value of 180.43 mg%. Phosphorus is an essential component of adenosine triphosphate (ATP), a precursor to energy in the body and also they are precursors of nucleic acids that make up the genetic code of organisms (Liang et al., 2010; Devi et al., 2011).

Zn deficiency is common in underdeveloped countries and is mainly associated with malnutrition, affecting the immune system, wound healing, the senses of taste and smell, and impairing DNA synthesis (Zago and Oteiza, 2001). Zn seems to support normal growth and development in pregnancy, childhood and adolescence. Zn has been recognized to act as an antioxidant by replacing metals that are active in catalyzing free radical reactions, such as Fe (Oteiza et al., 2004). *ICMA 00888 X HSD 7193* had significantly high zinc content of 1345.5 ppm while *GACAATIVIL-6 (ICRISAT)* exhibited the lowest amount of 57.0 ppm. The other genotypes had zinc contents below 378 ppm. The range was from 57.1 - 1345.5 ppm with a mean of 193.4 ppm. This particular variety can be explored in breeding programs so as to develop varieties with high zinc content.

Potassium is important to keep the body parts running smoothly and is involved in maintaining water and electrolyte balance and regulating nerve and muscle functions (Oniango et al., 2003). The potassium levels in most genotypes had no big significant difference. The range was from 13.6–432 ppm with a mean of 160.4 ppm. *Tsholotsho bearded*, *ICMA 00888 X HSD 7193, 863 A X HSD 3508*, and *ICMA 93222 X DEMBI YELLOW* are some of the genotypes with high potassium while *GACAATIVIL-6 (ICRISAT), MIXTURE (KIRAKA/GACALI Vil 2)* and *CMA 93222 X ICMV 221* had the lowest potassium levels. Determining the amounts of various micro and micro nutrients in pearl millet is essential to ascertaining its importance in food security.

Correlation studies showed a significant positive correlation of 0.78, $p \leq 0.05$ between starch and amylopectin. Amylose also exhibited a positive association with phosphorous 0.28, $p \leq 0.05$. The other parameters had no significant relationship with each other. These associations hint on the possible genetic associations. The macro and micro nutrient analysis showed significant variations between the genotypes analyzed and this was also reported by Singh and Raghuvanshi (2012). Hence, finger millet has shown good potential to supply these much needed nutrients to help curd food insecurity.

Principal component analysis

The genetic diversity of 60 pearl millet varieties was observed for their biochemical makeup which is a requirement for the pre-selection of varieties for future breeding programs for better varieties to enhance food security. The principal component analysis grouped the characteristics into starch, amylose, amylopectin, Zn, Ph and K that accounted for the entire (100%) variability, however only four principal components were significant. According to Hair et al. (1998) Eigen value greater than 1 are considered significant and component loadings greater than ± 0.3 were deemed meaningful. As a result, only the first four principal components were used for the study and traits with loadings greater than ±0.3 were taken to represent the corresponding principal axis.

PC1 (principal component 1) alone explained 28.4% of the total variety among the varieties and was mainly due to the influence of the carbohydrates that is starch, amylose and amylopectin with amylose having a negative loading. The sign indicates the direction of the relationship between the components and the variables (Johnson, 1998). The 2nd principal component accounted for 20.4% of the total variation was predominantly a function of starch, amylose, potassium, zinc and phosphorous all with

Table 1. Proximate analysis of macro and micro nutrients of 60 pearl millet genotypes.

Genotype	Starch (%)	Amylose (%)	Amylopectin (%)	Protein (%)	P - ppm	Zn- ppm	K - ppm
Tsholotsho bearded	46.6^{a}	13.8^{defghijkl}	32.8^{abcd}	6.1^{cde}	341.3^{fghijkl}	61.0^{gh}	432.1^{a}
CIAKAUNGE - Vil 3	45.3^{ab}	6.5^{qr}	38.7 ^{a}	9.9^{a}	119.4^{ijklm}	161.8^{defgh}	34.7^{qrstu}
ICMV 93771	44.4^{ab}	8.2^{nopqr}	36.2^{ab}	7.6^{abcd}	285.8^{ghijklm}	135.2^{defgh}	148.1^{fghijklmnopqrstu}
KIRAKA ORIGINAL	41.7^{bc}	8.2^{nopqr}	33.5^{abc}	7.6^{abcd}	1181.2^{bc}	195.9^{bcdefgh}	72.6^{opqrstu}
IP 8773	41.6^{bc}	12.8^{efghijklmn}	28.7^{cdefg}	5.3^{de}	135.2^{ijklm}	178.9^{cdefgh}	124.1^{fghijklmnopqrstu}
IP 8772	39.9^{cd}	10.1^{ijklmnopqr}	29.7^{bcdef}	6.1^{cde}	293.7^{fghijklm}	288.5^{bcd}	124.2^{fghijklmnopqrstu}
OKOA	39.8^{cd}	9.8^{jklmnopqr}	29.9^{bcde}	4.5^{e}	935.6^{cd}	284.1^{bce}	213.4^{cdefghijklm}
ICMV 96603	39.5^{cde}	11.5^{ghijklmnop}	28.0^{cdefgh}	6.1^{cde}	48.1^{lm}	113.4^{defgh}	35.0^{qrstu}
ICMV 91450	39.2^{cdef}	9.7^{klmnopqr}	29.4^{bcdef}	9.1^{ab}	182.8^{hijklm}	107.3^{defgh}	299.6^{abcd}
IP 8767	38.6^{cdefg}	14.7^{bcdefghij}	23.8^{efghijklmnop}	5.3^{de}	400.7^{fghi}	259.6^{bcdef}	44.0^{qrstu}
IP 8766	38.4^{cdefg}	21.6^{a}	16.8^{opqrst}	7.6^{abcd}	159.0^{hijklm}	147.4^{defgh}	226.5^{bcdefghij}
SDMV 96063	38.4^{cdefg}	11.0^{ghijklmnopq}	27.4^{cdefghi}	6.1^{cde}	135.2^{ijklm}	285.0^{bcde}	234.8^{bcdefghi}
IP 10470	38.4^{cdefg}	19.5^{ab}	18.8^{lmnopqrst}	7.6^{abcd}	376.9^{fghijk}	161.0^{defgh}	220.9^{cdefghijk}
IP 8764	38.2^{cdefg}	11.3^{ghijklmnopq}	26.9^{cdefghijk}	6.1^{cde}	151.1^{hijklm}	219.0^{bcdefgh}	248.7^{bcdefg}
SIUKU Vil 4B	38.0^{cdefgh}	11.2^{ghijklmnopq}	26.7^{cdefghijk}	7.6^{abcd}	270.0^{ghijklm}	250.0^{bcdefg}	82.1^{mnopqrstu}
IP 10471	38.0^{cdefgh}	12.9^{efghijklm}	25.0^{efghijklm}	8.3^{abc}	309.6^{fghijklm}	65.3^{fgh}	254.7^{bcdef}
KAT PM 2	37.2^{defghi}	10.8^{ghijklmnopq}	27.0^{cdefghij}	6.8^{bcde}	258.1^{ghijklm}	367.0^{bc}	233.1^{bcdefghi}
Okashani 2	37.2^{defghi}	10.1^{ijklmnopqr}	26.3^{cdefghijkl}	6.8^{bcde}	115.4^{ijklm}	119.5^{defgh}	208.0^{cdefghijklmn}
Tsholotsho	36.5^{defghij}	12.4^{efghijklmno}	24.0^{efghijklmnop}	6.1^{cde}	285.8^{ghijklm}	245.7^{bcdefgh}	94.0^{jklmnopqrstu}
ICMV 221 BRISTILED	36.1^{defghijk}	10.6^{ghijklmnopq}	25.5^{defghijklm}	7.6^{abcd}	198.6^{hijklm}	233.0^{bcdefgh}	65.0^{pqrstu}
GACAATI VIL -6 (ICRISAT)	35.2^{efghijkl}	10.7^{ghijklmnopq}	24.4^{efghijklmn}	6.8^{bcde}	238.3^{hijklm}	378.4^{b}	225.0^{bcdefghij}
IP 8783	35.2^{efghijkl}	18.6^{abcd}	16.6^{pqrst}	7.6^{abcd}	1391.2^{ab}	57.0^{h}	23.7^{tu}
KIRAKA Vil -b Vil - 1 (Irunduni)	35.1^{fghijkl}	11.9^{fghijklmnop}	23.2^{efghijklmnopq}	7.6^{abcd}	36.2^{m}	136.5^{defgh}	156.2^{efghijklmnopqrst}
IP 7390	35.1^{fghijkl}	14.2^{cdefghijk}	20.8^{ijklmnopqrst}	5.3^{de}	289.8^{fghijklm}	63.1^{gh}	235.2^{bcdefghi}
SDMV 94014	34.9^{fghijkl}	16.5^{bcdef}	18.4^{mnopqrst}	7.6^{abcd}	373.0^{fghijk}	246.1^{bcdefgh}	110.0^{hijklmnopqrstu}
SDMV 90031	34.8^{ghijkl}	16.6^{bcdef}	18.1^{mnopqrst}	6.1^{cde}	1359.5^{ab}	165.8^{defgh}	191.3^{defghijklmnop}
SHIBE	34.6^{ghijklm}	14.8^{bcdefghi}	19.7^{jklmnopqrst}	7.6^{abcd}	218.4^{hijklm}	173.6^{defgh}	32.6^{rstu}
IP 6791	33.7^{hijklmn}	13.6^{efghijklm}	20.1^{ijklmnopqrst}	6.8^{bcde}	87.7^{jklm}	160.1^{defgh}	86.5^{klmnopqrstu}
NKIRIGACHA Vil 8	33.2^{ijklmno}	8.8^{mnopqr}	24.4^{efghijklmn}	7.6^{abcd}	60.0^{lm}	139.6^{defgh}	64.3^{pqrstu}
ICMA 00888 X HSD 7193	33.2^{ijklmnop}	11.8^{fghijklmnop}	21.3^{ghijklmnopqrs}	8.3^{abc}	590.9^{ef}	1345.5^{a}	356.4^{ab}
DEMBI YELLOW	32.9^{ijklmnop}	17.3^{abcde}	15.5^{rstu}	8.3^{abc}	1343.7^{ab}	244.4^{bcdefgh}	138.2^{fghijklmnopqrstu}
SOSAT C 88	32.8^{jklmno}	9.8^{klmnopqr}	23.0^{efghijklmnopqr}	6.8^{bcde}	1173.3^{bc}	172.7^{defgh}	243.1^{bcdefh}
ICMV 221-1	32.6^{jklmno}	16.4^{bcdef}	16.1^{qrstu}	8.3^{abc}	1593.3^{a}	234.8^{bcdefgh}	203.4^{cdefghijklmno}
KITHARAKA Vil 9	32.5^{jklmno}	9.5^{klmnopqr}	23.0^{efghijklmnopqr}	7.6^{abcd}	163.0^{hijklm}	226.0^{bcdefgh}	196.2^{defghijklmnop}
ICMV 221	32.3^{jklmno}	8.8^{mnopqr}	23.5^{efghijklmnopq}	9.1^{ab}	107.5^{ijklm}	161.0^{defgh}	28.9^{stu}
ICMA 93222 X HSD 2163	32.1^{jklmno}	7.0^{pqr}	25.1^{efghijklm}	6.1^{cde}	83.7^{jklm}	127.8^{defgh}	219.0^{cdefghijkl}
KAIGONGI	31.8^{klmnopq}	7.5^{pqr}	24.3^{efghijklmno}	6.1^{cde}	123.4^{ijklm}	107.7^{defgh}	84.0^{lmnopqrstu}
ICMA 00888 X SERERE - IRAMBA	31.3^{lmnopqr}	8.9^{lmnopqr}	22.4^{fghijklmnopqrs}	6.8^{bcde}	277.9^{ghijklm}	203.8^{bcdefgh}	168.9^{defghijklmnopq}
SIUKU Vil 4a	31.3^{lmnopqr}	9.00^{lmnopqr}	22.3^{fghijklmnopqrs}	7.6^{abcd}	210.5^{hijklm}	147.4^{defgh}	128.4^{fghijklmnopqrstu}
ICMA 93222 X KIRAKA Vil 1	31.3^{lmnopqr}	11.0^{ghiklmno}	20.2^{ijklmnopqrst}	6.8^{bcde}	198.6^{hijklm}	121.7^{defgh}	288.0^{bcde}
MIXTURE (KIRAKA / GACALI Vil 2)	31.2^{lmnopqr}	7.8^{opqr}	23.4^{efghijklmnopq}	8.3^{abc}	147.1^{hijklm}	186.3^{cdefgh}	28.0^{stu}
863 A X FS VARIETY	31.2^{lmnopqr}	10.9^{ghijklmnopq}	20.2^{ijklmnopqrst}	4.5^{e}	230.3^{hijklm}	234.3^{bcdefgh}	142.9^{fghijklmnopqrstu}
ICMA 93222 X DEMBI YELLOW	30.9^{lmnopqr}	10.0^{ijklmnopqr}	20.9h^{ijklmnopqrst}	6.8^{bcde}	91.7^{jklm}	128.7^{defgh}	332.8^{abc}
KIRAKA Vil 1	30.9^{lmnopqr}	7.5^{pqr}	23.4^{efghijklmnopq}	7.6^{abcd}	325.4^{fghijklm}	251.8^{bcdefg}	67.6^{pqrstu}
SDEA 4L - 17 X HSD 7193	30.3^{mnopqr}	10.6^{ghijklmnopq}	19.7^{jklmnopqrst}	8.3^{abc}	186.8^{hijklm}	169.3^{defgh}	74.6^{nopqrstu}
KAT PM 1	30.3^{mnopqr}	15.1^{bcdefgh}	20.2^{ijklmnopqrst}	8.3^{abc}	135.2^{hijklm}	120.8^{defgh}	106.5^{ijklmnopqrstu}
ICMA 93222 X ICMV 221	30.3^{mnopqr}	10.0^{ijklmnopqr}	20.2^{ijklmnopqrst}	6.8^{bcde}	262.0^{ghijklm}	104.6^{defgh}	67.5^{pqrstu}
SDEA 4L - 17 X CIAKAUNGE Vil 3	30.3^{mnopqr}	10.0^{ijklmnopqr}	15.1^{stu}	6.8^{bcde}	115.4^{ijklm}	61.0^{gh}	13.6^{u}
863 A X HSD 3508	30.2^{mnopqr}	13.0^{efghijklmn}	17.1^{nopqrst}	6.8^{bcde}	210.5^{hijklm}	123.0^{defgh}	300.6^{abcd}
KIMBEERE	30.2^{nopqr}	8.6^{mnopqr}	21.5^{ghijklmnopqrs}	7.6^{abcd}	75.8^{jklm}	95.0^{defgh}	159.1^{efghijklmnopqrs}
ICMA 93222 X KAT PM 1	30.0^{nopqr}	10.9^{ghijklmnopq}	19.1^{lmnopqrst}	7.6^{abcd}	75.8^{klm}	370.6^{bc}	142.7^{fghijklmnopqrstu}

Table 1. Contd.

ICMA 93222 X CIAKAUNGE Vil - 3	29.9^{nopqr}	10.4^{ijklmnopqr}	19.5^{klmnopqrst}	6.1^{cde}	83.7^{jklm}	83.2^{efgh}	231.9^{bcdefghi}
253 X 254/KOG X NKIRIGACHA VIL 8	29.8^{nopqr}	9.5^{klmnopqr}	20.3^{ijklmnopqrst}	6.8^{bcde}	440.3^{fgh}	63.6^{gh}	166.7^{defghijklmnopqr}
ICMA 00888 X SIUKU Vil 4b	29.6^{nopqr}	5.32^{r}	24.3^{efghijklmno}	6.8^{bcde}	182.8^{hijklm}	109.0^{defgh}	225.2^{bcdefghij}
ICMA 93222 X OKOA	29.4^{opqr}	9.1^{lmnopqr}	20.3^{ijklmnopqrst}	6.8^{bcde}	28.3^{m}	161.8^{defgh}	257.0^{bcdef}
253 X 254/KOG X PMV 3	28.9^{opqr}	15.3^{bcdefg}	13.65^{tu}	6.8^{bcde}	547.3^{fg}	152.2^{defgh}	208.7^{cdefghijklmn}
ICMA 93222 XICMV 221-1	28.9^{pqr}	10.2^{hijklmnopqr}	18.6^{mnopqrst}	5.3^{de}	384.9^{fghij}	241.7^{bcdefgh}	117.4^{ghijklmnopqrstu}
ICMA 00111 X SUDAN 11	27.6^{qr}	18.9^{abc}	8.6^{u}	7.6^{abcd}	880.1^{cde}	161.4^{defgh}	168.7^{defghijklmnopq}
ICMA 00888 X MIXTURE (KIRAKA /GACALI VIL 2)	27.2^{r}	9.6^{klmnopqr}	17.5^{nopqrst}	7.6^{abcd}	856.3^{de}	190.7^{bcdefgh}	94.1^{jklmnopqrstu}
ICMA 00888 X HSD 2163	27.0^{r}	10.2^{ijklmnopqr}	16.8^{pqrst}	7.6^{abcd}	365.0^{fghijk}	102.0^{defgh}	143.0^{fghijklmnopqrstu}

Means with the same letter in the same column are not significantly different.

Table 2. Principal component analysis of starch, amylose, amylopectin, proteins, phosphorous, zinc and potassium in pearl millet varieties showing Eigen vectors, Eigen value and their percentage contribution to the total variations explained in the first two principal component axes.

Variables	PC1	PC2	PC3	PC4
Starch	0.50	0.50	0.13	-0.29
Amylose	-0.44	0.44	0.02	-0.36
Amylopectin	0.69	0.15	0.10	-0.02
Protein	-0.02	-0.22	0.74	0.16
Phosphorous	-0.27	0.30	0.44	-0.37
Zinc	-0.09	0.36	0.33	0.68
Potassium	-0.09	0.52	-0.35	0.40
Eigenvalue	1.99	1.43	1.14	1.02
Individual (%)	28.43	20.47	16.30	14.62
Cumulative (%)	28.43	48.90	65.19	79.81

positive loadings. Therefore varieties with high PC2 scores would have high amounts of these parameters. The 3rd principal component with 16.2% variance separated these varieties on protein, phosphorous, zinc, potassium with potassium having a negative loading. PC4 accounted for 14.6% of the variation that was attributed to amylose, phosphorous, zinc and potassium with positive and negative loadings. Protein concentration was important in only PC3 showing that it had little contribution to the variation among varieties unlike amylose that contributed to variation in PC1, PC2 and PC4 (Table 2). The micronutrients contributed to the variation in the 2nd, 3rd and 4th principal components, they contributed to a larger percentage in the variation of these varieties

A plot of the PC1 and PC2 showed that *CIAKAUNGE Vil 3 (#E)*, *ICMA 00888 X HBD 7193* (#J), *ICMA 00111 X SUDAN 11* (#14), *ICMA 93771* (#Z) and *Tsholotsho bearded (#7)* were the most divergent from the majority group centered on zero. The bi-plot would give a breeder the ability to visualize the distances between the varieties and point out the best varieties to be selected depending on several variables compressed in the two major principal components. Their divergence was attributed to their high contents of starch and amylose for *ICMA 93771* (#Z) and *CIAKAUNGE Vil 3 (#E)*, high zinc for *ICMA 00888 X HBD 7193* (#J), high phosphorous content for *ICMA 00111 X SUDAN 11 (#14)*, and high potassium for *Tsholotsho bearded (#7)*. Varieties with close proximity in the score plot are similar; those near the origin are distinctive while those far from the origin are extremes. The varieties which overlap in the principal component axis had some relationships in the concentration of the used traits. These extremes varieties are favorable for breeding programs due to their biochemical difference from the rest which makes it unique. In Figure 1, the loading plot shows the similarities and differences between the biochemical parameters.

Principal component loadings plots classified the varieties into four quadrants based on the concentration of the minerals, proteins and carbohydrates analyzed in this study. The varieties scattered in 3 quadrants

Table 3. Summary of cluster means of 60 pearl millet varieties biochemical characteristics.

Cluster	Starch	Amylose	Amylopectin	Protein	Phosphorous	Zinc	Potassium	Mean
1.0	38.4	11.6	26.8	6.3	439.9	212.3	89.5	103.2
2.0	46.7	13.8	32.9	6.1	341.3	61.0	432.2	117.0
3.0	33.7	17.1	16.6	7.2	636.8	186.1	172.3	134.1
4.0	43.8	7.6	36.2	8.4	528.9	164.3	85.2	109.8
5.0	37.6	11.1	26.5	7.3	169.8	185.7	233.6	84.6
6.0	30.8	9.6	21.2	7.1	237.9	157.8	150.9	77.7
7.0	33.2	11.9	21.3	8.4	590.9	1345.6	356.4	296.8
Mean	37.7	11.8	25.9	7.3	420.8	330.4	217.2	

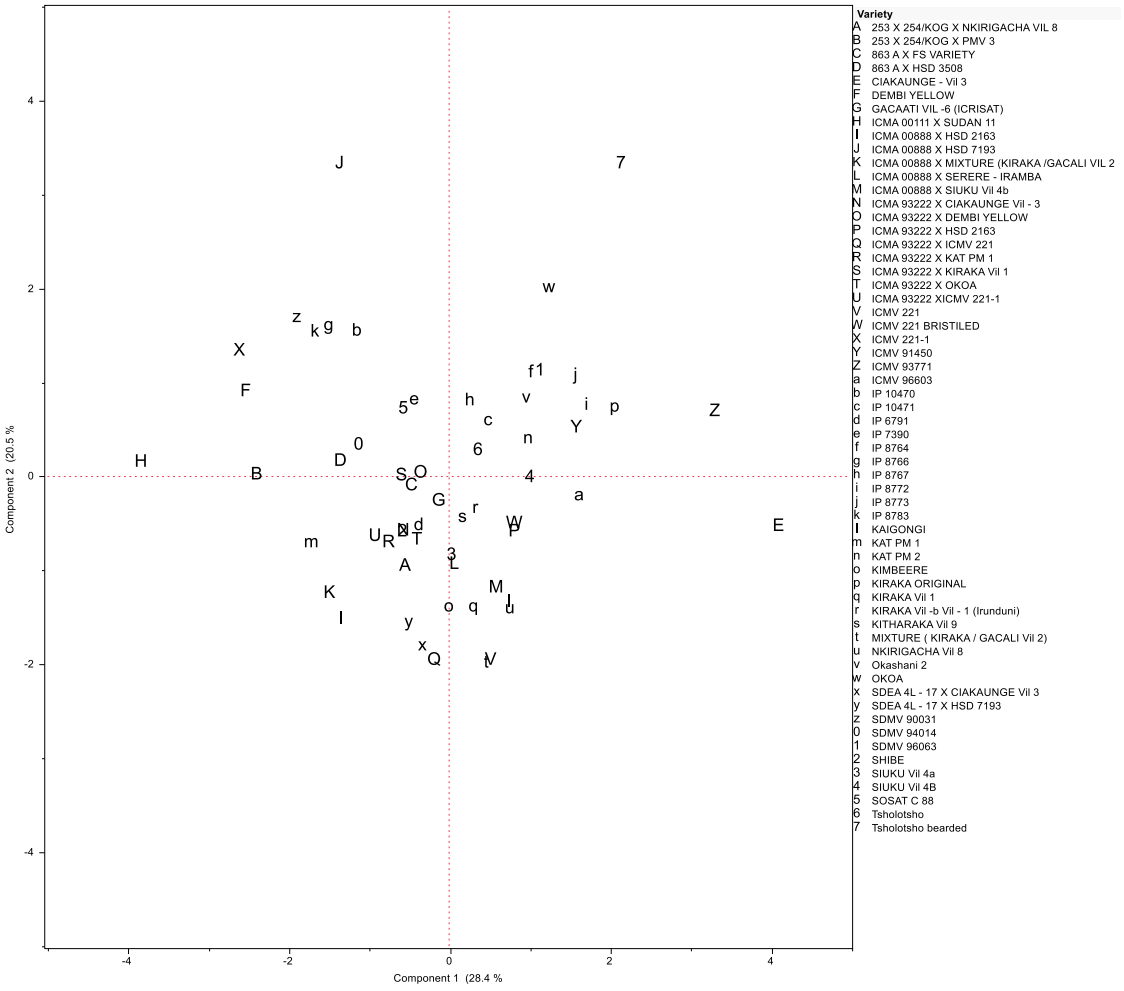

Figure 1. Principal component score plot of PC1 and PC2 describing the overall variation among pearl millet varieties estimated using biochemical character data.

demonstrating genetic variability in their composition. The varieties on the top left quadrant were related in amylose, phosphorous, zinc and potassium. The right top quadrant varieties were related in starch and amylopectin while the right bottom varieties did not show any associations in the measured traits. The left bottom varieties showed portrayed relations in their protein content. The distance between the locations of any two varieties on the score plot is directly proportional to the degree of similarity or difference between them in terms of their analyzed traits

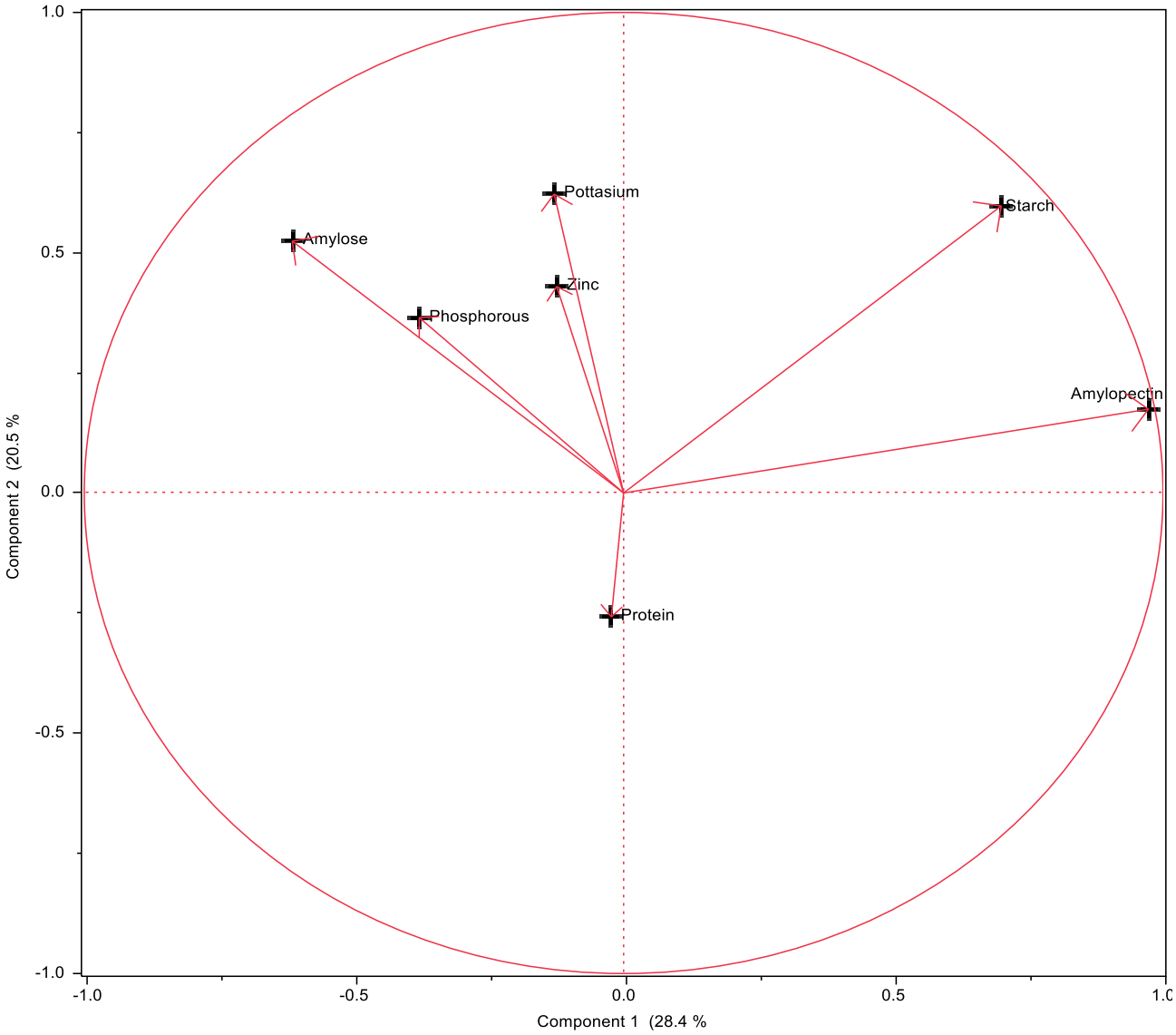

Figure 2. Principal component analysis loading plot for seven biochemical traits of 60 pearl millet varieties.

(Figure 2).

Cluster analysis and genetic distance

Estimates of genetic distance matrix was based on the nutritional traits for all pair wise combinations of (60×59)/2 =1770 for the 60 pearl millet varieties (data not shown). The observed genetic distance was from 0.37 - 8.73 pair wise combinations showing the diversity of the varieties in terms of their nutritional composition. The lowest genetic distance of 0.37 and 0.44 were recorded between *SDEA 4L 17 X HSD 7193* and *SDEA 4L – 17 X CIAKAUNGE Vil 3*, and between *IP 8764* and *SDMV 96063*, respectively. The highest genetic distance of 9.1 and 8.73 was between *CIAKAUNGE Vil 3* and *ICMA 00888 X HSD 7193* and between *Tsholotsho bearded* and *ICMA 00888 X HSD 7193*. The low genetic distance within the varieties points towards relatedness and thus confirms that there is enough genetic diversity in the measured mineral elements, carbohydrates and protein among the varieties despite the relatedness. The varieties with high genetic distance can be adopted for breeding programs.

Cluster analysis portrayed a clear differentiation between sorghum varieties. Table 3 reveal the difference among clusters by summarizing cluster means for the seven biochemical parameters. The highest cluster mean was recorded in phosphorous (420.8) and the lowest was in protein (7.3). Maximum cluster mean was recorded in cluster VII (296.8) and III (134.1). This showed the existence of maximum genetic divergence among the

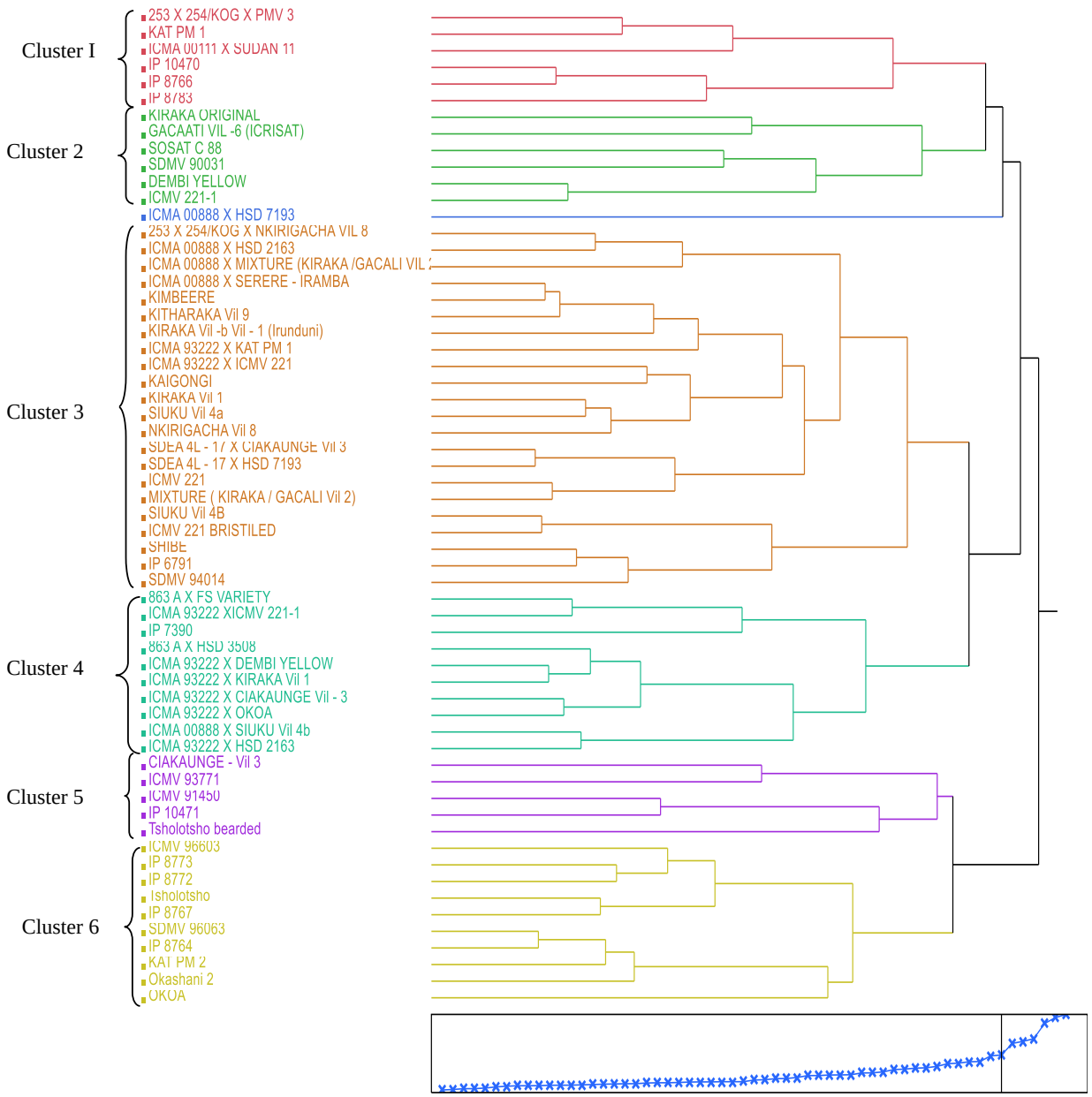

Figure 3. Hierarchical cluster dendrogram showing clusters 1 - 4 of 60 biochemical and morphological characteristics of pearl millet varieties.

varieties in these clusters. Based on these parameters, the varieties were grouped into clusters shown on the dendrogram. The dendrogram divided the varieties into 7 clusters and a singleton as shown (Figure 3). Cluster I was characterized by varieties with good amylose and phosphorous contents with other parameters in moderate amounts. Cluster II varieties were characterized by highest phosphorous and high amylose moderate amounts of starch and proteins. Cluster III had low starch, good protein and the lowest phosphorous amounts. Cluster IV had the high potassium and amylopectin, low starch and protein. Cluster V had the highest starch, protein, amylopectin and potassium. Cluster VI had the highest zinc, amylopectin and protein, medium phosphorous and low protein. Variety *ICMA 00888 X HSD 7193* was grouped as a singleton and this showed that it was dissimilar from other varieties in terms of its nutritional composition. As a result, this pearl millet has the capability of being adopted in plant breeding programs. The crossing of pearl millet varieties in different clusters will provide higher heterotic groups in breeding. Various authors including Shergo (2010)

demonstrated genetic diversity among sorghum varieties on the basis of their nutritional composition. In pearl millet quality improvement programs, it is vital to critically identify and quantify varieties to enhance their nutritional quality like minerals, proteins and carbohydrates.

Conclusion

Similar to many other cereals, pearl millets have high carbohydrate and other nutrients, making them useful components of dietary and nutritional balance in foods. The genotypes of the pearl millet analyzed, presented a broad variability in the studied contents and most of them are comparable to the contents found in the pearl millets cultivated worldwide. Dietary deficiencies, can be dealt with by encouraging the population to consume traditional foods like pearl millet especially women and children. Including these readily available cereals in the diet will improve nutrition status. Based on the observed variation for both qualitative and quantitative characteristics, it can be concluded that phenotypic diversity of pearl millet varieties is important to classify the genetic potential of varieties and increase the efficiency of the pearl millet breeding programs.

Conflict of interest

The authors did not declare any conflict of interest.

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Full Length Research Paper

Nutrients content, characterization and oil extraction from *Acrocomia aculeata* (Jacq.) Lodd. fruits

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Proximate composition, physical and chemical analysis of the pulp, kernel and oils of *Acrocomia aculeata* (Jacq.) Lodd. were investigated. The macauba pulp and the kernel represented 49 and 6.25% of the mass of the whole fruit, respectively. The main components present in the pulp and kernel are lipids (23.62 and 46.96%) and fibers (13.89 and 15.81%). Furthermore, the mineral analysis showed that the kernel had more micronutrients than pulp, principally Cu, Mn and Zn. The influence of extraction by pressing and Soxhlet extractor in different solvents regarding the quality and yield of the oils was evaluated. The highest yield in oil extraction was obtained by Soxhlet method, influenced by the type of solvent and part of macauba's fruit. For pulp, the better solvent is ethanol and ethanol 95%, and for kernel is ethanol, ethanol 95%, hexane and ethyl either. The analysis of the composition of fatty acids in the oil from the pulp and kernel showed that the monounsaturated fatty acids were predominant in the pulp oil, specially the oleic acid (C 18:1), and saturated fatty acids in the kernel oil, principally the lauric acid (C 12:0).

Key words: *Acrocomia aculeata* (Jacq.) Lodd., macauba oil, pulp, kernel, proximate composition, nutritional components, fatty acids.

INTRODUCTION

One of the major biomes of Brazilian biodiversity is the Cerrado, which harbors many native fruit trees and immense biological wealth, because of its climatic nuances and varied soils (Klink et al., 2005). In recent years, more than half of the Cerrado has been changed by deforestation (Ratter et al., 1997; Verburg et al., 2014). The exploitation of natural resources of this biome increases each year, therefore researchers have been increasingly seeking to study the potential of native plant species, for the development of new products, in order to foster a greater appreciation of plants with therapeutic effects and of fruits with important nutritional properties (Silva et al., 2009a). The macauba *Acrocomia aculeata* (Jacq.) Lodd., has a potential pharmaceutical, foodstuff and chemical applications among the fruit bearing species. *A. aculeata* (Jacq.) Lodd. is a palm tree belonging to the Arecaceae family (Caldas-Lorenzi et al., 2006), popularly known as macauba, bocaiuva, cocobabão, bacauva, mocajuba and macaiba.

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It is found in tropical regions and is abundant in the Brazilian Cerrado (Hiane et al., 2006). It bears fruit between the months of September and January depending on climatic conditions, planting site and maturation (Dessimoni-Pinto et al., 2010).

Macauba fruit is composed of epicarp, mesocarp, endocarp and endosperm. In the harvest time, the epicarp presents a yellowish color and an orange mesocarp or pulp.

The endocarp is hard and dense to protect the kernel, which is coated with a thin brown integument, and makes up the edible portion together with the pulp (Chuba and Sanjinez-Argandoña, 2011).

Pulp and kernel are consumed *in natura* or in ice creams, pastries, cakes and biscuits (Hiane et al., 2006; Bora and Rocha, 2004; Ramos et al., 2008). In the medicine folk, fruits of the macauba palm are used in the treatment of cardiovascular diseases and vitamin A deficiencies because of its high carotenoid content (Ramos et al., 2007).

Also, macauba contains galactoglucomannan which is a polysaccharide, reported to be a therapeutic agent in the prevention of inflammatory processes (Silva et al., 2009b).

Another interesting aspect of the fruit is the oleaginous potential. It can produce ten times more oil mass per hectare than traditional oilseed crops, such as soybeans (Roscoe et al., 2007). This potential makes its use beneficial in food products, pharmaceuticals, cosmetics, as well as in bioenergy.

Therefore, characterization of the fruit is interesting to improve its applicability. Considering this, the work aimed to characterize the fruit of *A. aculeata* (Jacq.) Lodd. regarding its biometric characteristics, nutritional composition and the physical and chemical properties of the pulp, kernel and oils.

MATERIALS AND METHODS

Harvesting and storage of fruits

Ripe fruits of *Acrocomia aculeata* (Jacq.) Lodd. were collected in the *Fundação MS,* in Maracaju, MS, Brazil latitude 21°36'52" and longitude 55°10'06", at an altitude of 384m, between December 2011 and January 2012, and transported to the Laboratory of Food Technology of the Federal University of Grande Dourados (UFGD). Fruits were selected to obtain a uniform batch regarding size and absence of injuries, and they were washed and sanitized with a solution of 0.66% sodium dichloroisocyanurate dihydrate (content of active chlorine 3%). Afterwards they were peeled, pulped, deseeded, and stored at -5°C until the use.

Physical and chemical characterization of fruits

The longitudinal and transversal diameters of 100 fruits were determined with the aid of a digital caliper (Mitutoyo). The mass of the whole fruit, peel (epicarp), pulp (mesocarp), endocarp and kernel, was determined in an analytical balance (Shimadzu-AUY220). Pulp and kernel were analyzed according to their pH, determined digital potentiometer, total soluble by solids, by refractometry, total titratable acidity, determined by titration with a solution of 0.1 N NaOH, moisture, by gravimetry in an oven at 105°C until constant weight, lipid content, determined by the Soxhlet method, ash, by gravimetry in a muffle furnace at 550°C, and carbohydrates according to the methods described by the Adolfo Lutz Institute (Lutz, 2008). Crude fiber was quantified by acid and alkaline hydrolysis and protein content by quantifying total nitrogen, determined by the microKjedahl method (AOAC, 1984).

Mineral levels were evaluated according to the methodology described by Salinas and Garcia (1985). The samples were crushed and homogenized, followed by organic digestion using a mixture of hydrochloric acid and hydrogen peroxide, both concentrated, at high temperatures, solubilizing macro and microelements. The elements were quantified by spectrometry, using the standard curve for each mineral. Concentration of calcium, iron, magnesium, manganese, zinc and copper were determined with an atomic absorption spectrophotometer (Varian-AA240FS) and acetylene gas.

Extraction and physical and chemical characterization of oils

The pulp and kernel were previously dehydrated at 40°C in a dryer (NG Scientific) with an air flow of 0.5 ms^{-1} for 72 h. The extraction of oil from the pulp and kernel was accomplished by two methods: (a) cold pressing in an "expeller" type press, model MPE-40P (Ecirtec) and the resulting oil centrifuged at 5000 rpm for 15 min; (b) by Soxhlet extraction, using different solvents (ethanol, ethanol (95%), methanol, ethyl ether, petroleum ether, hexane and acetone). The yields of both extraction methods were evaluated to evaluate the yield of extraction (Brumm et al., 2009).

The physical and chemical characterization of oils according to their density, iodine value, refraction index and acidity index, were carried out by official methods (AOAC, 1984) and standards of the Adolfo Lutz Institute (Lutz, 2008). To determine the viscosity of the oils, a Brookfield (Model LVDVIII +) viscometer was used equipped with a cylinder that has a Spindle of reference SC4-18. The viscometer was coupled to a thermostatic bath, which enabled measuring the viscosity of the oils at 40°C, with an accuracy in temperature of 0.5°C (Brock et al., 2008).

Characterization of the fatty acids, present in the oil, was carried out by the transmethylation. The reaction was performed according to the Hartman and Lago method (1973), using an ammonium chloride and sulfuric acid solution in methanol as esterifying agent. The treated samples were analyzed by a gas chromatograph (HP-6890), equipped with automatic sampler (HP-7683); split injector, 75:1 ratio; CP-SIL 88 capillary column (100 m x 0.25 mm i.d., 0.20 mm of film); and flame ionization detector (FID). The chromategraphic conditions were as follows: initial temperature of 120°C/2 min, heating from 120 to 220°C on a scale of 2.2°C/min and from 220 to 235°C; hydrogen carrier gas (flow rate of 1 ml/min); make-up gas, nitrogen at 30 ml/min; injector temperature of 270°C; detector temperature of 310°C; injection volume of 1 ml. The identification of fatty acids was performed by comparing the standard retention time of fatty acids with those of the sample. The quantification was performed by area normalization and the results were expressed in g/100 g of sample.

Statistical analysis

The results of oil extraction yields from the pulp and kernel of the macauba palm were submitted to analysis of variance (ANOVA), and to compare the means, the Tukey test was used ($p< 0.05$).

RESULTS AND DISCUSSION

Physical and chemical characterization of fruits

Table 1 shows the biometric characteristics of the fruits. The transversal and longitudinal diameters characterize the macauba as a rounded shape. The pulp represented 49%

Table 1. Biometric characteristics of the *Acrocomia aculeata* (Jacq.) Lodd. fruits.

Biometric characteristic	Present work	Reference Values	
		Hiane et al. (2006)	Chuba et al. (2011)
Transversal diameter (mm)	34.17 ± 2.32	-	33.39 ± 1.26
Longitudinal diameter (mm)	34.68 ± 2.62	-	34.68 ± 1.55
Whole Fruit (g)	22.07 ± 3.69	21.83 ± 2.48	21.83 ± 1.49
Peel (g)	4.22 ± 1.00	4.68 ± 1.07	4.54 ± 0.48
Pulp (g)	11.00 ± 2.19	9.61 ± 1.17	8.98 ± 1.07
Seed (g)	5.36 ± 0.97	6.72 ± 1.23	8.31 ± 0.61
Kernel (g)	1.38 ± 0.44	0.83 ± 0.23	1.35 ± 0.09

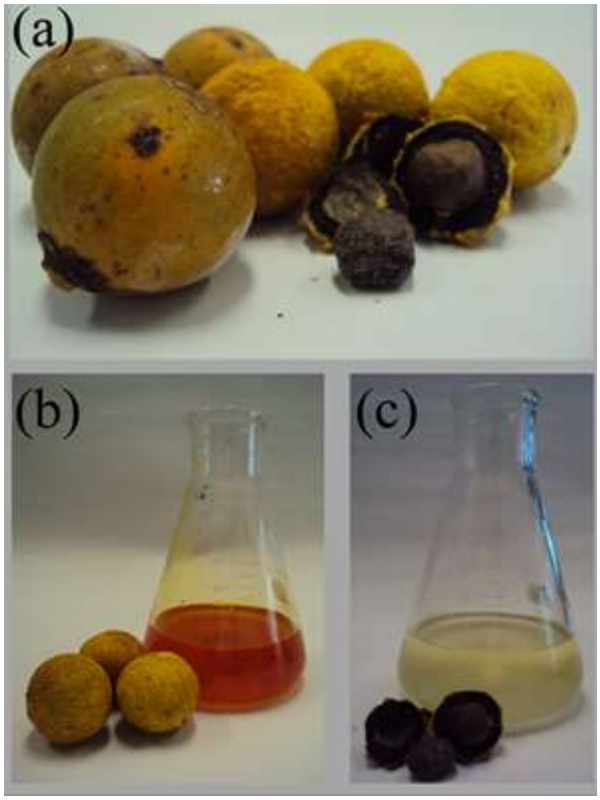

Figure 1. (a) Acrocomia aculeata (Jacq.) Lodd. fruits showing the whole fruit and its parts. **(b)** Macauba pulp oil. **(c)** Macauba kernel oil.

of the mass of the whole fruit (22.07±3.69 g), for this reason, its use in technology and agroindustry is potentialized. The other parts of the fruit were the peel (12%), endocarp (24.28%) and kernel (6.25%) (Figure 1). These values are similar to those obtained by other authors for the same species (Hiane et al., 2006; Chuba and Sanjinez-Argandoña, 2011). Machado et al. (2014) revealed lower values (10. 49 g) for pulp from macauba fruit collected in the Paraná state. This difference in the values of biometric fruits of the same species can be justified by edaphoclimatic difference between the two regions of harvest.

Components of pulp and kernel are shown in Table 2, and the main components observed were lipids and fibers, besides moisture and protein for kernel. Usually, palm tree fruits such as licuri (*Syagrus coronata* (Martius) Beccari), guariroba (*Syagrus oleracea*) and pindo (*Syagrus romanzoffiana*) have high lipid content (Coimbra and Jorge, 2011; Crepaldi et al., 2001). Although, it is less expressive, the protein content of the pulp was similar to the value found by Coimbra and Jorge (2011). On the other hand, the protein content of kernel was lower than that obtained by the same author and by other authors (Hiane et al., 2006;

Table 2. Nutritional components of the *Acrocomia aculeata* (Jacq.) Lodd. fruit.

Components	Pulp	Kernel
Moisture (%)	48.76 ± 1.92	12.87 ± 1.44
Ash (%)	1.50 ± 0.23	1.86 ± 0.43
Total lipids (%)	23.62 ± 1.10	46.96 ± 0.81
Proteins (%)	5.31 ± 0.77	16.44 ± 0.93
Carbohydrates (%)	6.92 ± 1.26	6.06 ± 0.40
Fibers (%)	13.89 ± 1.00	15.81 ± 0.30
Ascorbic acid (mg/100 g)	15.41 ± 0.34	1.18 ± 0.30
Titratable acidity (%)	0.27 ± 0.03	0.07 ± 0.00
pH	6.00 ± 0.00	6.00 ± 0.00
Water activity	0.988 ± 0.00	0.677 ± 0.00

Values are means ± SD (*n* = 3).

Table 3. Mineral levels in the pulp and kernel of the *Acrocomia aculeata* (Jacq.) Lodd. fruit.

Minerals	Pulp	Kernel
Macronutrient		
Calcium (mg g^{-1})	1.13 ± 0.03	0.92 ± 0.09
Magnesium (mg g^{-1})	1.23 ± 0.02	1.72 ± 0.07
Micronutrient		
Copper (µg g^{-1})	1.37 ± 0.01	15.80 ± 0.51
Manganese (µg g^{-1})	3.21 ± 0.02	18.78 ± 0.21
Iron (µg g^{-1})	41.34 ± 0.06	26.08 ± 1.49
Zinc (µg g^{-1})	< LQ	45.14 ± 0.84

Values are means ± SD (*n* = 3). LQ, limit of quantification = 3.33 µg g^{-1} (Zn).

Silva et al., 2008) for the same species. The quantification of carbohydrates content showed that both, pulp and kernel have similar relative values of these compounds, about six percent of its proximate composition. The fruits were characterized as slightly acidic according to its pH, which is 6.0 and its low acidity (0.27%). These results are similar to those found in the literature (Chuba and Sanjinez-Argandoña, 2011). The ascorbic acid content (15.41 mg/100 g) was higher than the licuri (*Syagrus coronata* (Martius) Beccari), which showed traces of vitamin C (Crepaldi et al., 2001), and higher than the buriti (0.7b mg/100 g) and bacuri (0.5 mg/100 g) (Barreto et al., 2009). The variations found in fruits of the same species can be attributed to the soil type, climatic conditions, harvesting period and other edaphoclimatic factors (Kim et al., 2003).

Table 3 presents the results of mineral composition found in the pulp and kernel of macauba fruits. Among the analyzed macronutrient elements, magnesium showed the highest concentration, followed by calcium. In the kernel, the Mg concentration is almost twice the Ca content.

Moreover, the Ca content found in this work (0.62 mg g^{-1}) is higher than the content found by Ramos and collaborators (2008). Although, the mineral concentration is less than the recommended daily intake for adults (Anvisa, 1998), the pulp of macauba can be considered a source of minerals. Whereas, it showed significant levels of some micronutrients, such as copper, iron and manganese, which participate in important protein transport ways, corroborating the importance of the intake of these nutrients. Furthermore, mineral deficiencies have been implicated as causes of several chronic diseases (Ba et al., 2009).

The main cellular electrolytes in the human body are sodium, potassium, magnesium, phosphate and, to a lesser degree, calcium. These nutrients are easily acquired by the ingestion of fruit as well as milk and its derivatives (Clereci and Carvalho-Silva, 2011). In this context, this study provides information that enhance the prospects of consumption of macauba fruits, in addition supports and enable future studies for prevention of malnutrition and degenerative diseases.

Extraction and physical and chemical characterization of oils

Figure 2 shows the methods evaluated and the values regarding the oil extraction yield from the pulp and kernel from macauba fruits. Among the conventional oil extraction methods, the high temperature extraction method (Soxhlet method), presented the greatest efficiency in yields. The results are compatible with those found in the literature. This is resulted of the immersion of the sample in the solvent and the flow of the solvent through the sample, increasing the effectiveness of the method (Brumm et al., 2009). However, cold pressing has some advantages. For example, no solvents are used, which minimizes the risk of intoxication of the handler and the environment (Pighinelli et al., 2008). In addition, it does not involve high temperatures, which can result in less loss of degradable compounds such as carotenoids and tocopherols (Coimbra and Jorge, 2012), because increased temperatures are associated with the degradation of bioactive compounds (Rodriguez-Amaya et al., 2008). The levels of oil in the pulp, ranged between 19.3 and 47.3%, indicating that the yield of oil depends on the solvent, given that some solvents are able to remove vitamins, steroids, resins and pigments as well as triacylglycerides (Galvão et al., 2008). In the kernel, the values varied from 35.4 to 46.9%. The extraction with hexane by Soxhlet method is commonly applied in research laboratories and it was used in this work to compare with the pressing method. Table 4 presents the physical and chemical characteristics of the oils from the pulp and kernel extracted by pressing and Soxhlet method. The density was kept at the same value (0.919 g cm^{-3}) for all of the analyzed oils; in other words, the extraction method makes no difference for the density of the oil. The values for the iodine value and refraction index observed in

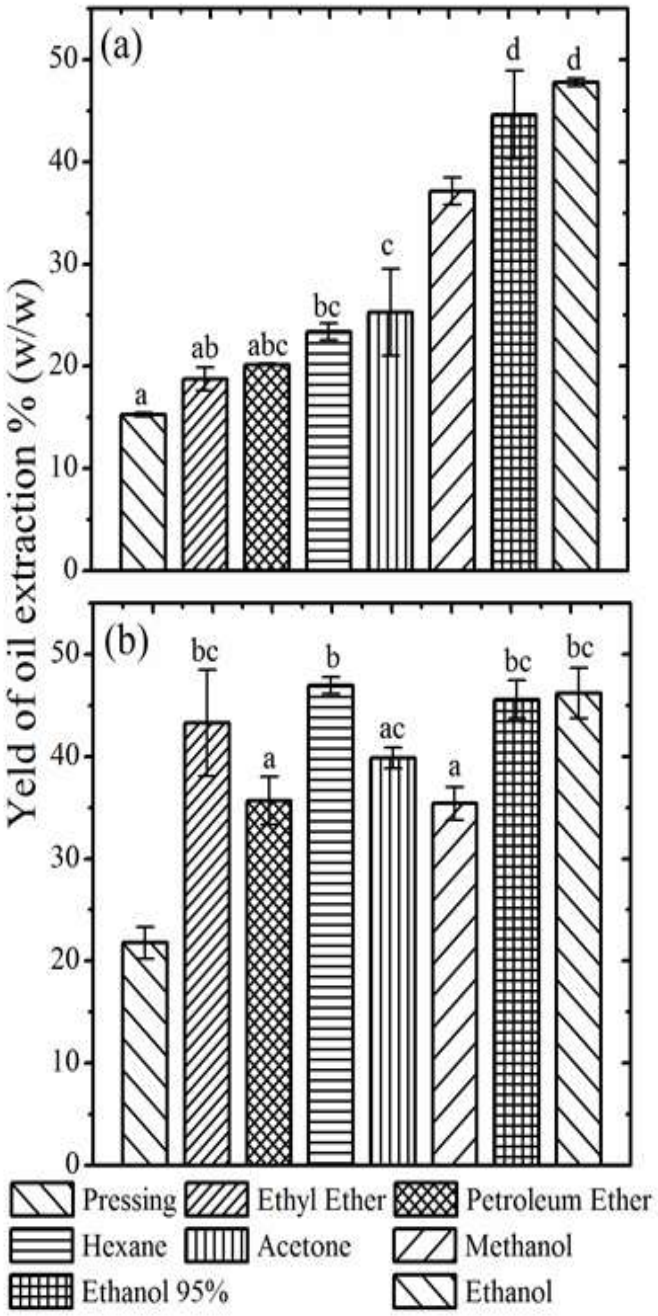

Figure 2. Yield of oil extraction from the pulp (a) and kernel (b) of the *Acrocomia aculeata* (Jacq.) Lodd. fruit. Same letters do not differ significantly among themselves at the level of 5% of Tukey's test. Values are means ± SD (n = 3).

the samples is typical for oils, as has been observed in other studies (Ferreira et al., 2008; Jorge et al., 2005). A significant difference can be seen in the determination of acidity value of oils from the pulp when compared with oils from the kernels, which shows that there are more acidic compounds in the pulp oil. However, the kernel oils presented the same determination of acidity values, independently of the extraction process. The same did not occur with the oils from the pulp, which presented higher acidity with the Soxhlet method than with pressing. The viscosity results showed that the extraction methods were similar with regard to this parameter for the kernel oils. However, the oil viscosity of the pulp oil obtained by pressing was 27% higher than the oil obtained by solvent. The explanation is based on the fact that, solvent extraction does not drag the water present in the sample.

Composition of fatty acids

The fatty acids found in the pulp and kernel of macauba´s oils are presented in Table 5. The characterization of fatty acids of the oil obtained by pressing (cold method) and hexane solvent were done. The pulp oil was composed of saturated fatty acids (25.01%) and unsaturated fatty acids (74.99%), of which 68.51% were monounsaturated and 6.48% polyunsaturated. For the kernel oil, the values for saturated fatty acids were 67.15% and for unsaturated fatty acids 32.85%, of which 29.39% were monounsaturated and 3.46% polyunsaturated.

Navarro-Díaz et al. (2014) analyzed the fatty acids from macauba and found similar values of this work, about 70% of the total composition of acids. Unsaturated fatty acids have important functions, such as maintaining the immune system in inflammatory processes and an anti-microbial effect (Menendéz et al., 2006; Weatherill et al., 2005; Debmandal and Mandal, 2011). The fatty acids profile of pulp and kernel evidenced the amount of polyunsaturated acids, thus it is possible to suggest that both oils in the present study are important sources of these bioactive compounds.

In Table 5, the predominance of oleic, palmitic and palmitoleic acids can be observed. The predominant poly-unsaturated fatty acids in the pulp were linoleic acids (5.46%) and linolenic acids (1.02%). Santoso et al., (1996) obtained values of 7.18% for linoleic acids and of 1.59% for linolenic acids in the oil from the *Cocos nuciferas* (Arecaceae), which is close to the values found in this study. Medicinal properties, such as antibacterial, antifungal, anti-viral, anti-parasite, antioxidant, hypoglycemic, immuno-stimulant and liver protecting effects have been attributed to the oil of *C. nuciferas*, which is correlated with its fatty acids (Debmandal and Mandal, 2011).

The linoleic fatty acid represents the omega 6 (ω-6) family and it is essential in a diet. Its deficiency results in adverse clinical symptoms, such as scaly skin rashes and reduced growth. It is a precursor of arachidonic acid, a component of the membrane of structural lipids (Hohl and Rosen, 1987). Linolenic acid, of the omega 3 family (ω-3), is a precursor of eicosapentaenoic acid and docosahexaenoic acid. A deficiency in linolenic acid in a diet can result in neurological abnormalities and poor growth. Among other functions, linoleic and linolenic acids are important for the formation of prostaglandins, thromboxanes,

Table 4. Physical and chemical characterization of the pulp and kernel oils of *Acrocomia aculeata* (Jacq.) Lodd.

Parameters	Pulp oil		Kernel oil	
	Solvent*	Pressing	Solvent*	Pressing
Density (g cm^{-3})	0.91 ± 0.00	0.91± 0.00	0.91± 0.00	0.91 ± 0.02
Iodine value (gI_2/100 g)	189.25 ± 3.70	182.37 ± 2.41	193.67 ± 1.84	195.95 ± 3.34
Acidity value (mg KOH/ g)	0.87 ± 0.00	0.97 ± 0.01	0.05 ± 0.01	0.07 ± 0.01
Refraction index (40ºC)	1.45 ± 0.00	1.46 ± 0.00	1.45 ± 0.00	1.45 ± 0.00
Viscosity (mPa.s)	29.00 ± 0.01	36.80 ± 0.08	27.90 ± 0.02	30.46 ± 0.02

Values are means ± SD (*n* = 3). *Hexane was the solvent employed.

Table 5. Composition of fatty acids in the oil from the pulp and kernel of the *Acrocomia aculeata* (Jacq.) Lodd. fruit.

Fatty Acids (mg/100 g)	Pulp oil		Kernel oil	
	Solvent*	Pressing	Solvent*	Pressing
Caproic acid (C 6:0)	0.24 ± 0.01	0.14 ± 0.01	0.36 ± 0.04	0.48 ± 0.00
Caprylic acid (C 8:0)	0.25 ± 0.01	0.14 ± 0.00	4.49 ± 0.37	5.71 ± 0.09
Capric acid (C 10:0)	0.16 ± 0.01	0.11 ± 0.01	3.85 ± 0.11	4.96 ± 0.13
Lauric acid (C 12:0)	0.85 ± 0.01	0.59 ± 0.00	38.98 ± 0.05	41.88 ± 1.61
Myristic acid (C 14:0)	0.61 ± 0.12	0.39 ± 0.01	8.84 ± 0.08	7.70 ± 0.60
Pentadecanoic acid (C 15:0)	0.04 ± 0.04	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.01
Palmitic acid (C 16:0)	17.65 ± 0.15	20.11 ± 0.26	7.22 ± 0.08	6.05 ± 0.19
Palmitoleic acid (C 16:1)	2.44 ± 0.11	2.56 ± 0.01	0.06 ± 0.01	0.04 ± 0.03
Heptadecanoic Acid (C 17:0)	0.08 ± 0.01	0.08 ± 0.01	0.05 ± 0.02	0.04 ± 0.01
Heptadecaenoic acid (C 17:1)	0.13 ± 0.01	0.11 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
Stearic acid (C 18:0)	3.15 ± 0.11	3.09 ± 0.34	3.01 ± 0.02	3.63 ± 0.01
Oleic acid (C 18:1)	70.28 ± 1.33	65.71 ± 1.05	29.13 ± 0.39	25.85 ± 1.82
Linoleic acid (C 18:2)	2.84 ± 0.65	5.46 ± 0.35	3.42 ± 0.03	3.08 ± 0.26
α-Linolenic acid (C 18:3)	0.85 ± 0.04	1.02 ± 0.07	0.04 ± 0.01	0.05 ± 0.03
Eicosanoic acid (C 20:0)	0.18 ± 0.03	0.21 ± 0.00	0.20 ± 0.01	0.25 ± 0.01
Eicosenoic acid (C 20:1)	0.19 ± 0.08	0.16 ± 0.01	0.17 ± 0.00	0.16 ± 0.00
Docosanoic acid (C 22:0)	0.04 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.06 ± 0.02
Lignoceric Acid (C 24:0)	0.06 ± 0.01	0.08 ± 0.02	0.10 ± 0.01	0.05 ± 0.01

Values are means ± SD (*n* = 2). *Hexane was the solvent employed.

prostacyclins and leukotrienes, which play an important role in the mediation of immunological and inflammatory reactions (Hohl and Rosen, 1987; Bora and Moreira, 2003). The quantities found in these fatty acids in the pulp and the appropriate consumption of this part of fruit can assist in dietary enrichment and reinforce its importance for human health.

Conclusion

The biometric characterization demonstrates that the pulp is the main component, representing 49% of the fruit's mass. From a nutritional point of view, the pulp and kernel of the macauba have significant lipid, fiber and protein contents, confirming its potential as food. The extraction by the pressing method resulted in the lowest yields. The solvent parameter significantly influenced oil extraction from the pulp by the Sohxlet method.

The physical and chemical analyses of the pulp and kernel oils of the macauba indicated properties that are comparable to good quality vegetable oils, given the predominance of monounsaturated fatty acids, in particular of oleic acid. For the polyunsaturated fatty acids essential to the human diet, the macauba presented a significant percentage for linoleic and linolenic acids.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Storage stability of tomato paste as influenced by oil-citric acid and packaging materials

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Tomato pulp was concentrated by boiling at 90°C for 1 h. A mixture of 0.1% citric acid and 25 ml of vegetable oil were added to 100 g of the paste and packaged in aluminum foil (AF), low density polyethylene (LDP), plastic containers (PC) and stored under ambient temperature (30±0.1°C). Chemical analyses were carried out to determine pH, total solids, total acidity and refractive index as well as viscosity. Microbial analyses were also carried out after 8 weeks of storage. Results obtained showed significant ($p<0.05$) decreased in the pH value and increase in the titrable acidity (TTA) with storage time in all the packaging materials and samples treated with oil and citric acid (WOC) and those with no oil and citric acid (NOC). However, the decrease in pH and increase in TTA was more rapid in NOC than WOC. The total solid, vitamin A and C decreased significantly ($p<0.05$) with storage time following the trend as pH. Yeast and total viable count increased significantly ($p<0.05$) as the storage time increased. Tomato paste stored using AF had lower total plate count, ranging from 1.25×10^5 to 4.51×10^5 as compared to LDP (1.21×10^5-4.41×10^5) and PC (1.21×10^5-6.02×10^5). Generally, tomato paste in AF retained higher quality after 8 weeks of storage as compared to samples stored in LDP and PC. Also, NOC samples were more prone to spoilage than WOC. These findings can be applied for better preservation of tomato paste in rural communities where there is no electricity for cold storage.

Key words: Packaging materials, tomato paste, citric acid, vegetable oil, storage period.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is one of the most widely consumed fresh vegetable in the world (Thybo et al., 2006). Fresh-market tomatoes are a popular and versatile fruit vegetable, making significant contributions to human nutrition throughout the world (Simonne et al., 2006). Tomato is rich in water soluble vitamin such as carotenoids, vitamins B and C; it could provide 12.2% recommended daily allowance of vitamin C (Smith and Hull, 2004). It has 20-25 mg ascorbic acid per 100 g (Ahmed and Shivhare, 2001). Tomato is also a rich source of natural lycopene. Tomato

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contains diseases fighting phytochemical (Robert et al., 2002) and antioxidant agent (Ahmed and Shivhare, 2001). The fruit consist of 98.1 g water/100 g, 0.7 protein/100 g, 3.1 g carbohydrate/100 g, 1.3 g dietary fibre/100 g and 0.3 g fat/100 g (Ahmed Shivhare, 2001).

FAO reports indicate that tomato production is on the increase globally (FAO, 2007), leading to rapid development of tomato processing industries with a series of inter-linked activities such as production of salad, soup, juice, puree, paste and powder and extraction of oil from the pulp. The demand for tomato pastes is increasing rapidly both in domestic and in international market (Tyssandie et al., 2004).

Tomato production in Nigeria at present is estimated at about one million tonnes (FAO, 2007). Due to its highly perishable nature, huge wastage and losses occur during the harvesting period. Therefore, the prevention of this losses and wastage is of major interest especially in Nigeria where there is imbalance in supply and demand at the harvesting and offseason (Akanbi et al., 2006). The imbalance is largely due to inability of the large population of Nigerians, especially the rural dwellers, to have access and use readily available and appropriate packaging materials for storage of tomato pastes, rather depending mainly on fresh tomato.

Packaging of the pastes is very important because of protection of the product from contamination by macro or micro-organism, reduction of oxidation (Matsuoka et al., 2002). Appropriate packaging of tomato pastes locally will serve purposes which are essential to extend the shelf life of the product and create a brand that is appealing to the consumers as well as add value to the product (Matsuoka et al., 2002).

Several works have been reported in literature on the use of plastic containers and low density polyethylene in the packaging of tomato paste (Famurewa et al., 2013; Akanbi and Oludemi, 2004); information is lacking on the use of Aluminum foil which are readily available and cheap. This work seeks to investigate the use of Aluminum foil in tomato paste packaging and storage.

Citric acid and oil has being reported to have inhibitory effect on the growth of microorganism especially in canned tomato (USDA, 2009). There is however scanty information on the use of citric acid and oil to extend the shelf life of packaged tomatoes. Thus, oil and citric acid were used in this study to investigate the effect of the pre-treatments on shelf life of packaged tomato pastes.

The objective of this study was to evaluate the effect of three different storage materials on the shelf stability of tomato paste pre-treated with citric acid and vegetable oil.

MATERIALS AND METHODS

Source of materials

The tomatoes used in this study were ripe and free from any form of mechanical injury, visible disease or rot. The fresh tomatoes were purchase from a farm on the bank of River Benue at Wurukum Makurdi, Benue State, Nigeria. Aluminum foil, low density polyethylene and plastic container were purchased from Modern Market Makurdi.

Sample preparation

Tomato paste was processed by the traditional methods described by (Iwe, 2002) with slight modifications. The ripe and wholesome tomatoes were sorted out, selected and washed thoroughly to remove dirt and contaminants. The fresh tomatoes were blanched in hot water at about 90°C for 3-5 s to inactivate enzymes and easy peeling of the skin. The blanched tomatoes were sprayed with cold water and the skin was peeled off by pulling it back from the blossom end. The seed cavity was removed neatly with a knife and pulped in a blender. The pulp was heated in an open pot at 90°C for 1 h to concentrate the paste.

The paste was divided into three lots of 100 g each and filled into plastic containers, low density polyethylene and aluminium foil respectively, to a head space of about 10 mm in each case. Each lot was divided into two sublots. The headspace of one of the sublots was overlaid with vegetable oil and citric acid, while the sub lots without oil and citric acid act as control. The plastic container, low density polyethylene and aluminium foil were covered with air tight-lid and stored at room temperature 30 ± 1°C. Samples were checked at two weeks intervals for chemical and microbiological changes.

Determination physicochemical analysis

Total acidity

The total acidity was determined according to AOAC (2005) official method by direct titration of 2 g of the puree with 0.1 M sodium hydroxide using phenolphthalein as indicator. The total acidity (as percentage citric acid) was calculated as follows:

Citric Acid=(mole of NaOH x volume of base x molar mass of acid x 100)/(Milli equivalent of acid x volume of sample)

pH

Twenty grammes of samples were dissolved in water and made up to 100 ml. The pH of the pastes was estimated using the electrode of a portable pH meter. The pH meter was standardized using buffer solution prior to sample analysis.

Vitamin A

This was determined according to the method of James (1995)

Ascorbic acid content

5 ml of standard solution of ascorbic acid was pipetted into 100 ml conical flask. 10 ml of oxalic acid was added and the solution titrated against the dye (V1 ml) until a pink colour persisted for 15 s.

The dye consumed is equivalent to the amount of ascorbic acid. Also, 0.5g of the sample was extracted in 4% oxalic acid and made up to 100 ml. The solution was filtered. 10 ml of oxalic acid was added to 5ml of the filtrate above. The solution was then titrated against the dye solution (2,6-dichlorophenol indophenol). The volume of dye used was recorded as (V2 ml) (Ibitoye, 2005).

$$\text{Ascorbic acid (mg/100 g)} = \frac{0.5\ \text{mg} \times V_2 \times 100\ \text{ml}}{V_1 \times 5\text{ml} \times W} \times 100$$

Where W = sample weight.

Table 1. Physicochemical parameters and vitamin content of tomatoes paste stored in aluminum foil (AF) without and with oil/citric acid for varying period of time.

Storage time	PH		Total solid		Viscosity (centipoise)		Total Acidity		Refractive index		Yeast count (cfu/g)		TVC (10^5) (cfu/g)		Vitamin C		Vitamin A	
	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	WOC	NOC	NOC	WOC
0 week	4.51^{a}	4.22^{a}	0.90^{a}	0.90^{a}	91.25^{a}	80.10^{a}	0.006^{d}	0.071^{c}	1.35^{a}	1.33^{a}	0.62^{d}	0.39^{c}	1.81^{d}	1.21^{e}	2.31^{a}	2.31^{a}	1.61^{a}	1.32^{a}
2 weeks	4.48^{a}	4.21^{a}	0.87^{b}	0.90^{a}	90.10^{a}	80.20^{a}	0.05^{c}	0.06^{d}	1.35^{a}	1.33^{a}	0.61^{d}	0.31^{d}	1.51^{e}	1.22^{d}	2.22^{b}	2.12^{b}	1.51^{b}	1.31^{b}
4 weeks	4.41^{a}	4.20^{a}	0.85^{c}	0.87^{b}	90.01^{a}	80.10^{a}	0.05^{c}	0.07^{c}	1.35^{a}	1.35^{a}	0.65^{c}	0.39^{c}	2.92^{c}	1.82^{c}	1.96^{c}	2.11^{c}	1.49^{c}	1.31^{b}
6 weeks	4.35^{a}	4.11^{a}	0.81^{d}	0.85^{c}	85.15^{b}	75.10^{b}	0.01^{b}	0.17^{b}	1.36^{a}	1.34^{a}	1.21^{b}	0.73^{b}	3.41^{b}	2.52^{b}	1.86^{d}	2.06^{e}	1.32^{d}	1.30^{c}
8 weeks	4.05^{b}	3.99^{b}	0.78^{e}	0.81^{d}	80.10^{c}	70.20^{c}	0.12^{a}	0.18^{a}	1.35^{a}	1.34^{a}	2.22^{a}	1.81^{a}	4.52^{a}	3.31^{a}	1.86^{d}	2.07^{d}	1.30^{e}	1.31^{b}
P-Value	0.210		0.001		0.787		0.004		0.224		0.001		0.001		0.004		0.002	

Mean in the same column with different superscripts differ significantly (P<0.05). NOC, Without oil and citric acid; WOC, with oil and citric acid.

Viscosity

The viscosity of the tomato was measured using A Brook field viscometer (Lv – 8 viscometer) with spindle No. 4 at a speed of 6 rpm.

Total solid

This was determined by the use of a refractometer (IHP series Brownel London, Belligham and Stanly Limited). Ten grammes of the paste were mixed with 20g of water and filtered through muslin. The total solid was then determined by a drop of the filtered paste on the splint with the corresponding reading on the refractometer as percentage total solid AOAC (2005).

Refractive index

Ten grammes of the puree was mixed with 20 ml of the water and strained directly through muslin. The refractive index of the drop of the filtrate at 30°C was obtained by taking the corresponding reading on the refractometer (Okanlawon et al., 2002).

Total soluble solids

The total dissolved solids of the various samples were analyzed by weighing 10 g of the sample into 20ml of small Whatman filter paper. The refractive index of a drop of the filtrate was taking at 20°C (AOAC, 2005).

Microbiological analysis

Preparation of media

Acidified potato dextrose agar for mould count was prepared according to the method of Ogbulie et al. (2001).

Microbial culturing and examination

Methods of standard pour plate technique were used as according to Ogbulie et al. (2001). After autodialing and cooling of the agar media to a temperature of 45°C about 20-25 ml of agar was poured into each dried plate.

1 ml portion of each sample serial dilution was added to the plate and the plate tilted from side to side until the liquid was evenly distributed across the surface of the agar. The culture plates were incubated at 37°C for 24 h for bacteria and mould count respectively prior to counting of colonies.

Statistical analysis

Determination were done in triplicate and all data were subjected to analysis of variance (ANOVA) and the mean separated using Duncan's multiple range test (DMRT) using version 16.0.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 respectively showed that the pH values of samples treated with oil and citric acid (WOC) were generally lower than that with no oil and citric acid (NOC), indicating that WOC samples were more acidic. Addition of citric acid may have increased acidity of WOC samples. Also, the pH of samples stored in aluminum foil (AF) was higher than that stored in low density LDP and PC. The differences could be due to variation in rate of permeability of oxygen in the packaging materials. Aluminium foil has higher resistance to oxygen permeability (Smith and Hull, 2004) and therefore experienced less oxidation, evidenced by low pH values compared with LDP and PC.

There was general decrease in the pH value as the storage time increased for both WOC and NOC samples. pH values decreased from 4.51- 4.05; 4.51-3.95 and 4.51-3.98 for NOC samples stored in AF, LDP and PC respectively within the storage time of 8 weeks. Similarly, WOC samples showed more slow decrease in pH values from 4.22-3.99; 4.44-3.55 and 4.46-3.20 for AF, LDP and PC respectively. Values were not significantly different ($p<0.05$) from 0-6 weeks but differed significantly ($p>0.05$) at the 8^{th} week across storage period.

Table 2. Physicochemical parameters and vitamin content of tomatoes paste stored in Low density polyethylene (LDP) without and with oil/citric acid for varying period of time.

Storage time	pH		Total solid		Viscosity (centipoise)		Total Acidity		Refractive index		Yeast count (cfu/g)		TVC (10^5) (cfu/g)		Vitamin C		Vitamin A	
	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	WOC	NOC	NOC	WOC
0 week	4.51^{a}	4.44^{a}	0.90^{a}	0.90^{a}	91.15^{a}	87.00^{a}	0.006^{d}	0.07^{c}	1.34^{a}	1.34^{a}	0.85^{c}	0.14^{e}	1.81^{e}	1.21^{e}	3.21^{a}	2.85^{a}	1.37^{a}	1.31^{a}
2 weeks	4.35^{a}	4.31^{ab}	0.88^{b}	0.90^{a}	91.15^{a}	80.10^{b}	0.05^{a}	0.06^{d}	1.36^{a}	1.35^{a}	0.73^{e}	0.56^{c}	2.52^{d}	1.72^{d}	3.21^{a}	2.83^{b}	1.30^{b}	1.31^{a}
4 weeks	4.22^{b}	4.20^{b}	0.87^{c}	0.88^{b}	85.20^{b}	80.20^{b}	0.01^{c}	0.02^{e}	1.36^{a}	1.35^{a}	0.82^{d}	0.43^{d}	4.11^{c}	2.81^{c}	2.96^{b}	1.76^{d}	1.30^{b}	1.31^{b}
6 weeks	4.21^{b}	4.00^{b}	0.83^{d}	0.86^{c}	80.20^{c}	70.25^{d}	0.01^{c}	0.17^{b}	1.37^{a}	1.37^{a}	2.01^{b}	1.61^{b}	4.51^{b}	3.01^{b}	2.72^{c}	1.77^{c}	1.28^{d}	1.24^{d}
8 weeks	3.95^{c}	3.55^{c}	0.70^{e}	0.76^{d}	70.25^{d}	75.15^{c}	0.02^{b}	0.80^{a}	1.37^{a}	1.35^{a}	4.15^{a}	2.62^{a}	4.91^{a}	4.41^{a}	2.72^{c}	1.77^{c}	1.29^{c}	1.25^{c}
P-Value	0.210		0.001		0.787		0.004		0.224		0.001		0.001		0.004		0.002	

Mean in the same column with different superscripts differ significantly (P<0.05). NOC, without oil and citric acid; WOC, with oil and citric acid.

Table 3. Physicochemical parameters and vitamin content of tomatoes paste stored in Paste container (PC) without and with oil/citric acid for varying period of time.

Storage time	pH		Total solid		Viscosity (centipoise)		Total Acidity		Refractive index		Yeast count (cfu/g)		TVC (10^5) (cfu/g)		Vitamin C		Vitamin A	
	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	NOC	WOC	WOC	MOC	NOC	WOC
0 week	4.51^{a}	4.46^{a}	0.90^{a}	0.93^{a}	91.15^{a}	80.06^{a}	0.006^{d}	0.009^{e}	1.33^{a}	1.34^{a}	0.63^{e}	0.39^{d}	1.81^{e}	1.21^{e}	2.31^{b}	3.03^{a}	1.51^{a}	1.31^{a}
2 weeks	4.46^{a}	4.24^{b}	0.89^{b}	0.91^{b}	91.10^{a}	70.10^{b}	0.006^{d}	0.09^{c}	1.35^{a}	1.34^{a}	1.71^{d}	0.83^{c}	3.31^{d}	1.82^{d}	2.32^{a}	2.83^{b}	1.50^{a}	1.31^{a}
4 weeks	4.31^{ab}	4.19^{b}	0.86^{c}	0.90^{c}	91.02^{a}	65.15^{c}	0.07^{c}	0.08^{d}	1.36^{a}	1.34^{a}	1.41^{c}	0.73^{d}	4.21^{c}	3.71^{c}	2.22^{d}	2.79^{c}	1.50^{a}	1.31^{a}
6 weeks	4.21^{b}	3.99^{b}	0.85^{d}	0.88^{d}	90.02^{b}	60.10^{d}	0.16^{b}	0.18^{b}	1.36^{a}	1.34^{a}	2.81^{b}	2.22^{b}	5.71^{b}	4.21^{b}	2.22^{d}	2.66^{d}	1.49^{b}	1.30^{b}
8 weeks	3.98^{c}	3.20^{c}	0.79^{e}	0.84^{e}	88.10^{c}	60.05^{d}	0.17^{a}	0.19^{a}	1.37^{a}	1.34^{a}	4.21^{a}	3.72^{a}	6.81^{a}	6.02^{a}	2.23^{c}	2.52^{e}	1.49^{b}	1.30^{b}
P-Value	0.210		0.001		0.787		0.004		0.224		0.001		0.001		0.004		0.002	

Mean in the same column with different superscripts differ significantly (P<0.05). NOC, without oil and citric acid; WOC, with oil and citric acid.

The decrease in the pH values with storage time may be due to increase in microbial activities with storage time (Giordano et al., 2000). According to Campos et al. (2006), pH is a key element in tomato quality and for tomato paste, pH below 4.5 is appropriate for tomato paste, but above 4.5 is undesirable trait, because it will not halts the proliferation of microorganisms in the final product.

Total solid (TS) content is a measure of the solid particles after concentration. The results of the total solid as presented in Tables 1, 2 and 3 for samples stored in AF, LDP and PC respectively revealed that WOC samples had higher TS values compared with NOC samples. Similarly, samples stored in PC were higher in TS than those stored in AF while those in LDP had lower in values. Generally, there was significant ($p<0.05$) decrease in TS as the storage period increased. From Table 1, for samples packaged in AF, the TS value of NOC decreased significantly ($p<0.05$) from 0.90 - 0.78 during the period of storage (0-8 weeks). On the other hand, the values for WOC decreased more steadily from0.90-0.81. Similar trend were also observed for samples stored in LDP and PC (Tables 2 and 3). Smith and Hull (2004) reported that AF, LDP and PC permit the diffusion of gases, vapour and volatile flavour of stored products. Therefore, the

observed trend could be due to differences in the rate of diffusion of gasses through the packaging materials.

According to earlier researchers (Saliba-Colombani et al., 2001), there is a connection between sugar content and total solid in tomato fruit. Soluble solid content measurements may give a fair estimate of the sugar level in tomato fruit, thus, the reduction in TS over storage time may be a function of the degradation of sugar by microorganism which was more noticeable in NOC than WOC samples. This gives indication of the inhibitory effect of citric acid and oil on microbial activities.

The viscosity of tomato products depends on fibre, fat, protein, and total solids (Tamburini et al., 1999). The viscosities of NOC samples stored in AF were higher and decreased more steadily than WOC samples (Tables 1, 2 and 3). Viscosity was observed also to decrease significantly ($p<0.05$) with storage time especially from the 2nd week of storage. Similar trend was also observed in samples stored in LDP and PC packaging materials, attributable to microbial activity on the stored tomato paste

The total titrable acidity (TTA) increased significantly ($p<0.05$) with the storage period for both NOC and WOC samples stored in AF, LDP and PC packaging materials. In Tables 1, 2 and 3, the TTA of WOC samples were generally higher than NOC, owing to the presence of citric acid and oil used in the treatment procedure. The TTA of NOC samples stored in PC were higher (0.006-0.17) than LDP (0.006-0.05) and AF (0.006-0.12). On the other hand, TTA of WOC sample was higher in LDP (0.07-0.80) compared to the other packaging materials.This result is in line with the work of Okanlawon et al. (2002). The authors observed that, decrease in acidity is probably due to the effect of organisms responsible for the spoilage, some of which can release basic substances into the samples.

The refractive index of WOC samples were generally lower than NOC, although values were not significantly different ($p<0.05$) across the packaging materials and storage period (Tables 1, 2 and 3). Refractive index is a measure of the soluble solid (Famurewa et al., 2013), hence the presence of citric acid and oil in WOC samples reduced the breakdown of solids leading to less available soluble solids compared to NOC samples.

Tables 1, 2 and 3 show that .the yeast counts in WOC samples were generally lower than NOC in the three packaging materials namely AF, LDP and PC. The yeast count of WOC samples ranged from 0.39-1.81; 0.14-2.62 and 0.39-3.72 for AF, LDP and PC respectively, while that of NOC was higher ranging from 0.63-4.21; 0.85-4.15 and 0.62-2.22. Samples were observed to increase significantly ($p>0.05$) in yeast count with storage time in all the packaging materials. This trend could be attributed to the effect of the citric acid and oil which inhibited the multiplication of the microorganism in WOC samples. Samples stored in LDP were higher in yeast count than AF and PC for NOC and WOC. This higher population of the microorganisms in LDP may be due to higher permeability of O_2, CO_2 and other gases (Smith and Hull, 2004). The growth of yeast and mould increased with storage time in accordance with the report of (Mozumber et al., 2012)

Tables 1, 2 and 3 also show that the total viable count (TVC) followed the same trend as the yeast and mould count. Total viable count was higher in NOC samples than WOC across all samples stored in AF, LDP and PC.This is also attributable to the inhibitory action of oil and citric acid.

Values obtained for samples stored in AF, were lower than those stored in LDP and PC, indicating the relative resistance of AF to microbial growth. The total viable count values generally increased significantly ($p<0.05$) as the storage time increased in according with (Mozumber et al., 2012) who reported that microbial growth increases as the storage time prolongs.

The results presented in Tables 1, 2 and 3 show that there was significant difference ($p>0.05$) in the vitamin C content among the pre-treatments and the storage period.

The vitamin C values of samples treated with oil and citric acid (WOC) were generally higher than samples with no citric acid and oil (NOC). Higher retention of vitamin C in WOC may be as a result of inaction of the endogenous enzymes such as ascorbic acid oxidase, cytochrome oxidase and peroxidase as reported in similar by (Okorie et al., 2004).

The vitamin C of NOC samples was lower than WOC. Vitamin C was higher in LDP compared to the other packaging material. Generally, vitamin C decreased significantly ($p<0.05$) with storage period owing to the reactive nature of vitamin C, to storage environment (Davey et al., 2000). The result of this work agrees with earlier work by Leonardi et al. (2001) who reported that storage condition has significant impact on the loss of ascorbic acid.

The data obtained for vitamin A showed higher values in NOC than WOC samples. This may be due to the concentration of citric acid used in the pre-treatment procedure of the samples. Vitamin A samples stored in PC degraded more slowly than those stored in LP and AF; this may be as a result of heat generated internally by the PC. Vitamin A values were observed to decrease more gradually, relative to the other parameters as the storage time increased. This may be due to degradation of vitamin A by light and heat in the storage environment.

Conflict of interest

The authors did not declare any conflict of interest.

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Influence of fish smoking methods on polycyclic aromatic hydrocarbons content and possible risks to human health

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Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants representing an important group of carcinogens that have been detected in smoked fish. This work investigated the effect of fish smoking methods on dietary exposure to PAHs and potential risks to human health. The smoking methods considered accounted for differences in smoked catfish/solefish content of 16 PAHs. The results revealed traditional method of smoking had 7 genotoxic PAHs. Traditionally smoked catfish/solefish were 18 - 24 times higher than those measured by modern method. Risk assessment conducted using benzo[a]pyrene carcinogenic and mutagenic toxicity equivalency factors (TEF and MEF, respectively) showed low risk (2.01×10^{-8} - 2.86×10^{-8} and 1.09×10^{-8} - 1.83×10^{-8}, respectively for carcinogenicity and mutagenicity) associated with consuming smoked catfish/solefish and below the USEPA guideline (1.0×10^{-5}) for potential cancer risk. Mean hazard indexes were below 1 (below an acceptable cumulative threshold) ranging from 1.43×10^{-6} - 9.96×10^{-8}. A significantly high accumulation of PAHs was found in the smoked fish as compared to the non-smoked fish control samples. This study indicates that there is no adverse health effect of PAHs content on consumers of smoked fish species but levels of PAHs present in smoked catfish/solefish prepared using traditional methods may pose elevated cancer risks if consumed at high consumption rates over many years.

Key words: Smoked fish, polycyclic aromatic hydrocarbons, mutagenic, carcinogenic, human health, hazard index.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) compounds are groups of potent carcinogens that are present in the environment; traces of these substances have been found in various food products (Guillen and Sopelana, 2003). PAHs are formed by incomplete combustion processes which occur whenever wood, coal or oil are burnt. The possible sources of PAHs in food are environmental contamination, as well as thermal treatment of varying severity which is used in the preparation and manufacturing of foods (Guillen, 1994), (Guillen, 1994), the absorption and deposition of particulates during food processing such as smoking, grilling, boiling and toasting, the pyrolysis of fats and the incomplete combustion of charcoal (Larsson et al., 1983; Guillen, 1994; Moret et al.,

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1997). Regarding food of animal origin, one hypothesis suggests that the lipophilic character of PAHs is responsible for the accumulation in the fat of animals which eat contaminated plants (Guillen et al., 1997). PAHs occur as contaminants in different food categories and beverages including water (Belykh et al., 1999), fruit, cereals, oils (Dennis et al., 1983, 1991; Moret and Conte, 2002), smoked meat (Potthast, 1977; Simko, 2002) and smoked fish (Simko, 1991; Akpan et al., 1994; Lodovici et al., 1995; Moret et al., 1999). Non-processed fish contains low PAHs concentration even when it comes from contaminated water because fishes rapidly metabolize PAHs, resulting in low steady-state level in the tissue (Moret et al., 2000; Chen and Chen, 2005; Wretling et al., 2010; Essumang et al., 2013). The health effects resulting from PAH exposure have recently been discussed extensively in the literature (Shen et al., 2008). These include growth retardation, low birth weight, small head circumference, low IQ, damaged DNA in unborn children and the disruption of endocrine systems, such as estrogens, thyroid and steroids (Essumang et al., 2012).

Skin changes (thickening, darkening and pimples) and reproductive-related effects such as early menopause due to destruction of ova have also been identified with PAHs (Essumang et al., 2011, 2012). It is known that in mammalian cells, PAHs undergo metabolic activation to diol, and epoxides that bind covalently to cellular macromolecules, including DNA, thereby causing errors in DNA replication and mutations that initiate the carcinogenic process (Rodriguez et al., 1997; Schoket, 1999; Lightfoot et al., 2000; Essumang et al., 2012, 2013). Polymorphisms causing glutathione transferase deficiencies (GSTM1) may result in elevated breast cancer, lung cancer and other forms of human cancer risk from PAHs (IARC, 1999; Van der Hel et al., 2003). Because of their mutagenic and carcinogenic effects, PAHs have been included in several priority pollutant lists of the Agency of Toxic Substances and Disease Register (ATSDR), the International Agency for Research on Cancer (IARC), the European Community (EC) and the Environmental Protection Agency (USEPA). Several studies have been carried out to determine the levels of exposure of humans to PAHs (De Vos, 1990).

Smoking is one of the oldest food preservation technologies and can be used to achieve the characteristic taste, colour and aroma for food (especially meat and meat products, fish and fish products) (Djinovic et al., 2008). In Europe, about 15% of the total quantity of fish for human consumption is smoked prior to release to the market (Stolyhwo and Sikorski, 2005). However, foods are nowadays smoked for sensory quality rather than for the preservative effect. Yanar et al. (2006) reported that the acceptance of smoked fish in developed countries is based primarily on the sensory characteristics it imparts to the product while Akintola et al. (2013) confirmed the nutritional qualities and adequacies. In addition to this, smoking enhances preservation due to the dehydrating bactericidal and antioxidant properties of smoke such as phenol derivates, carbonyls, furan derivates, organic acids and their esters (Simko, 2002).

The actual levels of PAHs in smoked foods depend on several variables in the smoking process, including type of smoke generator, combustion temperature, and degree of smoking (Moret et al., 1997). Smoke is generated by thermal pyrolysis of a certain kind of wood when there is limited access of oxygen. Temperature of smoke generally plays a very important role, because the amount of PAHs in smoke formed during pyrolysis increases linearly with the smoking temperature within the interval 400-1000°C (Toth and Blaas, 1972).

In modern industrial ovens, the smoke is usually generated in a separate chamber cleaned by using various techniques, such as electrostatic filters or smoke washing, and then led into the smoking chamber. This, together with the control of some important parameters such as temperature, humidity, smoke concentration, and circulation rate, can contribute to the minimization of PAHs contamination (Moret et al., 1999).

Incomplete wood combustion during smoking can produce considerable amounts of PAHs which can penetrate through the surface of products (Jira et al., 2006). In a study performed by Gomma et al. (1993), the total PAH concentrations were detected between 2.6-29.8 and 9.3-86.6 µg/kg in smoked meat and fish, respectively.

In another study conducted by Panalaks (1976) in Canada, smoked fish and meat samples were analyzed and PAH compounds were detected in 18 out of 25 smoked fish samples (maximum of 141 µg/kg) and in 19 out of 43 smoked meat samples (maximum of 13 µg/kg). Petrun and Rubenchik (1966) found the levels of BaP ranged from 4.2 to 60 µg/kg in hot and cold-smoked fish samples. In their study, Storelli et al. (2001) reported that the concentration of total PAHs in seafood varied from 46.5 to 124 µg/kg.

Reinik et al. (2007) found the highest total PAH concentrations in smoked meat, sausage and chicken samples as 16, 19 and 6.5 µg/kg, respectively. In another study, Djinovic et al. (2008) stated that there are differences in PAH contents between final smoked beef ham samples from traditional smokehouse (3.9 µg/kg) and industrial smokehouse (1.9 µg/kg).

This study seeks to determine the effect of smoking process on PAHs content in smoked fish samples (catfish and solefish) in Nigeria. The data from the study will also be used to assess dietary intake of PAHs and the carcinogenic health hazards via smoked fish consumption.

MATERIALS AND METHODS

Sample collection and preparation

Fresh fish and commercially smoked fish of two different species commonly consumed in Nigeria, namely catfish and solefish were purchased from 3 different local fish vendors in Lagos. The fresh

fish was gutted, cleaned and a part was placed over a wire gauze that was on burning hardwood charcoal (15 cm away from the hot hardwood charcoal ember). The catfish and solefish were allowed to cook for 90 min on both sides to obtain a greater level of drying. Fresh, laboratory smoked and commercially smoked fish samples from different vendors were pooled together to obtain representtative samples for each of 2 types of fish species. The fish samples were separately composited, homogenized, packed in amber bottles and kept in the freezer prior to analysis.

Reagents

Methanolic 2 M-KOH (methanol/water 9 + 1) and hexane analytical grade were redistilled in glass before use. Methanol (analytical grade), Silica gel (mesh: 70 – 230), glass wool and potassium hydroxide pellets (Purity: 86.1%) were obtained from Sigma Aldrich. PAH standard mixture containing 16 PAHs compounds (purity: 95.9-99.9%) including naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Py), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenzo[a,h]anthracene (DBahA) and benzo[g,h,i]perylene (BghiP) in a 80 mg/L mixture solution were obtained from AccuStandard Chem. Co. (New Haven, CT, USA). Deuterated PAH internal standard solutions (naphthalene-d8, acenaphthene-d10, phenanthrene-d10, and chrysene-d12) at 4,000 mg/L and surrogate standard solutions (2-fluorobiphenyl and 4-terphenyl-d14) at 2,000 mg/L were obtained from AccuStandard Chem. Co. PAHs working standards, internal standard mixture solutions and surrogate standard mixture solutions were properly diluted in *n*-hexane and prepared daily before the analysis. Glassware were washed with detergent, soaked 24 h in dichromic acid rinsed severally with tap water, deionized distilled water, acetone and dried in an oven at 105°C. Helium and nitrogen gases were obtained from Air Liquid Nigeria Plc.

Extraction and sample clean-up

PAH extraction was carried out by applying the method described by Wretling et al. (2010). All the samples were analyzed in duplicate. Aliquot of 10 g of homogenized smoked fish were weighed into a 250 mL Erlenmeyer flask and spiked with 1 mL of a perdeuterated PAH internal standard mixture. Saponification was achieved by adding 60 mL of methanolic 2 M–KOH, the sample was extracted under reflux for 2 h. 50 mL of n-hexane was added and the refluxing was continued for another 5 min. The extract was cooled to ambient temperature and transferred to a 250 mL separating funnel using 30 mL of methanol/water (4+1). The funnel was shaken and the layers were allowed to separate. The aqueous layer was drained into a second 250 mL separating funnel and shaken with another 30 mL of n-hexane. The aqueous layer was discarded and the hexane phases were combined and washed successively with 30 mL of methanol/water (4+1), 30 mL of methanol/water (1+1) and 2 x 30 mL of water. The washed hexane solution was transferred to a 250 mL round-bottomed flask and concentrated to about 1 mL in a rotary evaporator under reduced pressure at 40°C and cleaned by silica gel column chromatography. The eluent was re-concentrated to 0.5 ml in a rotary evaporator (at 40°C) and concentrated further under a nitrogen flow to 200 µL before transferring to a GC sample vial with a conical glass insert.

GC–FID analysis

The polycyclic aromatic hydrocarbon analysis was carried out by an Agilent 7890A gas chromatograph system coupled with a flame ionisation detector. 2 µL of sample solution was injected in the pulsed splitless mode onto a 30 m x 0.32 mm i.d. fused capillary column with a film thickness of 0.25 µm (HP-5). Helium gas was used as the carrier gas. Other operating conditions were: pulse pressure 10.74 psi, purge time 0.75 min, purge flow 15.0 mL. An injection temperature was set at 300°C. The column temperature was initially held at 80°C for 1 min, and ramped to 320°C at a rate of 20°C/min and then 320°C was held for 20 min. Identification of PAHs in the samples was based on comparison of the retention times with those in a standard solution, and quantification on the corresponding areas of the respective chromatograms. Procedural blanks were analyzed and quantified.

Analytical quality control

A spiking procedure was used to calculate recoveries. The recoveries (mean of 2 replicate analyses) were calculated by comparing the difference between spiked (4.6 – 8.1 µg/kg) and unspiked sample with the known amount of PAHs added. Recoveries obtained for different PAH standards ranged from 72 and 108% and their relative standard deviation ranged from 15.9 to 21.3%.

Benzo[a]pyrene equivalent estimation

Toxic equivalency factors (TEFs) have been developed for a number of individual PAHs to expresses its potency relative to benzo(a)pyrene, which has a TEF of unity. The concentration of each of the individual PAH compounds is multiplied by its TEF proposed by (Nisbet and LaGoy, 1992) (Table 1), and these values are summed to yield benzo(a) pyrene equivalent concentrations, TEQ_{BaP} (AFSSA, 2003). This technique has been applied successfully to smoked and fresh seafood monitoring studies, and other wider monitoring programmes (Law et al., 2002). The mutagenicity of individual PAHs relative to B(a)P had also been computed using the mutagenic equivalency factor (MEF) proposed by Durant et al. (1996, 1999) as shown in Table 1. The sum of the concentration of each individual PAH multiplied by the corresponding MEF gives the mutagenic equivalents (MEQ).

$$TEQ_{BaP} = \sum(TEF_i \times C_i) \quad (1)$$

$$MEQ_{BaP} = \sum(MEF_i \times C_i) \quad (2)$$

where Ci is the measured individual PAHs concentrations for the 'ith' compound with the assigned TEFi or MEFi.

Dietary exposure to PAHs

Estimates of human dietary PAH exposure doses (mg kg^{-1} BW d^{-1}) occurring over a lifetime were determined. The daily BaP equivalent dose of mixtures of carcinogenic (mutagenic) PAH compounds was calculated for carcinogenicity and mutagenicity using the following equation.
Average daily dose of carcinogenic (mutagenic) PAHs is:

$$\frac{\text{TEQ (or MEQ)} \times \text{IR} \times \text{CF}}{\text{BW}} \quad (3)$$

These exposure assumptions were made to be consistent with EPA guidance on default assumption on ''reasonable maximum exposure'' (USEPA, 1991). Where IR is the ingestion or intake rate of carcinogenic (mutagenic) PAHs based on average fish consumption rate set to 68.5 g day^{-1} per person from the annual per

Table 1. Proposed benzo(a)pyrene equivalent factors for carcinogenic (TEF) and mutagenic toxicity (MEF).

PAH compound	TEF (Nisbet and LaGoy, 1992)	MEF Durant et al. (1996, 1999)
Naphthalene	0.001	
Acenaphthylene	0.001	
Acenaphthene	0.001	
Fluorene	0.001	
Phenanthrene	0.001	
Anthracene	0.01	
Fluoranthene	0.001	
Pyrene	0.001	
Benzo(a)anthracene	0.1	0.082
Chrysene	0.001	0.017
Benzo(b)Fluoranthene	0.1	0.25
Benzo(k)fluoranthene	0.01	0.11
Benzo(a)pyrene	1.0	1.0
Dibenzo(a,h)anthracene	1.0	0.29
Indeno(1,2,3-cd)pyrene	0.1	0.31
Benzo(g,h,i)perylene	0.01	

Table 2. Toxicity values for PAHs contaminants

PAHs	RfD (mg / kg-d)		CSF (USEPA, 2004) (1/mg / kg-d)
Naphthalene	2.00×10^{-02}	Chrysene	7.30×10^{-3}
Acenaphthylene	2.00×10^{-02}	Benzo(a)anthracene	7.30×10^{-1}
Acenaphthene	6.00×10^{-02}	Benzo(b)Fluoranthene	7.30×10^{-1}
Fluorene	4.00×10^{-02}	Benzo(k)fluoranthene	7.30×10^{-2}
Phenanthrene		Benzo(a)pyrene	7.30
Anthracene	3.00×10^{-01}	Dibenzo(a,h)anthracene	7.30
Fluoranthene	4.00×10^{-02}	Indeno(1,2,3-cd)pyrene	7.30×10^{-1}
Pyrene	3.00×10^{-02}		
Benzo(g,h,i)perylene	4.00×10^{-02}		

capita fish consumption of 25 kg for Nigeria (FAO, 2008). CF is the conversion factor (0.001 mg μg^{-1}) and BW represents body weight which was set at 70 kg.

Non-cancer hazard, carcinogenic and mutagenic risk calculations

Risk associated with dietary exposure to non-carcinogenic PAHs was evaluated using a hazard quotient approach. Hazard quotients represent a ratio of the exposure dose for each PAH divided by an oral chronic reference dose (RfD).

Hazard quotient (HQ) = Average daily dose (ADD) / RfD (4)

Pertinent RfD values (mg / kg day) are listed in Table 2. Summation of individual hazard quotients results in hazard index.

Hazard index (HI) = $\Sigma(HQ_1 + HQ_2 + HQ_3 + \ldots\ldots HQ_n)$ (5)

The calculated TEQ_{BaP} and MEQ_{BaP} for the seven USEPA classified carcinogens (mutagens) were used to estimate carcinogenic and mutagenic risk involved in ingestion of smoked fish used herein for life time of 70 years (USEPA, 2000). The total risk due to exposure to mixtures of carcinogenic (or mutagenic) PAHs is the product of the dietary carcinogen exposure dose (mg kg^{-1} BW d^{-1}) and benzo[*a*]pyrene's slope factor value in Table 2.

Risk (carcinogenic or mutagenic) = average daily dose x slope factor (6)

Statistical analysis

Analysis of variance (ANOVA)) for $\alpha = 0.05$ were performed to estimate the significance of the differences between the means of total and individual PAHs content in traditionally and modern smoked fish using both SPSS and Microsoft Excel.

RESULTS AND DISCUSSION

PAHs levels in fresh fish samples

The PAHs concentrations in non-smoked fresh catfish-

Table 3. Concentration (µg/kg) (mean± SD) of PAHs for catfish / solefish smoked with different smoking methods.

PAHs	Traditional smoked fish		Modern smoked fish		Fresh fish	
	A1	B1	A2	B2	A3	B3
Naphthalene	12.89±0.22	5.48±0.68	0.03	0.02	0.01	0.01
Acenaphthylene	0.41±0.15	0.19±0.05	0.93±0.04	0.31±0.24	0.02	0.01
Acenaphthene	1.05±0.32	2.02±0.32	0.29±0.01	0.41±0.15	0.01	nd
Fluorene	4.04±1.19	2.62±0.82	0.02	nd	nd	nd
Phenanthrene	15.94±1.30	9.85±2.22	0.99±0.06	0.38±0.18	nd	nd
Anthracene	2.09±0.39	0.27±0.13	0.01	0.02	nd	nd
Fluoranthene	2.89±2.33	5.07±0.84	nd	nd	nd	nd
Pyrene	1.30±0.01	1.10±0.39	nd	nd	nd	nd
Chrysene	nd	nd	nd	nd	nd	nd
Benzo(a)anthracene	0.29±0.06	0.16±0.13	nd	nd	nd	nd
Benzo(b)Fluoranthene	0.11±0.09	0.12±0.15	nd	nd	nd	nd
Benzo(k)Fluoranthene	nd	nd	nd	nd	nd	nd
Benzo(a)pyrene	nd	nd	nd	nd	nd	nd
Dibenzo(a,h)anthracene	nd	nd	nd	nd	nd	nd
Benzo(g,h,i)perylene	0.26	0.35	nd	nd	nd	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd	nd	nd
Total PAHs	41.27	27.23	2.27	1.14	0.04	0.02
Carcinogenic PAHs	0.40	0.28	0.00	0.00	0.00	0.00
Non-Carcinogenic	PAHs 40.87	26.95	2.27	1.14	0.04	0.02

A = catfish; B = solefish; Non- carcinogenic PAHs = Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene; Anthracene, Pyrene, Fluoranthene, Benzo(ghi)perylene; Carcinogenic PAHs = Chrysene, Benzo(a)anthracene, Benzo(b)Fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Indeno(1,2,3-cd)pyrene, nd = no data.

/solefish samples used as control were below detection limits as presented in Table 3 (0.002 – 0.005 µg kg^{-1}). This is in conformity with the statement made by Stołyhwo and Sikorski (2005) that fish and marine invertebrates may naturally contain small or undetectable amounts of different PAH absorbed from the environment. The lack of PAHs in fresh catfish/solefish samples indicates that the PAHs measured in smoked catfish/solefish samples were wholly attributable to smoking processes as affirmed by Forsberg et al. (2013) The results in non-smoked fresh fish samples were considerably lower than those reported by other authors (Silva et al., 2011; Wretling et al., 2010).

PAHs levels in smoke fish samples

The mean values of PAHs content measured in traditional and modern smoked catfish/solefish as presented in Table 3 were predominantly those with ≤4 rings. Similar studies were reported in traditional Nigerian smoked fish (Akpambang et al., 2009) and fish prepared using traditional German smoking kilns (Karl and Leinemann, 1996). The two smoked fish had different PAH (levels) contribution from the smoking process. This could be attributed to the differences in fat and moisture contents and the nature of skin cover (Nakamura et al., 2008). Smoked catfish on the average recorded the highest mean levels of PAHs for both traditional and modern techniques. Individual PAH levels ranged from 0.01– 15.94 µg kg^{-1}. Phe was the most abundant PAH followed by Naph, Flu, Fla, Ant, Py, Ace, Acy and BghiP. The summation of these 9 analytes accounted for 98% of the total mass of PAHs measured across all smoked catfish and solefish. Together, 2-ring, 2+3-ring, and 2+3+4-ring PAHs accounted for roughly 36, > 75 and > 98% of the total PAHs mass measured across all smoked catfish and solefish samples, respectively. The individual PAHs of lower molecular weight found in high level could be attributed to the lower average wood temperature used in the smoking process (Nakamura et al., 2008).

This shows that smoking process contributed to the increase percentage composition of these PAHs. Irrespective of the smoking method applied, benzo[a]pyrene used as biomarker in monitoring carcinogenic PAHs recorded mean concentrations below detection (Table 3) limit which was much lower than maximum tolerable limit of 5.0 and 2.0 µg/kg in smoked fish established by the European Commission (Regulation (2005) and Turkish Codex Regulation (2008), respectively.

The results obtained in this work therefore indicated that the smoked fish may contribute no levels of cancer and cancer-related cases in the study area, because BaP is widely known for its carcinogenicity and mutagenicity; further epidemiological studies may be required to prove

Table 4. Source characterization and assessment of PAHs.

PAH ratios	Petroleum	Wood	This study	Reference
[Ant/(Ant + Phe)]	<0.10	>0.10	0.01-0.12	Yunker et al. (2002), Zhang et al. (2004), Li et al. (2006) Pies et al. (2008) and Placha´ et al. (2009)
(Fla/(Fla + Py)]	0.40	>0.5	0.69-0.82	Yunker et al. (2002), Zhu and Wang (2003) and Placha´ et al. (2009)
BaA/(BaA + Chr)]	<0.20	1.2–5.0	1.00	Maher and Aislabie (1992), Gilbert et al. (2006), Zhang et al. (2006), Pies et al. (2008), Essumag et al. (2012)
[BaP/(BghiP)]	>0.6	1.2–5.0	-	Park et al. (2002), Yin et al. (2008), Maliszewska-Kordybach et al. (2008), and Essumag et al. (2012)
[IcdP/(IcdP + BghiP)]	<0.5	>0.5	-	Maliszewska-Kordybach et al. (2008) and Yin et al. (2008)

Ant = anthracene, Phe = phenanthrene, Fla = fluoranthene, BaA = benz[a]anthracene, Chr= chrysene, BaP = benzo[a]pyrene, BghiP = benzo[g,h,i]perylene, and IcdP = indeno[1,2,3-cd]pyrene.

this conclusion. Fish species smoked by traditional method was 18 times greater than corresponding modern smoked fish samples. The observed differences probably reflect the highly controlled and standardized smoking systems used in modern smoking (Vaz-Velho, 2003). Furthermore, in order to increase the shelf-life of the product, fish vendors may re-smoke the product many times until they are sold, thus contributing to increase PAH formation (Akpambang et al., 2009).

One-way ANOVA conducted at 95% confidence level on the numbers of aromatic rings showed no statistical significant differences ($p > 0.05$) between the numbers of aromatic rings with respect to each of the fish type smoked. Thus the number of aromatic rings was species independent. Further analysis of variance (one-way ANOVA) conducted on the data at 95% CL showed significant difference ($p < 0.05$) in PAH levels between fish type with respect to the smoke type used. Thus PAHs levels in smoked fish were species dependent.

Sources of PAHs in smoked fish

PAH ratios of selected compounds are generally considered to be a good indicator of the pollution and the mechanism of PAH distribution in foods. Yunker et al. (2002) have summarized the literature on PAH ratios (Table 4). The ratio of [An/(An + Phen)] in this study ranged from 0.01 to 0.12 with a mean of 0.05 (Table 5). This indicates a predominance of petroleum as a source for PAHs (ratio < 0.1) in the smoked fish. The [Fl/(Fl + Py)] ratio in this work also ranged from 0.69 to 0.82 which is an indication of wood or coal combustion as a source of the PAHs in the smoked fish samples. The results from the [BaA/(BaA + Chry)] ratio again confirm wood combustion as the primary and major source of PAH contamination in smoked fish. These PAH ratios reveal that the major source of PAHs in the smoked fish is the wood combustion with vehicular traffic source contributing a comparatively insignificant amount.

Cancer and non-cancer risk assessment of PAHs in smoked fish

The carcinogenic toxicity (TEQ_{BaP}) and mutagenic toxicity (MEQ_{BaP}) relative to B(a)P were calculated for the carcinogenic and mutagenic risk associated with ingestion of the smoked fish (Tables 1 and 3). While TEQ_{BaP} is directly associated with carcinogenicity, MEQ_{BaP} (mutagenic activity) may not be directly associated with cancer (Zeiger, 1998, 2001; Essumang et al., 2013) and may have implications for other non-cancerous adverse health effects like pulmonary diseases, birth defects, impotency, low intelligent quotient, etc. (DeMarini et al., 2004; Essumang et al, 2013). From the result in Table 6, the TEQ for the seven USEPA priority carcinogens were 0.040 and 0.028 for catfish and solefish smoked traditionally. Known carcinogenic PAHs were not found in smoked fish prepared by modern method (Tables 6 and 7).The corresponding EQ_{BaP} daily dose and carcinogenic risk for an adult involved in life time of 70 years ingestion of the smoked fish products were also calculated to be 3.92×10^{-8} and 2.75×10^{-8} mg kg^{-1} day^{-1} for a risk of 2.86×10^{-8} and 2.01×10^{-8}, respectively (Tables 2 and 6). These risk values mean that for ingestion of catfish prepared by traditional smoking, 3 out of 10,000,000 adults are likely to suffer from cancer in their life time and for ingestion of solefish prepared by traditional smoking, 2 out of 10,000,000 people are likely to suffer from cancer in their life time. This means that the consumption of catfish and solefish prepared by traditional smoking pose

Table 5. Fish species and PAH isomer ratios for source assessment in smoked fish sampled.

Fish species/ Isomer ratio	Ant/ Ant + Phe	Fla/ Fla + Py	BaA/ BaA + Chr	BaP/ BghiP	Ind/ IcdP + BghiP
S1	0.12	0.69	1.00	-	-
S2	0.03	0.82	1.00	-	-
S3	0.01	-	-	-	-
S4	0.05	-	-	-	-

S1 = traditional smoked catfish; S2 = traditional smoked solefish; S3 = modern smoked catfish; S4 = modern smoked solefish

Table 6. Risk assessment based on carcinogenic equivalency, average daily dose and risks (Mean±SD) for traditional and modern smoked fish species.

Carcinogenic equivalency	T. smoked Catfish	T. smoked Solefish	M. smoked Catfish	M. smoked Solefish
Benzo(a)anthracene	0.029	0.016	nd	nd
Benzo(b)Fluoranthene	0.011	0.012	nd	nd
Benzo(k)fluoranthene	nd	nd	nd	nd
Benzo(a)pyrene	nd	nd	nd	nd
Dibenzo(a,h)anthracene	nd	nd	nd	nd
Chrysene	nd	nd	nd	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd
∑BaP-TEQ	0.040	0.028		
BaPEQ daily dose ($mgkg^{-1}day^{-1}$)	3.92×10^{-08}	2.75×10^{-08}		
LECR	2.86×10^{-08}	2.01×10^{-08}		

T. = traditional; M. = modern; LECR = life-time excess carcinogenic risk, nd = no data.

Table 7. Risk assessment based on mutagenic equivalency, average daily doses and risks (Mean±SD) for traditional and modern smoked fish species.

Mutagenic equivalency	T. smoked Catfish	T. smoked Solefish	M. smoked Catfish	M. smoked Solefish
Benzo(a)anthracene	0.024	0.013	nd	nd
Benzo(b)Fluoranthene	0.019	0.002	nd	nd
Benzo(k)fluoranthene	nd	nd	nd	nd
Benzo(a)pyrene	nd	nd	nd	nd
Dibenzo(a,h)anthracene	nd	nd	nd	nd
Chrysene	nd	nd	nd	nd
Indeno(1,2,3-cd)pyrene	nd	nd	nd	nd
∑BaP-TEQ	0.0259	0.015	0	0
BaPEQ daily dose ($mgkg^{-1}day^{-1}$)	2.51×10^{-08}	1.49×10^{-08}		
LECR	1.83×10^{-08}	1.09×10^{-08}		

T. = traditional; M. = modern; LECR = life-time excess carcinogenic risk, nd = no data.

no risk, because it is lower than the USEPA (1993, 2009) carcinogenic unit risk of 1×10^{-5} (carcinogenesis threshold). Generally, relatively lower $\sum TEQ_{BaP}$ and cancer risk values below the acceptable USEPA (1993, 2009) carcinogenic unit risk of 1×10^{-5} (carcinogenesis threshold) were recorded for the fish samples prepared by traditional smoking. Also, the mutagenic equivalent for these PAHs calculated were 0.026 and 0.015 for catfish and solefish prepared by traditional smoking (Table 7). The corresponding EQ_{BaP} daily doses were also calculated to be 2.52×10^{-8} and 1.49×10^{-8} mg kg^{-1} day^{-1} for catfish and solefish prepared by traditional smoking respectively (Tables 2 and 7). Hence, the mutagenic risk involved in ingestion of these smoked fish products of

Table 8. Risk assessment based on non-carcinogenic equivalency, average daily doses and hazard index (Mean±SD) for traditional and modern smoked fish species.

Non-Carcinogenic equivalency	T. smoked Catfish	T. smoked Solefish	M. smoked Catfish	M. smoked Solefish
Naphthalene	0.013	0.0055	0.00001	0.00001
Acenaphthylene	0.0004	0.00019	0.00093	0.00031
Acenaphthene	0.00105	0.00202	0.00029	0.00041
Fluorene	0.00404	0.00262	0.00002	nd
Phenanthrene	0.016	0.0099	0.00099	0.00038
Anthracene	0.0209	0.0027	0.00001	0.00002
Fluoranthene	0.0029	0.0051	nd	nd
Pyrene	0.0013	0.0011	nd	nd
Benzo(g,h,i)perylene	0.00026	0.00035	nd	nd
∑BaP-TEQ	0.060	0.029	0.002	0.001
BaPEQ daily dose $mgkg^{-1}day^{-1}$	5.86×10^{-08}	2.88×10^{-08}	2.20×10^{-09}	1.11×10^{-09}
Hazard index	1.43×10^{-06}	9.02×10^{-07}	9.96×10^{-08}	4.08×10^{-08}

nd = no data.

70 years was calculated to be 1.83×10^{-8} and 1.09×10^{-8}, respectively. This imply that for adult's life time ingestion of catfish prepared by traditional smoking; 2 out of 10,000,000 and 1out of 10,000,000 people are like to suffer from non-cancer and other cancer related disease in their life time, respectively. Generally, relatively lower $\sum MEQ_{BaP}$ and mutagenic risk values below the acceptable USEPA (1993, 2009) unit risk of 10^{-5} were recorded for catfish and solefish samples prepared by traditional smoking. Catfish prepared by traditional smoking produced the largest observed values for carcinogenic and mutagenic PAHs. From these results, it may be said that catfish and solefish prepared by traditional smoking had low cancer and mutagenic risk and may be considered safe for consumption.

Exposure to non-carcinogenic PAHs resulted in hazard indexes ($\sum PAH_{16}$ HQs) ranging from 9.02×10^{-7} to 9.96×10^{-8} across the two smoking methods (Tables 2 and 8). The non-carcinogenic PAHs produced hazard indexes less than 1; a level described by the EPA as generally having no appreciable risk for the development of non-cancer health effects through the ingestion of these hazardous PAHs from smoked fish in their diets. Taken together, risks associated with carcinogenesis pose the largest threat to human health.

Conclusion

From the results discussed above, it may be concluded that smoked catfish/solefish could be deemed fit for human consumption. Smoked catfish/solefish from comercial fish mongers (traditional method of smoking) showed elevated levels of polycyclic aromatic hydrocarbons (PAHs) as compared to modern method of smoking, and this may result in cases of cancer and cancer-related ailments in Nigeria. The high levels of PAHs in smoked catfish/solefish prepared by traditional method is a result of uncontrolled fish-smoking practices that burn wood at higher temperatures, coupled with thermal pyrolysis of fat in fatty fish at higher temperatures to give the fish a longer shelf-life, but which also promotes PAH production.

This study found that fish smoking practices employed by fishmongers in Nigeria are similar throughout the nation. There is therefore a need to educate fishmongers about safe smoking practices, and also most importantly to adopt a fish smoking procedure that would reduce considerably the levels of toxins in fishes smoked with traditional kilns in order to ensure not only the health safety of consumers but also that of fishmongers exposed to smoke during
fish-smoking processes.

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

Proximate, anti-nutrient and sensory properties of *ogi*, a millet based gruel supplemented with treated African oil bean (*Pentaclethra macrophylla* Benth.) seed flour

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The proximate, anti-nutrient and sensory properties of millet *ogi* supplemented with treated African oil bean flour were evaluated. Millet *ogi* was produced using traditional method which involved soaking, wet milling, fermentation and drying. African oil bean seeds were decoated, roasted, fermented and defatted under controlled experiment. Flours obtained were substituted with millet *ogi* at 10 and 20% levels. The results show that there was significant (P ≤ 0.05) increase in fat, crude protein and energy value and a converse decrease in the carbohydrate content. Fermentation and roasting significantly (P ≤ 0.05) reduced oxalate and tannin contents of the blends. However, phytate and phytate phosphorus were not significantly (P ≥ 0.05) affected. In sensory attributes, samples containing fermented African oil bean flour compete favourably with the control while samples having roasted blends had the least acceptability.

Key words: Millet, African oil bean seed, *ogi,* roasting, fermentation.

INTRODUCTION

In order to meet the challenging food need of man, most especially those in the developing countries, various approaches on the use of under utilised locally found foods to supplement the daily staple such as millet, maize, corn, cassava, etc. has been advocated as a measure. To tackle the root cause of malnutrition and hunger in developing world, the introduction of an approach geared towards providing adequate and sufficient nutrition is paramount (FAO, 2013). The basic requirement of man is his right to adequate and sufficient nutrition. Inadequate nutrition early in life and amongst the elderly can result in irremediable damage to the developing brain and body of infants as well as complications in the elderly (Nathan, 2008). In order to have a healthy population sufficiently satisfied with adequate nutrition hence, the need to exploit unexploited and underutilized food resources is essential (Enujiugha and Agbede, 2000).

Ogi is a gruel considered as a special transitional food cherished by infants and is characterised by its homogeneity and its liquid or semi-liquid consistency (Treche, 1998). Cereals grains such as maize, millet, sorghum, etc, are usually utilised locally in *ogi* production due to their

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high gelling capacity, availability, affordability and ease of processing. Millet, a predominantly cross pollinated crop found mostly in the semi-arid tropical region of Asia and Africa, is a starchy cereal crop used in gruel preparation (Rai et al., 1999). Though high in carbohydrate, millet has been found to be a good source of essential amino acids excluding lysine and threonine (Saleh et al., 2013) and phytochemicals and micronutrients (Mal et al., 2010; Singh et al., 2012). It is classified as the principal source of carbohydrate, vitamins and minerals for millions of the poorest people in the semi-arid tropics of Asia and Africa. Furthermore, millet is also used as temporary summer pasture crop and is considered one of the most important cereals in the tropics alongside rice, maize and sorghum (Mal et al., 2010).

Ogi production involves soaking the grains in water for 3 h until soft. Softened grains are then ground in to a slurry and wet-sieved through cheese cloth. The starch is allowed to sediment and ferment in a liquid menstrum (Ojokoh, 2009). Ready to serve *Ogi* is prepared by diluting the starch sediment in little amount of water after which boiling water is added to form an aqueous porridge (Ojokoh, 2009). It is considered a common staple food in most African countries. The product serves different categories of people in terms of its uses, such as weaning food for babies, breakfast cereal for adults, a meal to enhance breast milk production for nursing mothers, and recovery diet for the sick (Afolayan et al., 2009)

African oil bean seed (*Pentaclethra macrophylla* Benth.) is a leguminous woody plant belonging to the subfamily Mimosoideae which occurs naturally in the humid lowlands of west tropical Africa (Enujiugha, 2003). Its fermented form *Ugba* amongst the Igbos, is used for the preparation of many delicious African delicacies including African salad (abacha), soups and sausages for eating with different staples (Enujiugha, 2003). The seed serves as a source of edible oil and the residue after oil extraction is locally used for candles and soap making. African oil bean seed contains essential amino and fatty acids making it a good source of protein and calories (Enujiugha, 2003). It also serves as a good source of vitamin (Enujiugha and Olagundoye, 2001). This protein rich legume has been found to contain sufficient amounts of lysine and tryptophan, lacking in cereals (Ikediobi, 1981) and this unique attribute of the oil seed makes it a suitable and convenient raw material for supplementation to improve the protein quality of cereal-based products.

Despite the nutritive relevance of the African oil bean seed, its high oil content, short shelf life and the presence of antinutrients are some of the reasons limiting its use as a food supplement. Treatments such as soaking, fermentation, roasting, cooking and malting have been found to not only increase the nutritive quality of foods but also reduce antinutritional content (Ajeigbe et al., 2012; Enujiugha and Olagundoye, 2001). Enujiugha (2006) supplemented *ogi* with raw African oil bean seed for infant weaning. The high an-tinutrients found in the resultant gruel serve as its greatest limitation in spite of the increase in the nutrient composition of the blend.

This study therefore was aimed at subjecting African oil bean seed to roasting and fermentation treatments and then blending the treated flour obtained with millet flour. Inclusion of treated African oil bean seed flour in to millet *ogi* would enhance the nutritional composition of the blend most especially, the essential amino acid and fatty acid compositions as well as protein and calorie values. This would address problems of micro nutrient deficiency and protein energy malnutrition.

MATERIALS AND METHODS

Source of material

Millet and African oil bean seeds were purchased from the Kure Ultra-Modern Market Minna, Niger State, Nigeria.

Production of millet flour

Millet flour (MF) was produced using the method described by Enujiugha (2006). The grains were manually cleaned to remove stones and extraneous materials after which they were soaked for 72 h at 25°C. Soaked grains were dehulled and wet milled using laboratory Kenwood blender (Mini-Processor Model A90LD). The resultant slurry was fermented for 24 h at 25°C after which it was dewatered and then oven dried for 4 h at 65°C. The dried fermented flour lumps were broken down and milled into finer flour particles and passed through 0.25 mm sieve.

Production of treated African oil bean seed flour

Method described by Enujiugha and Olagundoye (2001) was adopted with slight modification. African oil bean seeds were manually sorted, cleaned and washed in cold tap water. Washed seeds were parboiled for 4 h and worked in a mortar to dehull the seeds and increase their surface area for easy fermentation and even penetration of heat during roasting. The kernels were then recooked for 5 h and washed in three changes of water, then soaked for 6 h.

Fully soaked kernels were drained and washed again in three changes of water. Washed kernels were then divided into two equal portions (500 g each). One portion was oven dried at 100°C for 90 min and then roasted at 200°C for 90 min. The other portion was subjected to natural fermentation after inoculating for 16 h by spreading the cotyledons on a jute bag and covered with the same material.

The cotyledons were fermented for 72 h at 30°C. At the end of fermentation, the mash was oven dried at 100°C for 90 min. Roasted and fermented kernels were separately milled using a Laboratory Hammer Mill and defatted using Soxhlet extractor. The resultant flour samples were oven dried to eliminate residual solvent and ground into fine flour and passed through 0.25 mm sieve. The two flour samples were packaged inside high density polyethene bag and stored in the refrigerator at 4°C prior to further analysis.

Formulation of blends

Millet and treated African oil bean seed flours were blended at ratios 90:10, 80:20, 90:10 and 80:20% for roasted and fermented

Table 1. Proximate composition of composite flour samples.

Proximate (%)	A	B	C	D	E
Moisture	4.26^{a}±2.00	4.11 ^{a} ±2.00	5.89 ^{a} ±2.00	3.43 ^{a} ±2.00	4.21^{a} ±2.00
Crude fat	3.26^{b}±2.00	5.49^{ab}±2.00	8.64^{a}±2.00	3.10^{b}±2.00	4.03^{b}±2.00
Crude protein	8.32^{b}±2.00	14.37^{a}±2.00	12.92^{a}±2.00	13.45^{a}±2.00	12.32^{a}±2.00
Crude fibre	2.27^{a}±2.00	3.81^{a}±2.00	4.02^{a}±2.00	3.52^{a}±2.00	4.11^{a}±2.00
Ash	1.07^{a}±2.00	1.07^{a}±2.00	1.22^{a}±2.00	1.31^{a}±2.00	1.32^{a}±2.00
Carbohydrate	81.43^{a}±2.00	71.76^{c}±2.00	67.93^{d}±2.00	75.81^{b}±2.00	74.62^{bc}±2.00
Energy value (Kcal,100g)	388.34^{c}±2.00	393.75^{b}±2.00	401.10^{a}±2.00	384.90^{cd}±2.00	384.01^{d}±2.00

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same row are significantly different ($p<0.05$). A = 100% millet flour; B = 90% millet flour and 10% roasted African oil bean seed flour; C = 80% millet flour and 20% roasted African oil bean seed flour; D = 90% millet flour and 10% fermented African oil bean seed flour; E = 80% millet flour and 20% fermented African oil bean seed flour.

flours, respectively, with 100% millet flour as the standard. A Kenwood mixer at speed 6 for 5 min was used for mixing flour samples to achieve uniform blending.

Proximate analysis

The moisture content, fat, crude protein, crude fibre, ash and carbohydrate of the flour blends and the control were determined using the method described by AOAC (2000).

Antinutrient determination

Phytin-phosphorus was determined as described by Wheeler and Ferrel (1971). Phytic acid was quantified as described by Aletor and Fasuyi (1997). Total cyanide was analyzed according to the method of AOAC (1990).

Determination of tannin was done according to the method outlined by Makkar et al. (1993). Oxalate content was quantified as described by Oke (1969). Antinutrients were determined in both the control and the blends.

Determination of sensory properties

A thirty-member panel consisting of students and staff of Food Science and Nutrition Option, Department of Animal Production of Federal University of Technology, Minna, Niger State were involved in the sensory evaluation. Ready to serve *Ogi* from various blends and the control were prepared using the traditional method of mixing the flour with a little amount of water and then adding boiling water to the paste until a porridge-like consistency is obtained. After preparation, the porridges were served and presented in coded white plastic plates.

The order of presentation of samples to the panel was randomized. Tap water was provided to rinse the mouth between evaluations. The panellists were instructed to evaluate the coded samples for appearance, texture, aroma, taste, colour and overall acceptability. Each sensory attribute was rated on a 9-point Hedonic scale (1=disliked extremely while 9-liked extremely).

Statistical analysis

Torrie, 1980). The difference between mean values was determined by least significant difference (LSD) and accepted at 5% probability level.

RESULTS AND DISCUSSION

Effect of treatment on proximate composition of millet and African oil bean seed flour blends

Table 1 shows the proximate composition of the flour blends. The result shows that the flour blends and the control were not significantly ($p > 0.05$) different in moisture content, crude fibre and ash. However, the samples were significantly ($p < 0.05$) different in crude fat, crude protein, total carbohydrate and energy value.

The fat content of the control (sample A) and blends containing 10 and 20% fermented African oil bean flours (samples B and C) were not significantly ($p>0.05$) different from each other. However, samples D and E containing 10 and 20% roasted African oil bean flour were signify-cantly ($p<0.05$) high in fat content. The increase in the fat content of blends with roasted African oil bean flour might be attributed to the contribution of fat globules extracted from the flour matrix during roasting. Unlike the blends that had fermented flour, the low fat content might be due to the utilisation of the available fat by microbes during fermentation. This increase shows a similar trend in roasted unripe plantain, corn and ripe plantain reported by Adetunde et al. (2012). Furthermore, loss of moisture during roasting could concentrate other macro molecules hence, increasing their concentration. From the stand point of storage, blends with high fat will be more susceptible to rancidity.

The protein content of blends containing both fermented and roasted African oil bean flours (samples B, C, D and E) were found to be significantly ($p < 0.05$) high. Fermentation and roasting both have beneficial effects on Data obtained were analysed using one way ANOVA (Steel and protein digestibility. The complex peptides were

Table 2. Anti-nutrient composition of flour composite.

Anti-nutrient (%)	A	B	C	D	E
Phytate	20.14^{b}±2.00	25.20^{a}±2.00	22.92^{ab}±2.00	22.85^{ab}±2.00	21.71^{ab}±2.00
Oxalate	22.50^{a}±2.00	15.96^{b}±2.00	17.94^{b}±2.00	17.92^{b}±2.00	16.35^{b}±2.00
Tannin	220.00^{a}±2.00	185.00^{b}±2.00	160.00^{d}±2.00	181.00^{c}±2.00	152.50^{e}±2.00
Cyanide	6.99^{c}±2.00	14.41^{b}±2.00	20.52^{a}±2.00	11.35^{b}±2.00	23.58^{a}±2.00
Phytate phosphorus	5.52^{a}±2.00	7.18^{a}±2.00	6.28^{a}±2.00	6.26^{a}±2.00	6.05^{a}±2.00

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same row are significantly different at ($p < 0.05$). A = 100% millet flour; B = 90% millet flour and 10% roasted African oil bean seed flour; C = 80% millet flour and 20% roasted African oil bean seed flour; D = 90% millet flour and 10% fermented African oil bean seed flour; E = 80% millet flour and 20% fermented African oil bean seed flour.

broken down to low molecular weights and constituent amino acids thereby increasing the active sites for protease activities during digestion (James and Nwabueze, 2013a). There was a significant ($p < 0.05$) reduction in the carbohydrate content of flour blends containing treated African oil bean flour at different ratios with sample E which contains 20% roasted flour having the lowest value (67.93%). This result agreed with report by Enujiugha (2006). The reduction could be attributed to the replacement of millet flour (carbohydrate source) with treated African oil bean flour at different ratios. Also, action of micro-organisms which degrade starch into sugars during fermentation could cause an increase in sugar content and a decrease in starch level (Huang and Zhang, 2011). This degradation would improve the nutritive value and further enhance absorption and digestibility of the gruel produced.

Roasting brought about significant ($p < 0.05$) increase in energy values with sample E having the highest increase (401 Kcal). This shows that roasting African oil bean flour and 20% level of substitution had the highest energy value. It could be suggested that roasting being a heat process could results in increase concentration of certain nutrients.

Effect of treatment on antinutrients in millet and African oil bean seed flour

The antinutrient composition of the control and the blends is shown in Table 2. The result shows that fermentation, roasting and replacement of millet at 10 and 20% were not significantly ($p > 0.05$) affected the phytate and phytate phosphorus content of all the samples. The oxalate contents of blends containing fermented and roasted African oil bean flour at different replacement levels were found to be significantly ($p<0.05$) low. This shows that fermentation and roasting the African oil bean flour have the potency in bringing down the oxalate content of the blends at replacement levels used. This is in line with the research conducted by Enujiugha and Olagundoye (2001) who reported that fermentation and roasting reduced the phytate content of African oil bean. Oxalate in the body combines with divalent cations Ca^{++}, Fe^{++}, forming their insoluble salts. These insoluble salts obstruct kidney tubules leading to kidney stones (Coe, 2005). Hence, it reduction implies increase mineral bioavailability and reduction of renal dysfunction. The high oxalate content in the raw millet flour (22.50%) agreed with report by Enujiugha (2006). Leaching out of phytate present in the outer (aleurone) layer of seeds during soaking brings about phytate reduction. Phytic acid forms insoluble complex with certain trace elements, zinc, iron and copper. The complex formed reduces the mineral bioavailability which in turn deplete tune over of haemoglobin production and impair metabolic process (James and Nwabueze, 2013b). There was significant ($p < 0.05$) decrease in tannin content of blends at different substitution levels. This shows that application of heat during roasting and activities of microbes during fermentation of African oil bean flour have reducing effect on tannin. The result is in line with that of Vadivel et al. (2010) who reported that fermentation of soybean brings about breakdown and decrease in tannin content. Reduced tannin content in the blends means increased bioavailability of macromolecules notably, proteins; increased palatability; reduced pathogenesis of cancer development and reduced damage to intestinal tract (Makkar and Becker, 1996; Uzeochina, 2007). The cyanide content of the control was found to be signify-cantly ($p < 0.05$) low as compared to the blends containing treated African oil bean seed flour. It was observed that, blends containing 20% treated African oil bean seed flour were found to be significantly ($p < 0.05$) high in cyanide. This means that, treated African oil bean seed flour has high cyanide content hence, its inclusion at 10% level is preferable.

Sensory attributes of prepared *ogi* from different flour blends

The sensory properties of porridge prepared form the control and different blends are shown in Table 3. The result shows that all the samples were significantly ($p < 0.05$) different in the attributes measured (favour,

Table 3. Sensory attributes of composite flours.

Sensory property	A	B	C	D	E
Flavour	8.73^{a}±2.00	7.58 ^{a} ±2.00	5.73^{ab} ±2.00	3.77^{b}±2.00	2.88^{b}±2.00
Colour	8.59^{a} ±2.0	7.62 ^{a} ±2.00	5.81^{ab} ±2.00	3.31^{b}±2.00	2.87^{b}±2.00
Mouth feel	8.31^{a} ±2.0	7.27 ^{a} ±2.00	5.46 ^{ab} ±2.00	3.35^{b}±2.00	2.88^{b}±2.00
Texture	8.31^{a} ±2.0	7.19 ^{a} ±2.00	5.62^{ab} ±2.00	3.15^{b}±2.00	2.91^{b}±2.00
Taste	8.69^{a} ±2.0	7.69 ^{a} ±2.00	5.96^{ab} ±2.00	3.62^{b}±2.00	3.23^{b}±2.00
Overall acceptance	8.36^{a} ±2.0	7.35 ^{a} ±2.00	5.65^{ab} ±2.00	3.54^{b}±2.00	2.92^{b}±2.00

Values are means and standard deviations of three determinations. Values not followed by the same superscript in the same row are significantly different ($p<0.05$). A = 100% millet flour; B = 90% millet flour and 10% roasted African oil bean seed flour C = 80% millet flour and 20% roasted African oil bean seed flour; D = 90% millet flour and 10% fermented African oil bean seed flour; E = 80% millet flour and 20% fermented African oil bean seed flour.

appearance, mouth feel, taste, texture and overall acceptability). Blends containing fermented African oil bean flour compete favourably with the control in all the sensory attributes measured.

Samples A, B and C were found to be more acceptable in all parameters of sensory properties analysed. However, samples D and E which contained 10 and 20% roasted African oil bean flour were significantly ($p < 0.05$) low.

This might be possibly as a result of roasting which could have impacted unpleasant characteristic attributes. Omemu (2011) reported that supplementation of fermented cereal gruel with protein-rich food in a bid to upgrade their nutrient composition sometimes bring about undesirable sensory properties when substituted with cereal. The acceptability of the flour blend with 10 and 20% in cooperation with fermented African oil bean were better preferred by the test panellists.

Conclusion

Inclusion of treated African oil bean flour significantly increased the crude fat, crude protein and energy values of the blends. However, moisture content and crude fibre were not significantly affected. Replacement of millet with treated African oil bean flour was significantly reduced, and tannin contents of the blends and phytate and phytate phosphorus were not significantly affected. Blends containing fermented African oil bean flour had superiority in proximate, antinutrients and sensory property over the control and the blends containing roasted African oil bean flour.

Conflict of interest

The authors did not declare any conflict of interest.

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A survey on existing practices adopted in *Dambu* production and utilization in some northern states of Nigeria

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A survey was conducted in relation to the production process of *dambu*. *Dambu* is a staple food for the Fulanis and Hausas. A majority of the *dambu* dealers (42.7%) were less than 25 years old. A proportion of 78.7% of processors of *dambu* were females. The processors/consumers (37.4%) indicated that millet was the major raw material for *dambu* production. A significant difference (p = 0.01) existed among respondents on the variety of millet chosen for *dambu* production. The study reveals that 'gero' is the variety in common use. Spices are indispensable as an ingredient in *dambu* production with ginger being the single most important spice (p = 0.07). The traditional pounding method for processing millet into flour is still very much used. The processors (58.9%) and retailers (41.0%) generally agreed that the method of marketing *dambu* is by hawking in transparent low density polyethylene package. Sun drying as indicated by 35.3% of the respondents was the most common method of storing left-over *dambu*. Total percentage of 27.4, 39.2 and 33.4% were deduced for the poor, the middle and the rich class consumption of *dambu*, respectively. With increasing influence of advertising upon customers, small food processing enterprises making *dambu* will have to improve the packaging and preservation of their products as to survive the competition. The implications of these are highlighted and a possible solution of optimizing the *dambu* production process is recommended.

Key words: Ginger, cloves, *dambu*, millet, packaging, shelf-life.

INTRODUCTION

In tropical Africa, cereal grains are milled and used to produce different types of food which are known by various names in different parts of the African continent as thin porridge (ogi) (Apena et al., 2006), thick porridge

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fura (Jideani et al., 1995; Umoh, 2003; Filli et al., 2007), baked or fried fermented bread such as *mawè* (Hounhouigan et al., 1993), *masa* (Ayo et al., 2008), snacks (Vidyavati et al., 2004), alcoholic beverage (burukutu) (Igyor et al., 2006) and non-alcoholic beverages such as *kunun zaki* (Gaffa and Jideani, 2001; Ayo and Obeya, 2004; Gaffa et al., 2004). In West Africa, particularly in the Northern part of Nigeria, one of such cereal-based products is called *dambu* - a steamed non-fermented granulated dumpling generally made from pearl millet (Nkama et al., 1999; Agu et al., 2007, 2008). It is known as *dambu* among the Hausas while among the Fulanis, it is known as "nyamri" (Haruna, 2003). *Dambu* is similar to *fura* – a semi- solid dumpling cereal meal, but differs only in the production process (Jideani and Wedzicha, 1994).

Dambu is produced mainly from moistened pearl millet flour, blended with spices and steamed for 20 min. using two fold systems, which involves pouring the mixed flour with spices into a sieve and placed on an open pot that contains little water. Care is taken for the sieve not to touch the water. As the water in the pot boils, the steam cooks the *dambu* on the sieve. It has coarse particles resembling moistened couscous. It is sprinkled into fermented skimmed milk (*nono*), or fermented whole milk (*Kindirimo*) and sugar may be added to taste. It is a popular mid-day meal called "*dambu* da nono" in Nigeria (Nkama et al., 1999).

Dambu contains energy of 257 kcal, moisture of 37%, ash 1.1%, crude protein 10.7%, fat 3.4%, crude fibre 2.0%, and carbohydrate 45.9% per 100 g. It also contains essential and non- essential amino acids with major and minor mineral elements (Agu et al., 2007). *Dambu* has been produced with different grain types and pearl millet was rated high based on aroma, texture and overall acceptability (Agu et al., 2007) and based on this, using different pearl millet cultivars will be productive.

Dambu is produced at home both for family and commercial consumption. Most *dambu* producers use the traditional method involving wooden mortar to dehull or mill the grains. Pearl millet products like *fura* and *dambu* are sold from calabash containers to consumers without appropriate packaging (Jideani et al., 2001a; Agu et al., 2008). Good packaging not only serves as food container but protects and carries the necessary information about the food product. It is particularly important in countries with tropical and humid climates, where food deterioration is most rapid (UNIFEM, 1993). *Dambu* is hawked by the local producers. The processors and retailers of *dambu* are only concerned with having a calabash or enamel porcelain container for their food. The practice of opening and closing of the container during sales subjects the food to microbial contamination. *Dambu* has a limited shelf-life of one day at ambient temperature. Usually, a day after the production, *dambu* shows visible mould growth on the surface. The short shelf-life has always been a major deterrent to large scale production. Thus, improving the processing, packaging and storage life of *dambu* are of interest before food manufacturers can think of large scale production. This study was conducted to establish the materials and the ingredients as well as the production process for *dambu* from the processors and consumers. This will provide baseline information for food manufacturers and a basis for improving this process.

MATERIALS AND METHODS

Survey area

States in northern Nigeria chosen for the study included Bauchi, Plateau, Kaduna, Kano, Gombe, Jigawa, Borno, Adamawa and Katsina. These are the major *dambu* producing and consuming states in Nigeria (Jideani et al., 2001).

Methods

The study data was collected using a structured questionnaire. The questionnaires were designed and pretested before being distributed to the potential respondents. The questionnaires were divided into sections in order to collect information on:

1. Socio demographic particulars,
2. Raw materials for *Dambu* production,
3. Steps involved in primary processing,
4. Steps involved in secondary processing mode of display,
5. Equipment used and its cost details,
6. Cost of inputs, packaging, mode od display, shelf life storage methods product utilization,
7. By product utilization and,
8. Personnel needed

Data collection and analysis

The processors, retailers and consumers of *dambu* in Bauchi, Plateau, Kaduna, Kano, Gombe, Jigawa, Borno, Adamawa and Katsina states were distributed a total of 1000 questionnaires. The questionnaires were completed by the respondents in their homes, markets and offices. There was agreement on when the completed questionnaires could be picked up. Where the respondents could not speak English, interviews were conducted in the local language with the assistance of an interpreter. Questionnaires (827) representing 82.7% were retrieved. The remaining 17.3% were either reluctant to return their questionnaire or could not be located.

Data from the questionnaires were analyzed by tally and the number of the tallies for each question was entered into a MINITAB (version 11, 1996) and tabulated as percentages. The Kruskal-Wallis H test (a non-parametric one-way ANOVA) was used to compare the data.

RESULTS AND DISCUSSION

Demography of respondents

Demographic characteristics in relation to the status of

Table 1. Demographic Characteristics as related to the processor retailer/consumer status.

Question	Processor	Retailer	Processor/Retailer	Consumer	Processor/Consumer	Total	P-value
	n =216	n = 83	n = 211	n = 143	n = 174	n = 827	
Sex							
Male	46 (21.3)	22(26.5)	67 (31.8)	77 (53.8)	78 (44.8)	290 (35.1)	
Female	170 (78.7)	61 (73.5)	144 (68.2)	66 (46.2)	96 (55.2)	537 (64.9)	0.07
Age							
≤25	106 (49.1)	37 (44.6)	79 (37.0)	58 (40.6)	73 (41.9)	353 (42.7)	
26-35	70 (32.4)	27 (32.5)	43 (20.4)	49 (34.3)	40 (23.0)	229 (27.7)	
36-45	30 (13.9)	9 (10.8)	56 (26.5)	36 (25.2)	15 (8.6)	146 (17.7)	
>46	10 (4.6)	10 (12.0)	33 (15.6)	-	46 (26.4)	99 (12.0)	0.01
Education							
None	55 (25.5)	12 (14.5)	74 (35.1)	37 (25.9)	39 (22.4)	217 (26.2)	
Primary	59 (27.3)	28 (33.7)	64 (30.3)	26 (18.27)	34 (19.5)	211 (25.5)	
Sec/Tech/Com	36 (16.7)	7 (8.4)	40 (19.0)	33 (23.1)	12 (6.9)	128 (15.5)	
Vocational	42 (19.4)	20 (24.1)	28 (13.3)	21 (41.7)	44 (25.3)	155 (18.7)	
University/Poly	24 (11.1)	16 (19.3)	5 (2.4)	26 (18.2)	45 (25.9)	116 (14.0)	0.23
Ethnicity							
Kanuri	31 (14.4)	21 (25.3)	5 (2.4)	56 (39.2)	28 (16.1)	141 (17.0)	
Hausa	84 (38.9)	40 (48.2)	93 (44.1)	32 (22.4)	46 (26.4)	295 (35.7)	
Fulani	101 (46.8)	22 (26.5)	113 (53.6)	55 (38.5)	100 (57.5)	391 (47.3)	0.06

Figures in parentheses are percentages; Kruskal - Wallis one-way ANOVA test.

the respondents are presented in Table 1. A total of 827 respondents were used for the study. Processors /retailers were those who produced *dambu* and either hawked it or sold it on a wholesale basis. Retailers- those who do not process *dambu* but bought from processors to sell were also surveyed. Processors/consumers were those who produced *dambu* for consumption purposes. Consumers were those not involved in processing but purchased *dambu* for consumption (Jideani et al., 2001). The respondents were comparable in their demographic characteristics ($p < 0.05$) for any variable.

The respondents (35.1%) of those directly involved with processing and retailing of *dambu* did not have a formal education. This may contribute to improper hygienic practices during production and handling of *dambu* which could lead to product with potential health hazards to the consumers. A significantly ($p < 0.05$) greater proportion (53.6%) of the processor/retailers and consumers of *dambu* were Fulanis, followed by the Hausas (44.1%). *Dambu* is therefore a staple food for both Hausas and Fulanis. Majority of the *dambu* dealers were less than 25 years old. A proportion of 78.7% of processors of *dambu* were females. The processing and marketing of *dambu* is known to be a woman's trade.

The study reveals that no male considered himself to be a processor. Those men who know the processing method for *dambu* either watched their mothers make it when they are younger or watched their wives. It was however surprising to note that there were some men who were retailers (26.5%) and 44.5% were processors /consumers. More men (53.8%) consumed *dambu* compared to women (46.2%). This could be because men are usually the bread winners in Africa and in these cultures have the right to spend their money anyhow and whenever they want and for anything of their choice. The women who in this culture depend on what is made available to them by their husbands, are concerned primarily with the feeding of the family and therefore, would have limited money to purchase *dambu* for consumption except when the female has made it part of the family menu (Jideani et al., 2001).

Raw materials and ingredients for *dambu* production

The major raw materials and ingredients used for *dambu* production are shown in Table 2. The processors /consumers (37.4%) indicated that millet was the major raw material for *dambu* production. The processors /consumers (36.8%) indicated that there were three

Table 2. Major raw materials and ingredients used in *dambu* production.

Question		Status						P-value
		Processor	Retailer	Processor/ Retailer	Consumer	Processor/ Consumer	Total	
		n =216	n = 83	n =211	n =143	n = 174	n = 827	
(a)	**Major grain**							
	Millet	59 (27.3)	33 (39.8)	70 (33.2)	77 (53.8)	64 (36.8)	303 (36.6)	0.01
	Sorghum	72 (33.3)	21 (25.3)	64 (30.3)	45 (31.5)	39 (22.4)	241 (29.1)	
	Maize	48 (22.2)	25 (30.1)	59 (28.0)	15 (10.5)	65 (37.4)	212 (25.6)	
	“Acha”	37 (17.1)	4 (4.9)	18 (8.5)	6 (4.2)	6 (3.4)	71 (8.6)	
(b)	**How many varieties?**							
	1	48 (22.2)	49 (59.0)	32 (15.2)	71 (49.7)	29 (16.7)	229 (27.7)	0.48
	2	58 (26.9)	19 (22.9)	23 (10.9)	52 (36.4)	13 (7.5)	165 (19.9)	
	3	53 (24.5)	6 (7.2)	56 (26.5)	16 (11.2)	64 (36.8)	195 (23.6)	
	4	42 (19.4)	_	87 (41.2)	4 (2.8)	16 (9.2)	149 (18.0)	
	All	15 (6.9)	9 (10.8)	13 (6.2)	_	52 (29.9)	89 (10.8)	
(c)	**Variety commonly used**							
	“Gero”	89 (41.2)	21 (25.3)	66 (90.4)	53 (37.1)	54 (31.0)	283 (34.2)	0.01
	“Dauro”	58 (26.9)	15 (18.1)	73 (34.6)	32 (22.4)	45 (25.9)	223 (27.0)	
	“Maiwa”	17 (7.9)	29 (34.9)	49 (23.2)	21 (14.7)	19 (10.9)	135 (16.3)	
	Pearl Millet	50 (24.3)	18 (21.7)	23 (10.9)	13 (9.1)	38 (21.8)	142 (17.2)	
	No Response	2 (0.9)	_	-	24 (16.8)	18 (10.3)	44 (5.3)	
(d)	**Can a substitute be used?**							
	Yes	143 (66.0)	49 (59.0)	178 (84.4)	55 (38.5)	75 (43.1)	500 (60.5)	0.27
	No	73 (33.8)	34 (41.0)	33 (15.6)	88 (61.5)	99 (56.9)	327 (39.5)	
(e)	**If yes what is the substitute?**							
	Sorghum	63(44.1)	12(24.5)	66(37.1)	12(21.8)	34(45.3)	187 (37.4)	0.8
	“Acha”	-	-	26(14.6)	8(14.5)	29(38.7)	63 (12.6)	
	Millet	55(38.5)	20(40.8)	54(30.3)	14(25.5)	10(13.3)	153 (30.6)	
	Maize	25(17.5)	17(34.7)	32(17.9)	17(30.9)	-	91 (18.2)	
	No response	-	-	-	4(7.3)	2(2.7)	6 (1.2)	
	Total	143	49	178	55	75	500	
(f)	**Reason for Substitute.**							
	Unavailability of millet	173 (80.0)	34 (41.0)	99 (46.9)	51 (35.7)	120 (69.0)	477 (57.7)	0.04
	Choice	31 (14.4)	27 (32.5)	38 (18.0)	22 (15.4)	54 (31.0)	172 (20.8)	
	Economy	12(5.6)	22 (26.5)	74 (35.1)	70 (49.0)	_	178 (21.5)	
(g)	**Effect of substitute on quality**							
	Colour	59 (27.3)	17 (20.5)	32 (15.2)	46 (32.2)	32 (18.4)	186 (22.5)	0.17
	Texture	71 (32.9)	10 (12.0)	83 (39.3)	21 (14.7)	32 (18.4)	217 (26.2)	
	Taste	40 (18.5)	24 (28.9)	68 (32.2)	27 (18.9)	53 (30.5)	212 (25.6)	
	Colour, Taste, Texture	36 (16.7)	8 (9.6)	17 (8.1)	41 (28.7)	29 (16.7)	131 (15.8)	
	Texture, Texture	10 (4.6)	24 (28.9)	11 (5.2)	8 (5.6)	28 (16.1)	81 (9.8)	

Table 2. Contd.

(h)	**Effect of substitute on demand**							
	Increase	125 (57.9)	31 (37.3)	33 (15.6)	57 (39.9)	52 (29.9)	298 (36.0)	
	Decrease	65 (30.1)	29 (34.9)	74 (35.1)	52 (36.4)	65 (37.4)	285 (34.5)	0.85
	Remain the same	26 (12.0)	23 (27.7)	104 (49.3)	34 (23.8)	57 (32.8)	244 (29.5)	
(i)	**Cereal used in combination**							
	Yes	140 (64.8)	60 (72.3)	114 (54.0)	42 (29.4)	147 (84.5)	503 (60.8)	0.22
	No	76 (35.2)	23 (27.7)	97 (46.0)	101 (70.6)	27 (15.5)	324 (39.2)	
(j)	**Preferred combination**							
	Millet & Maize	81 (57.9)	15 (25.0)	22 (19.3)	33 (78.6)	66 (44.9)	217 (43.1)	
	Millet & Sorghum	46 (32.9)	22 (36.7)	75 (65.8)	9 (21.4)	81 (55.1)	233 (46.3)	0.08
	Maize & Acha	13 (9.3)	23 (38.3)	17 (14.9)	_	_	53 (10.5)	
	Total	140	60	114	42	147	503	
(k)	**Purpose of combination**							
	To increase test	69 (31.9)	15 (18.1)	17 (8.1)	23 (16.1)	35 (20.1)	159 (19.2)	
	To make product brighter	82 (38.0)	12 (14.5)	46 (21.8)	59 (41.2)	49 (28.2)	248 (30.0)	0.66
	Absence of required grain	13(6.0)	34(41.0)	84 (40.0)	34 (23.8)	62 (35.6)	227 (33.5)	
	No response	52(24.1)	22(26.5)	64 (30.3)	27 (18.9)	28 (16.1)	193 (23.3)	
(l)	**Effect of combination on quality**							
	Colour	52 (24.1)	18 (21.7)	52 (24.6)	21 (14.7)	38 (21.8)	181 (21.9)	
	Texture	45 (20.8)	23 (27.7)	40 (19.0)	33 (23.1)	39 (22.4)	180 (21.8)	0.81
	Taste	69 (31.9)	36 (43.4)	27 (12.8)	27 (18.9)	42 (24.1)	201 (24.3)	
	Others	50 (23.2)	6 (7.2)	92 (43.6)	62 (43.4)	55 (31.6)	265 (32.0)	
(m)	**Effect of combination on demand**							
	Increase	68 (31.5)	34 (41.0)	48 (22.7)	53 (37.1)	21 (12.1)	224 (27.1)	
	Decrease	108 (50.0)	28 (33.7)	96 (45.5)	39 (27.3)	100 (57.5)	371 (44.9)	0.18
	Remain the same	40 (18.5)	21 (25.3)	67 (31.8)	51 (35.7)	53 (30.5)	232 (28.0)	
(n)	**Other ingredients used**							
	Potatoes	21 (9.7)	19 (22.9)	28 (13.3)	18 (12.6)	92 (52.8)	178 (21.5)	
	Ginger	63 (29.2)	18 (21.7)	66 (31.3)	24 (16.8)	20 (11.5)	191 (23.1)	0.71
	Pepper	25 (11.6)	24 (28.9)	54 (25.6)	47 (32.9)	33 (18.9)	183 (22.1)	
	Cloves	107 (49.5)	22 (26.5)	63 (29.9)	54 (37.8)	29 (16.7)	275 (33.2)	
(o)	**Proportion added**							
	Cloves/ginger/pepper							
	1:01:01	73 (33.8)	80 (96.4)	192 (91.0)	74 (51.7)	82 (47.1)	501 (60.6)	
	2:01:02	45 (20.8)	3 (3.6)	7 (3.3)	18 (12.6)	43 (24.7)	116 (14.0)	0.002
	4:01:01	93 (43.1)	-	12 (5.7)	51 (35.7)	49 (28.2)	205 (24.8)	
	No response	5 (2.3)	-	-		-	5 (0.6)	
(p)	**Commonest spice used**							
	Ginger	46 (21.3)	57 (68.6)	53 (25.1)	43 (30.1)	84 (48.3)	283 (34.2)	

Table 2. Contd.

	Cloves	78 (36.1)	8 (9.6)	44 (20.9)	63 (44.1)	63 (36.2)	256 (31.0)	
	Pepper	84 (38.9)	13 (15.7)	62 (29.4)	26 (18.2)	21 (12.1)	206 (24.9)	0.07
	Ginger/Pepper	8 (3.7)	5 (6.02)	52 (24.6)	11 (7.7)	6 (3.4)	82 (9.9)	
(q)	**Is it added for any purpose?**							
	Yes	99 (45.8)	35 (42.2)	82 (38.9)	94 (65.7)	92 (52.9)	402 (48.6)	0.68
	No	117 (54.2)	48 (57.8)	129 (90.2)	49 (34.3)	82 (47.1)	425 (51.4)	

Figures in parentheses are percentages; Kruskal - Wallis one-way ANOVA test.

varieties of millet, while (7.5%) indicated only two varieties. The number of varieties of millet varies from one state to another. For instance, in Bauchi State, there are three varieties of pearl millet in the market. These are 'gero', 'dauro', or 'maiwa' and 'gayamba'. In the other states, 'gero' and 'dauro' are very common. A significant difference ($p = 0.01$) existed among respondents on the choice of variety of pearl millet for *dambu* production. Majority of the respondents indicated that 'gero' is the variety in common use.

Although millet is the major cereal for *dambu* production, some other substitute grain can also be used alone or in combination with millet (Table 2). The choice of a substitute grain was not significant ($p = 0.27$). Among other substitute grain, sorghum was a significant grain of choice. The present study results are in agreement with results of Agu et al. (2007, 2008) which stated that different cereal grains could be used for *dambu* production. The significant reason ($p = 0.04$) given for the choice of a substitute grain was unavailability of millet. Other reasons included (i) the processors choice (ii) not sufficient money to purchase millet.

Other ingredients added to cereal flour for *dambu* production were sweet potatoes, as sweeteners; ginger, pepper and cloves as flavouring, potatoes were added as a sweetener. There was no significant difference ($p = 0.71$) in the choice of these ingredients. The quantity of these spices added was generally small. However, some of the respondents who estimated the ratio of the mixture gave 1:1:1, 2:1:1 and 4:1:1 for cloves, ginger and pepper respectively. There was a significant ($p = 0.05$) difference in the proportion of these spices. According to Agu et al. (2007, 2008) spices like ginger, cloves and pepper are used for *dambu* production in various proportions.

Most of the respondent indicated that the spices were added mainly for flavour purposes and other reasons given where for its medicinal and sedative properties. According to Zaika (1988) and Norman (1990), spices are one of the various strongly flavoured or aromatic substance of vegetable origin obtained from tropical plants commonly used as condiments and possess significant antimicrobial activity. These spices include cloves, ginger, pepper and nutmeg (Norman, 1990). The levels of the spices added in *dambu* are obviously not sufficient to produce a preservative effect. However, the properties of these spices if used in an overall approach (Giese, 1994), could be effective in extending the shelf-life of *dambu*.

The production process for making *Dambu*

The production process for making *dambu* can be divided into primary and secondary processing (Figure 1). Primary processing are those to which the cereal grain is subjected but the product is still not directly consumable (Table 3). The objective is to perform a clean separation of the pericarp (bran) from the rest of the grain. In spite of the increasing number of dehulling machines, the producers of *dambu* prefer the traditional hand pounding of dehulling the grain and producing the flour. The respondents (25.8%) believed that decorticating (dehulling) of the grain was achieved by pounding the moistened grain with the butt end of a heavy wooden pole (pestle) in a mortar to knock off the outer seed coat. The beaten mixture was washed to separate the bran. The respondents (40.6%) indicated that the step of pounding was very important and needed improvement. The respondents (24.9%) suggested the use of a dehuller as alternative to local but wooden mortar and pestle approved by 45.1% of the respondents.

The endosperm obtained was allowed to drain in a sieve for 15 - 20 min, spread under the sun for 2 h as to further reduce the moisture content to 10 – 15% before grinding to flour by second pounding. Some of the respondents (51.4%) own grinding machines (metal but foreign) while 48.6% own metal but local. The respondents (processors) charge between 20 - 50 naira for grinding. The processors (55.1%) pay around N50 for grinding. The grinding of the flour was coarse then sieved using 600 µm.

Secondary processing are those processes to which the end product of primary processing (flour) undergoes to obtain *dambu* and it is shown in Table 3. This involved processes such as mixing, steaming, cooling and packaging. After mixing flour with the spices, it then poured into a sieve and placed on an open pot that contained little water. Care should be taken that the sieve

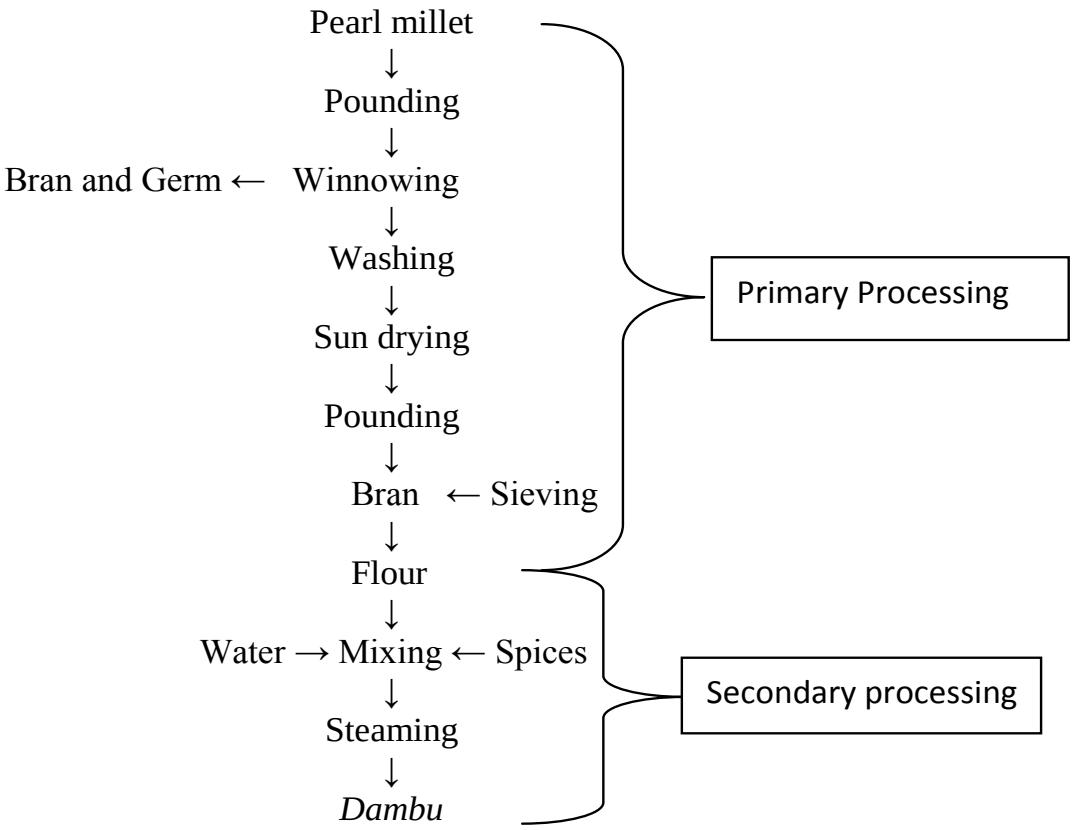

Figure 1. The Traditional Production of *dambu.*

does not touch the water. As the water in the pot boiled, the steam cooked the *dambu* on the sieve. The pot was covered and allowed to steam until the aroma was perceived. The respondents (28.3%) indicated that they mix between 30 – 60 S, while 32.6% indicated they steam between 20 – 45 min. The *dambu* after steaming was cooled between 1-5 min as indicated by 35.8% of the respondents.

The traditional pounding is a tedious task, which limits the use of the cereal. Enough pearl millet for a family meal (about 2.5 kg) takes two women about 1.5 h; converting the product into flour with a mortar and pestle requires an additional 2 h; sometimes more (NRC, 1996). Secondly, the moistening of the grain to facilitate removal of the bran and pounding into flour can result in slightly fermented flour leading to a modified flavour (Perten, 1983). Many of the respondents indicated that mechanical process needs to be improved in such a way to produce flour similar to that produced by the traditional method. The major criticism of the mechanical process is that dry grain must always be used, producing flour that is too dry and when used to produce *dambu,* does not result in the same taste as the traditionally processed flour (Jideani et al., 2001).

However, the production process for *dambu* can be optimized. Such optimization if achieved, would reduce the drudgery of women in *dambu* eating areas, improve health and family welfare and convert millet into a much more convenient grain and lead to food manufacturers getting involved in production of *dambu*. Moreover, with improvement in living standards in Nigeria and Africa at large, there has been an increasing demand for better quality food. Quality requirement for traditional *dambu* should consequently change. At present, the burden of the toil in millet processing is causing a silent rebellion against millet (Jideani et al., 2001) and the resulting *dambu*.

Packaging and distribution of *Dambu*

Packaging and distribution of *dambu* is shown in Table 4. The processors (58.9%) and retailers (41.0%) generally agreed that the method of marketing *dambu* is by hawking in a plastic (transparent low density polyethylene bag) while the processors (8.8%) and retailers (4.8%) indicated the use of calabash during hawking. At the vending point (market garage/park) the *dambu* are wrapped

Table 3. Primary and secondary processing of *dambu*.

Question		Status						
		Processor	Retailer	Processor/Retailer	Consumer	Processor/ Consumer	Total	P-value
		n = 216	n = 83	n = 211	n = 143	n = 174	n = 827	
(a)i	**Steps you follow in dehulling**							
	Winnowing, conditioning, pounding	64 (29.6)	0	91 (43.1)	16 (11.2)	42 (24.1)	213 (25.8)	
	Conditioning, pounding, drying	32 (14.8)	4 (4.8)	76 (36.0)	33 (23.2)	66 (37.9)	211 (25.5)	
	washing, winnowing, drying	88 (4.1)	56 (67.5)	28 (13.3)	15 (10.5)	19 (10.9)	206 (24.9)	
	No Response	32 (14.8)	23 (27.7)	16 (7.6)	79 (55.2)	47 (27.0)	197 (23.8)	1
(ii)	**Which step do you think improvement is Necessary?**							
	Winnowing	18 (8.3)	6 (7.2)	35 (16.6)	17 (11.9)	63 (36.2)	139 (16.8)	
	Pounding	83 (38.4)	59 (71.1)	76 (36.0)	44 (30.8)	74 (42.5)	336 (40.6)	
	Conditioning	32 (14.8)	_ (0)	25 (11.8)	36 (25.2)	_ (0)	93 (11.2)	
	Drying	8 (3.7)	_ (0)	19 (9.0)	21 (14.7)	28 (16.1)	76 (9.2)	
	No response	75 (34.1)	18 (21.7)	56 (26.5)	25 (17.5)	9 (5.2)	183 (22.1)	0
(b)i	**Steps in grinding to achieve desired quality**							
	Pounding & Sieving	93 (43.1)	45 (54.2)	65 (30.8)	43 (30.1)	69 (39.7)	315 (38.1)	
	Dehulling & Drying	78 (36.1)	8 (9.6)	31 (14.7)	72 (50.3)	28 (16.1)	217 (26.2)	
	Conditioning	42 (19.4)	30 (36.1)	87 (41.2)	13 (9.1)	47 (27.0)	219 (26.5)	
	Washing	3 (1.4)	_	28 (13.3)	15 (10.5)	30 (17.2)	76 (9.2)	0.04
(ii)	**Steps in which improvement is necessary to make grinding profitable**							
	Pounding & Sieving	36 (16.7)	43 (51.8)	65 (30.8)	88 (61.5)	83 (47.7)	315 (38.1)	
	Dehulling	79 (36.6)	32 (38.6)	83 (39.3)	35 (24.5)	46 (26.4)	275 (33.2)	
	Conditioning	56 (25.9)	8 (9.6)	33 (15.6)	16 (11.1)	27 (15.5)	140 (16.9)	
	Washing	45 (20.8)	_ (0)	30 (14.2)	4 (2.8)	18 (10.3)	97 (11.7)	0.01
(c)	**Steps in preparation of *dambu* with duration**							
i.	**Mixing**							
	30 - 60 S	53 (24.5)	15 (18.1)	72 (34.1)	31 (21.7)	63 (36.2)	234 (28.3)	
	1- 5 min	31 (14.4)	31 (37.3)	15 (7.1)	58 (40.6)	32 (18.4)	167 (20.2)	
	6 - 10 min	12 (5.6)	9 (10.8)	48 (22.7)	16 (11.2)	45 (25.9)	130 (15.7)	
	11 - 15 min	82 (38.0)	18 (21.7)	31 (14.7)	25 (17.5)	18 (10.3)	174 (21.0)	
	16 - 20 min	38 (17.6)	10 (12.0)	45 (21.3)	13 (9.1)	16 (9.2	122 (14.7)	0.22
ii.	**Steaming**							
	20 - 45 min	78 (36.1)	33 (39.8)	61 (28.9)	54 (37.7)	44 (25.3)	270 (32.6)	
	45 - 50 min	65 (30.1)	28 (33.7)	39 (.18.5)	68 (47.6)	33 (19.0)	233 (28.2)	
	50 - 60 min	59 (27.3)	12 (14.5)	83 (39.3)	13 (9.1)	15 (8.6)	182 (22.0)	
	1 - 12 h	14 (6.5)	10 (12.0)	28 (13.3)	8 (5.6)	82 (47.1)	142 (17.2)	0.44
iii.	**Cooling**							
	1 – 5 min	89 (41.2)	16 (19.3)	78 (37.0)	65 (45.4)	48 (27.6)	296 (35.8)	
	6 – 10 min	45 (20.8)	43 (51.8)	94 (44.5)	32 (22.4)	19 (10.9)	233 (28.2)	
	11 - 15 min	62 (28.7)	13 (15.7)	12 (5.7)	14 (9.8)	35 (20.1)	136 (16.4)	
	16 – 20 min	20 (9.25)	11 (13.3)	27 (12.8)	32 (22.4)	72 (41.4)	162 (19.6)	0.23
iv.	**Packaging**							
	10 – 20 min	4 1(19.0)	13 (15.7)	54 (25.6)	32 (22.4)	18 (10.3)	158 (19.1)	
	20 – 30 min	34 (15.7)	33 (39.8)	72 (34.1)	48 (33.6)	34 (19.5)	221 (26.7)	
	30 – 40 min	82 (38.0)	16 (19.3)	38 (18.0)	19 (13.3)	56 (32.2)	211 (25.5)	
	40 – 50 min	59 (27.3)	21 (25.3)	47 (22.3)	44 (30.8)	66 (37.9)	237 (28.6)	0.64

Figures in parentheses are percentages Kruskal - Wallis one way ANOVA test.

Table 4. Mode of marketing and storage stability of *dambu*.

Question		Status						
		Processor	Retailer	Processor/ Retailer	Consumer	Processor/ Consumer	Total	P-value
		n =216	n = 83	n =211	n =143	n = 174	n = 827	
(a)	**What packaging material is used for the product?**							
	Low density polyethylene	112 (51.9)	58 (69.9)	121 (57.3)	31 (21.7)	73 (42.0)	395 (47.8)	
	Paper	64 (29.6)	21 (25.3)	72 (34.1)	53 (37.1)	62 (35.6)	272 (32.9)	
	None	21 (9.7)	-	-	15 (10.5)	25 (14.4)	61 (7.4)	
	Calabash	19 (8.8)	4 (4.8)	18 (8.5)	44 (30.8)	14 (8.1)	99 (12.0)	0.01
(b)	**How do you market your product?**							
	Hawking	127 (58.8)	34 (41.0)	84 (39.8)	71 (49.7)	85 (48.9)	401 (48.5)	
	Whole sale	38 (17.6)	24 (28.9)	79 (37.4)	53 (37.1)	63 (36.2)	257 (31.1)	
	Consumption	46 (21.3)	25 (30.1)	48 (22.8)	19 (13.3)	26 (14.9)	164 (19.8)	
	No response	5 (31.3)	-	-	-		5 (0.6)	0
(c)	**Where do you sell your product?**							
	Market	102 (47.2)	136 (43.4)	116 (55.0)	44 (30.8)	93 (53.5)	391 (47.3)	
	Home	68 (31.5)	32 (38.6)	17 (8.1)	39 (27.3)	28 (16.1)	184 (22.2)	
	Garage/park	46 (21.3)	15 (18.1)	78 (36.8)	60 (42.0)	53 (30.5)	252 (30.5)	0.09
(d)	**How long it takes to sell the product?**							
	1 – 5 h	72 (33.3)	45 (54.2)	93 (44.1)	33 (23.1)	56 (32.2)	299 (36.1)	
	5 – 25 h	38 (17.6)	6 (8.4)	54 (25.6)	25 (17.5)	82 (47.1)	230 (27.8)	
	1 – 2 days	49 (22.7)	7 (8.4)	-	46 (32.2)	23 (13.2)	125 (15.1)	
	1 – 2 weeks	53 (24.5)	-	63 (29.9)	27 (18.9)	9 (5.2)	152 (18.4)	
	No response	4 (1.9)	-	1 (0.5)	12 (8.4)	4 (2.3)	21 (2.5)	0.01
(e)	**How is left over *dambu* preserved?**							
	Refrigeration	42 (19.4)	72 (86.8)	55 (26.1)	32 (22.4)	49 (28.2)	250 (30.2)	
	Sun-drying	97 (44.9)	5 (6.0)	82 (38.9)	45 (31.5)	63 (32.2)	292 (35.3)	
	Room Temperature	63 (29.2)	-	74 (35.1)	66 (46.2)	20 (11.5)	223 (27.0)	
	Others	-	-	-	-	42 (24.1)	42 (5.1)	
	No response	14 (6.5)	6 (7.2)	-	-	-	20 (2.4)	0
(f)	**How long can *dambu* be stored?**							
i.	**Refrigeration**							
	1 – 2 days	17 (16.2)	6 (35.3)	19 (23.2)	29 (40.0)	52 (57.4)	123 (34.4)	
	3 – 4 days	35 (33.3)	-	32 (39.0)	18 (28.6)	18 (19.8)	103 (28.8)	
	5 – 7 days	53 (50.5)	11 (64.7)	31 (37.8)	16 (25.4)	21 (23.7)	132 (36.9)	0.84
(ii)	**Sun-drying**							
	12 h	33 (45.2)	16 (36.4)	13 (24.5)	12 (31.6)	19 (17.0)	93 (35.8)	
	1 day	19 (26.0)	4 (9.1)	31 (58.5)	18 (47.4)	21 (39.6)	93 (35.8)	
	2 days and above	21 (28.8)	24 (54.6)	9 (17.0)	8 (21.1)	13 (24.5)	75 (28.8)	0.75
(iii.)	**Room Temperature**							
	1 day	15 (39.5)	3 (13.6)	34 (44.7)	16 (38.1)	12 (40.0)	80 (38.5)	
	2 days	18 (47.4)	19 (86.4)	17 (22.4)	5 (11.9)	3 (10.0)	62 (29.8)	

Figures in parentheses are percentages,Kruskal - Wallis one-way ANOVA test.

dambu migrating to towns and continents some distance from where *dambu* is made, a wider range of *dambu* packaging is required. Such packaging must be able to withstand transportation hazards, provide longer shelf-life and communicate necessary information about the product.

It takes about 1 - 5 h for 54.2% of the retailers to sell *dambu* worth N200.00. Some of the retailers (8.43%) would sell the same amount of *dambu* for 1 - 2 days. The rate of sale of *dambu* actually varies with the weather; peak of demand for *dambu* is usually the hot season (March to June) when the temperature range is 24.7 - 42.0°C.

Storage stability of *dambu*

Left over *dambu* can either be preserved by refrigeration, sun drying or at room temperature (Table 4). Sun drying as indicated by 35.3% of the respondents was the most common method of storing left over *dambu*. Choice of sun drying is not surprising since a majority of the processors/retailers do not have access to refrigerators. Storage of *dambu* under the sun was achieved by leaving the *dambu* in a desired container uncovered under the sun. Storage of *dambu* uncovered exposes the product to insects, flies and dust. The presence of dirt and dust on any food will increase the rate of spoilage because they carry microorganisms (UNIFEM, 1993). The habit of consuming the *dambu* without reheating can constitute a health hazard. The shelf-life of *dambu* stored under this condition was indicated by 35.6% of the respondents as 12 h, 35.6% of the respondents as 1 day (24 h) and 2 days and above by 28.7% of the respondents. It is known that shelf-life increases with decease in temperatures.

Storage at room temperature was achieved by leaving the *dambu* in a desired container (plate or calabash) uncovered. Storage of *dambu* without coverage predisposes the product to insect, flies and dust which will increase the rate of spoilage. This constitutes a health hazard. The variation in shelf-life depends on the ambient temperature. The best way to preserve *dambu* according to 36.9% of the respondents is by sun-drying. The respondents (33.5%) indicated it is by storing at room temperature and 28.8% at refrigeration temperature. Unfortunately, not many processors and consumers of *dambu* have access to refrigerators.

The two major signs of spoilage are off-odour and mould as indicated by 32.6 and 31.6% of the respondents respectively. Many of the respondents (32.9%) indicated that *dambu* stored under the sun was not safe for consumption after 1 day while majority indicated that it was after 4 - 7 days. Some of the respondents still believed that it can be consumed after this day.

***Dambu* Utilization Pattern**

The common liquid in which the *dambu* are crumbled are 'nono' (fermented skim milk) and 'Kindrimo' (fermented whole milk) as indicated by 35.7 and 37.7% of the respondents respectively. Water and milk seem to be the alternative media for eating *dambu* (Tables 5 and 6). *Dambu* can be eaten alone as indicated by 57.9% of the respondents' that is, in the absence of 'nono', 'kindrimo', water or milk. *Dambu* can be eaten as a main meal when accompanied with 'nono' or 'kindrimo'. Total percentage of 27.4, 39.2 and 33.4% was deduced for the poor, the middle and the rich class consumption of *dambu*, respectively. Majority of the respondents indicated that in a paper, low density polyethylene bag or mashed into fermented skim or whole milk for immediate consumption. *Dambu* is distributed with minimum packaging (Jideani et al., 2001). Processors and retailers of *dambu* are primarily concerned with reducing wastage and having a container for their food. Their choice of suitable packaging is to provide protection during a short shelf life and for local distribution. With population growth and consumers of infants at the age of 0 - 3 years consume *dambu*. The poor consumed less *dambu* as compared to other classes. A possible reason may be that they lack the money to meet up with the increasing cost of *dambu* and the corresponding 'nono' or 'kindrimo'. The respondents (55.5%) indicated that 30 - 60% of each class consumed *dambu*. The middle class ate more of *dambu* than all the classes. The rich does not eat as much as expected since money was not their problem. The problem of the rich and elites (particularly non - Fulani or Hausa) seemed to be the unsatisfactory method with which *dambu* was handled. The study reveals that there is a market potential for *dambu* if the processing method, packaging and storage are improved.

Economics of *dambu* processing

The economics of *dambu* processing is shown in Table 6. The cost of equipment for *dambu* production may be regarded as capital expenditure in the business of *dambu* production. Total cost was less than ₦2,000 depending on the size of the equipment purchased. Moreover, the equipment are not used for *dambu* production only. They find use in other household chores. This made them quite profitable to the processor.

The price of the grain varied, being low during the harvesting period. The cost of fuel (firewood), which was most common in use ranged from ₦35 - ₦40. The cost of outsourcing dehulling and grinding was about N10. Processing of 2.5 kg of millet grain will produce *dambu* worth ₦400 - ₦600 with by-product (bran) either sold for feedstuff or fed to animals as well as the water obtained from washing. *Dambu* production therefore was quite economical. Apart from the profit obtained from the marketing of the product, the by-products are of economic importance.

Labour cost in *dambu* production was fairly cheap. Majority employs assistants during *dambu* production.

Table 5. *Dambu* Product Utilization

Question		Status						
		Processor	Retailer	Processor/ Retailer	Consumer	Processor/Consumer	Total	P-value
		n = 216	n = 83	n = 211	n = 143	n = 174	n = 827	
(a)	**What is *dambu* eaten with?**							
	Soup	-	-	-	14 (9.79)	-	14 (1.7)	
	Water	-	-	-	4 (2.30)	-	4 (0.5)	
	"Madara"	86 (39.81)	16 (19.28)	6 (28.4)	35 (24.48)	59 (33.91)	202 (24.4)	
	"Nono"	73 (33.80)	45 (54.22)	77 (37.40)	17 (11.89)	81 (46.55)	295 (35.7)	
	"Kindirimo"	57 (26.39)	22 (26.51)	126(59.72)	73 (51.05)	34 (19.54)	312 (37.7)	0
(b)	**Can *dambu* be eaten alone?**							
	Yes	139 (64.35)	41 (49.40)	156(73.93)	90 (62.94)	53 (30.4)	479 (57.9)	
	No	77 (35.65)	42 (58.60)	55 (20.67)	53 (37.06)	121 (69.64)	348 (42.1)	0.36
(c)	**Is *dambu* eaten as a meal?**							
	Yes	138 (63.8)	31 (37.35)	95 (45.04)	41 (28.67)	80 (45.98)	385 (46.5)	
	No	78 (36.1)	52 (62.65)	116(54.98)	102 (71.33)	94 (54.04)	442 (53.4)	0.62
(d)	**If no, is it a snack?**							
	Yes	54 (69.23)	16 (30.77)	93 (80.17)	25 (24.51)	84 (84.3)	272 (61.5)	
	No	24 (30.77)	36 (69.23)	23 (19.83)	77 (75.49)	10 (10.63)	170 (38.5)	
	Total	78	52	116	102	94	442	0.3
(e)	**What classes of people eat *dambu*?**							
	Poor	74 (32.8)	19 (22.89)	44 (20.85)	32 (22.38)	61 (35.06)	227 (27.4)	
	Middle class	72 (33.53)	48 (57.83)	93 (44.08)	63 (44.06)	48 (27.59)	324 (39.2)	
	Rich	73 (33.80)	16 (19.28)	74 (35.07	48 (33.57)	65 (37.36)	276 (33.4)	
(f)	**Can you estimate the % of each?**							
	30 - 60%	118 (54.63)	60 (72.29)	163(77.15)	38 (26.57)	80 (45.98)	459 (55.5)	
	60 - 90%	68 (31.48)	15 (18.07)	42 (19.91)	71 (99.65)	66 (37.93)	262 (31.7)	
	90 - 100%	28 (12.96)	8 (9.69)	6 (2.81)	34 (15.87)	28 (16.99)	104 (12.6)	
	No response	2 (0.93)	_	_	_	_	2 (0.2)	
(g)	**Do infants eat *dambu*?**							
	Yes	115 (53.24)	45 (54.22)	142 (67.3)	78 (54.55)	92 (52.87)	472 (57.1)	
	No	101 (49.76)	38 (45.78)	69 (32.7)	65 (95.45)	82 (47.13)	355 (42.9)	0.26
(h)	**If yes at what age is *dambu* introduced?**							
	0 - 3 months	46 (40)	21 (46.67)	83 (58.45)	18 (23.08)	76 (82.61)	244 (51.7)	
	4 - 6 months	61 (53.04)	13 (28.89)	39 (27.46)	44 (56.04)	12 (13.04)	169 (35.8))	
	9 months - above	8 (6.96)	11 (24.44)	20 (14.08)	16 (20.51)	4 (4.35)	59 (12.5)	
	Total	115	45	142	78	92	472	
(i)	**If no what is the reason?**							
	Not ideal for them	34 (33.7)	7 (18.4)	23 (33.3)	9 (13.8)	3 (3.7)	76 (21.4)	
	Infant system cannot digest it	29 (28.7)	18 (47.7)	32 (36.4)	21 (32.3)	32 (39)	132 (37.2)	
	Constipation	23 (22.8)	13 (34.2)	14 (20.3)	35 (53.8)	47(57.3)	132 (37.2)	
	No response	15 (14.9)	_	_	_	_	15 (4.2)	
	Total	101	38	69	65	82	355	0.01
(j)	**Does *dambu* have any special attribute?**							
	Yes	120 (55.6)	53 (63.9)	93 (44.1)	71 (49.7)	89 (51.1)	426 (51.5)	
	No response	96 (32.4)	30 (36)	118 (55.9)	72 (50.3)	85 (51.1)	401 (48.5)	0.79

Figures in parentheses are percentages Kruskal - Wallis one-way ANOVA test.

Table 6. Economics of *dambu* processing.

Question		Status						
		Processor	Retailer	Processor/ Retailer	Consumer	Processor/Consumer	Total	P-value
		n = 216		n = 211	n = 143	n = 174	n = 827	
(a)	**If yes describe the attribute**							
	Medicinal	85 (70.8)	44 (83)	69 (74.2)	19 (26.8)	71 (79.8)	288 (67.6)	
	sedative	35 (29.2)	9 (16.9)	24 (25.8)	52 (73)	18 (20)	138 (32.4)	
	Total	120	53	93	71	89	426	0.06
(b)	**What kind of by-product is derived?**							
	Bran	103 (47.7)	48 (57.8)	145 (68.7)	61(42.7)	122 (70.0)	479 (57.9)	
	Chaf	113 (52.3)	35 (42.2)	66 (31.3)	82 (57.3)	52 (29.9)	348 (42.1)	0.28
(c)	**How is by product used?**							
	Animal feed	160 (74.1)	55 (66.3)	133 (63)	69 (48)	38 (21.8)	455 (55.0)	
	Fire wood	56 (25.9)	28 (33.7)	78 (36.9)	74 (51.7)	136 (78)	372 (45.0)	0.59
(d)	**Any monetary value to a produce**							
	Yes	81 (37.5)	14 (16.9)	182 (86.3)	5 (3.5)	89 (51.1)	371 (44.9)	
	No	135 (62.5)	69 (83)	29 (13.7)	138 (96.5)	85 (48.9)	456 (55.1)	0.67
(e)	**If yes indicate the value**							
	Per Mudu 2 - 5	32 (39.5)	12 (85.7)	93 (51.1)	5 (100)	68 (76.4)	210 (56.6)	
	Per bag 50 kg	45 (55.6)	2 (14.3)	82 (45.1)	-	21 (23.6)	150 (40.4)	
	No response	4 (4.9)	-	7 (3.8)	-	-	11 (3.0)	
	Total	81	14	182	5	89	371	0.61
(f)	**Is anyone assisting you in processing?**							
	Yes	146 (676)	72 (86.7)	182 (86.3)	78 (54.5)	96 (55.2)	574 (69.4)	
	No	70 (32.4)	11 (13.3)	29 (12.7)	65 (45.5)	78 (44.8)	253 (30.6)	0.03
(g)	**If yes, describe**							
	Relative	40 (27.4)	38 (52.8)	84 (46.2)	63 (80.8)	43 (44.8)	268 (46.7)	
	Employees	21 (14.4)	3 (4.2)	14 (7.7)	1 (1.3)	21(21.9)	60 (10.5)	
	Children	63 (43.2)	31 (43.1)	84 (46.2)	14 (17.9)	32(33.3)	224 (39.0)	
	Other	22 (15.1)	-	-	-	-	22 (3.8)	
	Total	146	72	182	78	96	574	0
(h)	**Which of these in Q. (f) do you pay?**							
	Relative	20 (16.5)	14 (42.4)	43 (58.9)	21(77.8)	12 (25)	110 (36.4)	
	Employees	21 (17.4)	12 (39.4)	14 (19.2)	1 (3.7)	21 (43.8)	60 (19.9)	
	Children	51 (12.4)	16 (48.5)	16 (21.9)	5 (18.5)	15 (31.3)	67 (22.2)	
	No response	65 (53.7)	-	-	-	-	65 (21.5)	
	Total	121	33	73	27	48	302	0.8

Figures in parentheses are percentages Kruskal - Wallis one-way ANOVA test.

Out of this number, majority employs their relatives' and friends. The cost of employing these workers is usually minimal.

When *dambu* quality is improved, consumers can have confidence in the products and the producers can obtain better prices. Moreover, with the increasing influence of

advertising upon customers, small food processing enterprises like *dambu* will have to improve the packaging and preservation of their products if they are to survive against competition (Jideani et al., 2001).

Conflict of interests

The authors did not declare any conflict of interest.

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Full Length Research Paper

An investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria

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The microbial profile of some concrete and earthen fish ponds within the Niger delta region was evaluated. The results of physico-chemical properties of the water samples showed that alkalinity values were significantly higher in concrete ponds (99.7±47.1 to 150±69.7) than (18±6.9 to 24±14.5 mg/L) in earthen ponds. Electrical conductivity values varied significantly between the ponds with (200±84.1 to 290±74.9 µs/m) in concrete ponds and (18±6.9 to 24±14.5 µs/m) in earthen ponds. Sulphate concentrations were higher in concrete ponds (0.25±0.36 to 1.53±14.9 mg/L) than (0.25±0.36 to 0.4±0.77 mg/L) in earthen ponds. The mean total heterotrophic bacteria count was higher in concrete ponds (6.5×10^5 to 7.4×10^5 cfu/ml) than in earthen (6.3×10^5 to 6.5×10^5 cfu/ml) ponds. The mean fungal count ranged from 2.11×10^5 to 2.25×10^5 cfu/ml in concrete pond and (1.8×10^5 to 2.4×10^5 cfu/ml) in earthen ponds. The bacterial genera isolated from the ponds were *Escherichia coli*, *Staphylococcus* sp., *Aeromonas* sp., *Streptococcus* sp., *Salmonella* sp., *Vibrio* sp., *Shigella* sp., *Proteus* sp., *Pseudomonas* sp., *Klebsiella* sp. *Enterobacter* species occurred only in concrete pond, while *Serratia species* occurred in earthen ponds. The fungal genera isolated from both the concrete and earthen ponds were *Aspergillus* sp., *Penicillum* sp. and *Cladosporium* sp. The genera, *Fusarium* sp. and *Mucor* sp were isolated only from earthen ponds. The study revealed that the ponds were grossly contaminated with pathogenic microorganisms which poses a risk to human health, thus of significant public health concern.

Key words: Heterotrophic bacteria, fish ponds, fungal isolates, pathogens, physico-chemical properties.

INTRODUCTION

Fish and its products are very important to human population all over the world. According to the Food and Agricultural Organization (2002), most of the world's population (56%) derives at least 20% of its animal protein intake from fish. This is because fish is the preferred source of much desired animal protein as compared to poultry, beef, mutton or pork. It is comparatively cheaper and highly acceptable with little or no religious bias which gives it advantage over other proteins (Philips, 2004).

In Niger Delta Region, fish pepper soup is one of their best delicacies leading to higher demand for fish in the region. This gave rise to the increasing number of fish farms in the region in order to meet the demand for fish

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and also offset the scarcity of fish gotten from the wild environment.

Fishes are reared in different culture media or controlled environment which could be ponds (concrete or earthen), vats (wooden or fiber glass) and plastics (Osawe, 2004). Among these culture systems, concrete and earthen ponds are widely used (Ezenwa, 2006). Earthen pond culture system has been the conventional method of fish culture in Nigeria, until recently; concrete tanks culture system is gaining grounds as land become costly, scarce and readily unavailable (Onome and Ebinimi, 2010). Research has shown that a higher number of fish farmers use concrete ponds (73%) as compared to 27% using earthen ponds in Nigeria (Ugwumba, 2010). Fishes cultivated in these controlled environments has been found to be contaminated by microorganisms (pathogenic and opportunistic organisms) (Fafioye, 2011; Nguyen et al., 2007). This contamination has been attributed to questionable water quality and high stocking densities (Okpokwasili and Ogbulie, 1998). The feed used for the fish in these ponds contain organic materials and introduces a wide variety of microorganisms into the ponds (Okpokwasili and Ogbulie, 1999).

Among the constraints of fish farming is the high cost of feeding, which resulted to the use animal manure to augment conventional feed in countries like China, India and also in Nigeria. However, organic manure also leads to the release of high concentration of opportunistic and pathogenic microorganisms into the ponds which are of public health concerns. According to Omojowo and Omojosola (2013), their presence in fish intended for human consumption may constitute a potential danger not only in causing disease but could act as reservoirs of antibiotic resistance organisms leading to treatment failures and high cost of treatment when improperly cooked fish is consumed. Fish is in direct contact with micro flora in the environment and the opportunistic pathogens already present in the water may invade the host under stress and undesirable water quality conditions. It is therefore, important to understand the micro flora associated with fish culture environment, since the microbial flora of a cultivated fish is a reflection of its aqueous environment (Erondu and Ayanwu, 2005). It is therefore significant to evaluate and compare the microbial quality and physiochemical parameters of some fish ponds within the Niger Delta region of Nigeria.

MATERIALS AND METHODS

Study area

The study area is within the Niger Delta region of Nigeria and lies between latitude 4-6°N and longitude 5-8°E. The region is characterized by high biodiversity, characteristic swamp, water ways, vast plains and mangrove forest. The farms combine fish production and livestock production, thus they operate integrated fish production having both concrete and earthen fish ponds stocked with tilapia and catfish.

Sample collection

Water samples were aseptically collected from the ponds biweekly using sterile screw capped bottles. Composites samples were obtained by collecting at different sampling points (four sides of the pond) and 10-15 cm depths from the water surface. The water samples were transported to the laboratory in an ice-packed container for microbiological and physiochemical analysis.

Isolation of total heterotrophic bacteria and fungi

Ten-fold serial dilution of the pond water samples were prepared aseptically in sterile physiological saline up to 10^{-5} and 0.1 ml aliquot of each dilution was inoculated on dried nutrient agar and Sabouraud dextrose agar (Oxiod Limited. Wade Road. Basingstoke Hampshire United Kingdom) plates, in triplicate using the spread plate technique for enumeration of total heterotrophic bacteria count and fungi count, respectively. The nutrient agar plates were incubated at 35°C for 24 h under aerobic condition, while the Sabouraud plates were incubated at room temperature for five days. Plates containing 30-300 bacterial colonies were selected and counted. The number of colony forming units per ml (cfu/ml) was calculated by multiplying the number of colonies per dilution factor. The fungi isolates were identified using lactophenol cotton blue stain while the bacterial isolates were identified using series of biochemical reactions. Cfu/ml = number of colonies per ml plated /total dilution factor.

Enumeration of total coliform

The most probable number (MPN) was adopted in the determination of the total coliform bacteria using MacConkey broth (Sigma-Aldrich, 3050 Spruce Street St. Louis, MO.) and the five tubes techniques. All positive tubes from the MPN tubes were sub-cultured on an EMB agar (Titan Biotech. Azadpur, New Delhi, India) plates in duplicate and incubated at 35°C for 24 h.

Isolation of *Salmonella*/*Shigella*

The *Salmonella/Shigella* agar (SSA) (Oxiod limited, Wade Road. Basingstoke Hampshire United Kingdom) was prepared according to the manufacturer's instructions and 0.1 ml aliquot of each water sample was transferred onto the surface of dried sterilized SSA plate. The plates were inoculated in triplicate and incubated at 37°C for 24 – 48 h. Thereafter, pure cultures were obtained by sub-culturing onto freshly prepared SSA plates and pure colonies were identified using biochemical reactions for example, motility indole – urea- tryptophan deamination test (MIUTDA), triple sugar iron test and o-nitrophenylbeta D-galactopyroside test (ONGP).

Isolation of *Vibrio* species

The thiosulphate citrate bile salt agar (TCBS) (Acumedia Neogen. Lasher Place, Lasing, USA.) was prepared and poured onto sterilized Petri dishes. On solidification, 0.1 ml of each pond water sample previously enriched in alkaline peptone water was transferred unto the dried agar plate in duplicate using a 1 ml pipette and spread evenly with a sterile hockey stick. It was incubated at 35°C for 24 – 48 h. After incubation, *Vibrio* colonies were enumerated for *Vibrio* count and identified using biochemical reactions.

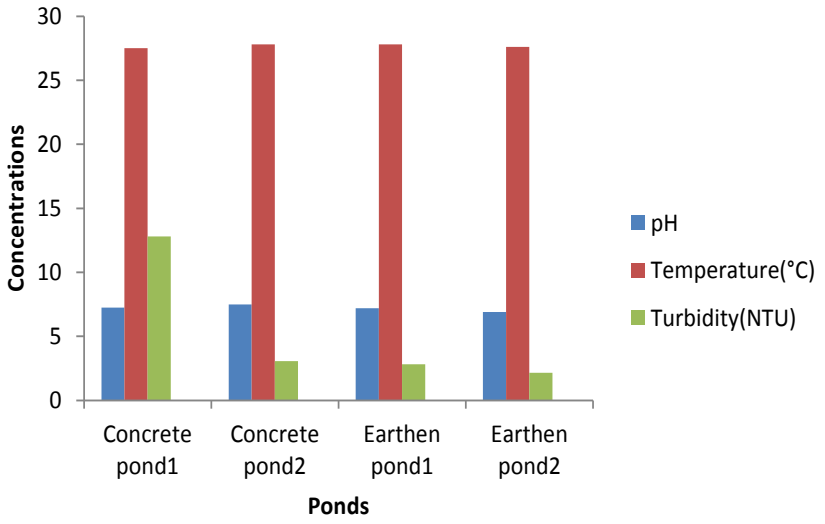

Figure 1. Concentration of pH, temperature and turbidity within the ponds.

Identification of bacterial isolates

The cultural, morphological and biochemical characteristic of the respective isolates were compared with the criteria in Bergey's manual of Determinative Bacteriology (1994). The biochemical test used in the identification and characterization of the isolates include: Gram staining, motility, indole production, methyl red – Voges proskauer, citrate utilization, oxidase, catalase, coagulase and sugar fermentation test.

Physico-chemical analysis of water samples

The water samples from the different ponds were examined for physico-chemical characteristics using standard procedures of the American Public Health Association (APHA). pH was measured *in situ* using a Phillips model of PW 9418 Ph meter after it has been calibrated with standard buffer 7. Temperature and conductivity was measured using Hach conductivity meter Model (CO150). The Winkler's iodometric titration method was adopted for dissolved oxygen determination, biochemical oxygen demand was detemined using the 5 days approach, while chemical oxygen demand was measured using Walkley and Black (1934) dichromate reflux condenser. Alkalinity was done by titrating 100 ml of samples with 0.02 ml of HCl solution using methyl orange as indicators, sulphate was determined using the barium chloride (turbidimetric) method, while the spectrophotometric method at 555 nm UV visible light PC UNICO 2102 USA was used in the determination of nitrite. Ammonia and phosphate were measured using Nessler reaction and ascorbic acid method, respectively.

Statistical analysis

Results collected were subjected to analysis of variance and the least significant difference (LSD) test was used to separate differences between means at 5% level of confidence.

RESULTS

The physico-chemical parameters of the water samples of the four ponds are presented in Figures 1 to 4. The parameters did not vary significantly within the ponds except for alkalinity, **(**99.7±47.1 to 150±69.7) which was higher in concrete ponds than in earthen ponds (18±6.9 to 24±14.5 mg/L). Conductivity values were higher in concrete ponds (200±84.1 to290±74.9 µs/m) than in earthen ponds (18±6.9 to 24±14.5 µs/m), sulphate was found to be higher in concrete ponds (0.25±0.36 to 1.53±14.9 mg/L) than in earthen ponds (0.25±0.36 to 0.4±0.77 mg/L). All the parameters were within the range that supports fish production except ammonia which was above the limit specified by the FAO for aquaculture. The microbial count did not vary significantly between the ponds (Table 1).

Total heterotrophic bacteria count was highest in concrete pond 1 ($9.5x10^5$ cfu/ml) and lowest in earthen pond 2 ($3.6 x10^5$ cfu/ml). Fungal count was also highest in concrete pond 1($5.5x10^5$ cfu/ml) and lowest in earthen pond1 ($1.2x10^5$). *Salmonella/Shigella* count was highest in earthen pond 1 ($8.5x10^5$) and lowest in concrete pond 1 ($1.0x10^5$cfu/ml). *Vibrio* count was higher in concrete pond 2 ($8.3x10^4$ cfu/ml) and lowest in concrete pond 1 ($1.0x10^5$ cfu/ml), while total coliforms was highest in concrete pond 1 (94 mg/100 ml) and lowest in earthen pond 2 (2 mg/100 ml). Bacteriological analysis of the water samples showed twelve different genera (Table 2) which are *Escherichia coli, Staphylococcus* sp.*, Salmonella* sp.*, Shigella* sp.*, Aeromonas* sp., *Vibrio* sp., *Pseudomonas* sp.*, Proteus* sp.*, Enterobacter* sp.*, Klebsiella* sp.*, Serattia* sp. and *Streptococcus* sp. *Enterobacter* sp. occurred only in concrete ponds 1 and 2, *Serattia* sp. occurred only in earthen pond 1, *Proteus* sp. and *Streptococcus* sp. occurred in all the ponds except earthen pond 2, while others occurred mainly in all the ponds.

The fungal genera isolated are presented in Table 3 and they include *Aspergillus* sp., *Penicillum* sp.*, Cladosporium* sp.*, Mucor* sp. and *Fusarium* sp.

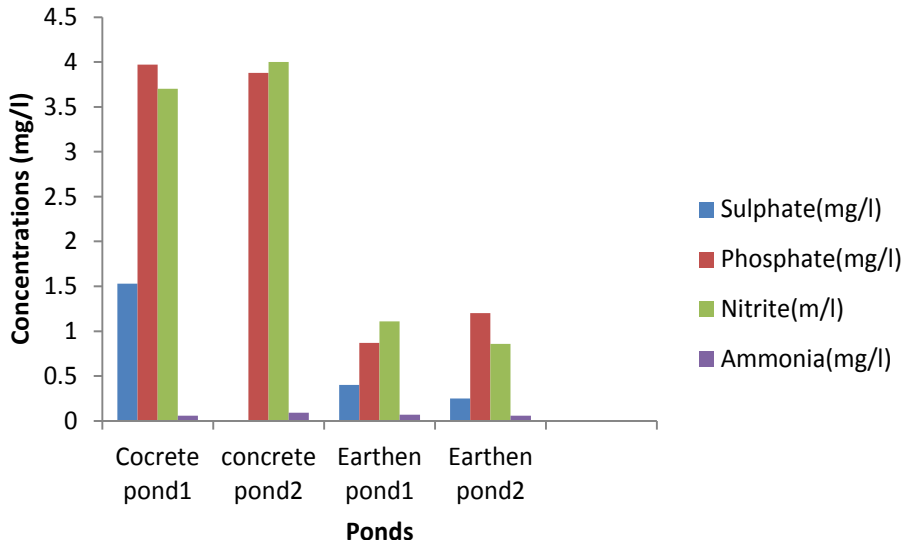

Figure 2. Concentration of sulphate, phosphate, nitrite and ammonia.

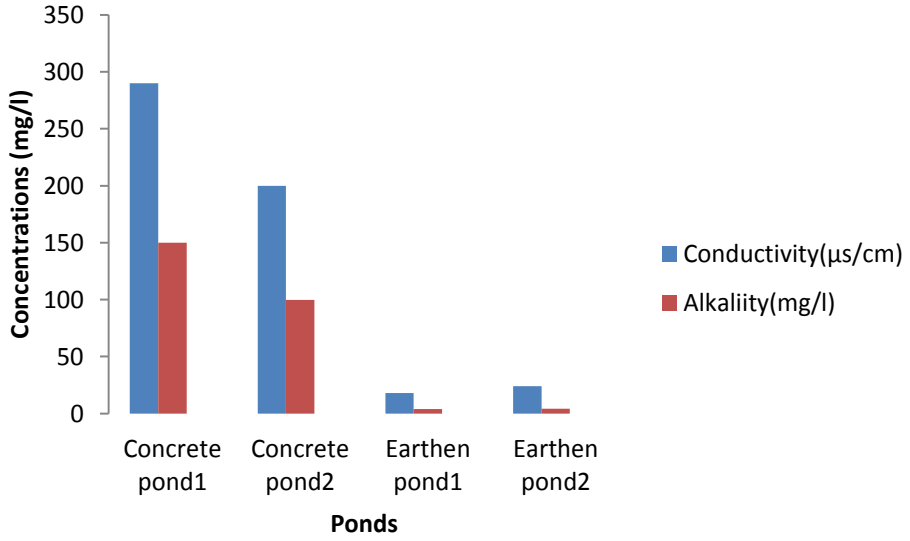

Figure 3. Concentration of conductivity and alkalinity.

DISCUSSION

The result of the microbiological characteristic showed that Gram negative bacteria were dominant in the bacteria isolated from the ponds. The microorganisms isolated were *E.coli*, *Salmonella* sp. *Shigella* sp. *Pseudomonas* sp. *Proteus* sp. *Klebsiella* sp. *Vibrio* sp., *Enterobacter* sp., *Serratia* sp., *Aeromonas* sp., *Staphylococcus* sp., and *Streptococcus* sp. The coliforms isolated were an indication of the contamination of the pond water with fecal materials which may result to the presence of pathogenic organisms in fish when their concentration is above (10^4-10^6) in the skin and (10^4-10^7 cfu/g). The fecal material may be as a result of fertilization of the ponds with animal manure which is discharged directly into the fish ponds, or excreted by the fish into the ponds (Kay et al., 2008). The presence of human pathogenic bacteria like *Vibrio* isolated from the concrete pond could be attributed to the fact that *Vibrio* spp. are regarded as indigenous bacteria in aquatic environment and naturally present in fish up to10^2-10^3 Cfu/g and multiply under favorable temperature condi-tions above 15°C. Thus, the temperature in the pond favored the growth and proliferation of *Vibrio* spp. According to Hay (2012), *Vibrio* cholera accounted for 75% of *Vibrio* associated diarrhea in some part of Niger Delta region. The presence

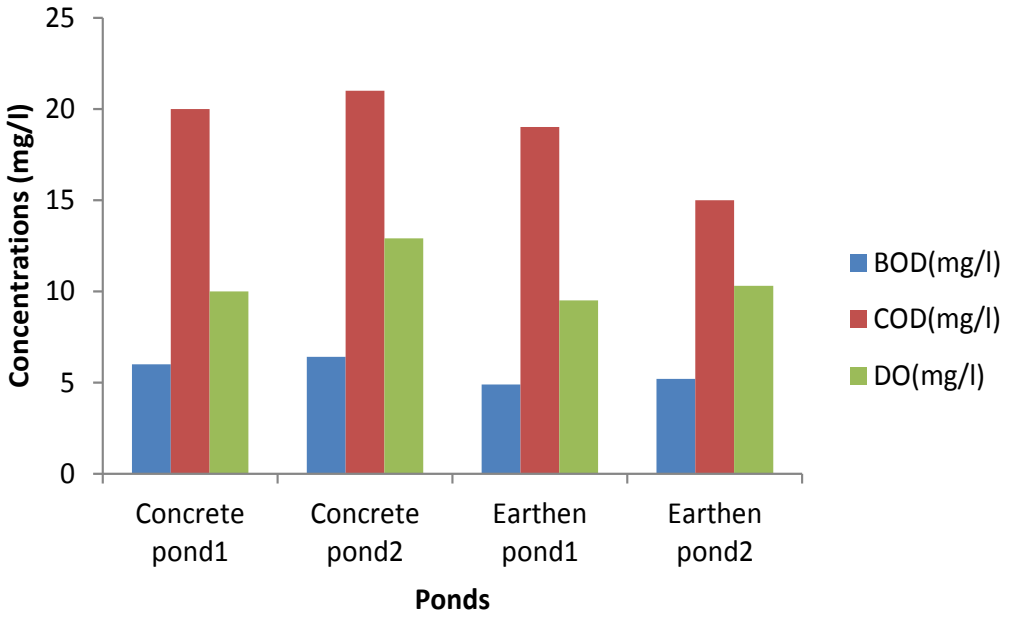

Figure 4. Concentration of BOD, COD and DO.

Table 1. Mean total heterotrophic bacteria count, fungal count, *Salmonella/Shigella* count, *Vibrio* count and coliforum count of the pond water.

	Total heterophic count (cfu/ml)	Fungal count (cfu/ml)	*Salmonella/Shigella* (cfu/ml)	Vibro count (cfu/ml)	Total coliform count (cfu/100ml)
Concrete Pond 1	6.5×10^5	2.11×10^5	3.2×10^5	4.9×10^4	59.6
Concrete Pond 2	7.4×10^5	2.5×10^5	1.9×10^5	5.9×10^4	21.0
Earthen Pond 1	6.4×10^5	1.8×10^5	3.1×10^5	5.1×10^4	23.0
Earthen Pond 2	6.3×10^5	2.4×10^5	1.7×10^5	4.9×10^4	70.3

of pathogenic microorganisms especially *E. coli, Salmonella, Shigella* and *Vibrio* can lead to the transmission of water borne diseases such as, typhoid fever, cholera, food poisoning and gastroenteritis (Piet, 2009) on consumption of improperly cooked fish cultivated in these ponds or through contact with the contaminated fish and water. The diverse groups of bacteria isolated from these ponds are in line with the report of Okpokwasili and Ogbulie (1999) who worked on pond water suggesting that allochthonous bacteria from feed added to the ponds are the principle source of bacteria of health importance and Dabbor (2008) who reported similar organisms in the microbiological study of El-quanter fish pond. *E. coli* was the most dominant organism occurring in both concrete and earthen ponds. The presence of *E. coli* in water or food indicates the possible presence of causative agents of many gastro-intestinal diseases (Ampofo and Clerk, 2010). *Pseudomonas*, *Proteus*, *Staphylococcus* species have been implicated in food poisoning (Oni et al., 2013). *Aeromonas* species were also predominantly present in both ponds. This organism is one of the most opportunistic pathogen for fresh water fish and the main etiological agents in disease outbreak were several mortalities were recorded in India (Das and Mukheyce, 1999).

Fungal infection is an important economic and limiting factor in intensive fish production. The fungi genera isolated from the ponds were *Aspergillus* sp., *Penicillum* sp., *Cladosporium* sp., *Mucor* sp., and *Fusarium* sp. *Aspergillus* and *Penicillum* species formed the dominant group of fungi in this study. The observation is consistent with the work of Obire and Anyanwu (2009) who noted that *Aspergillus* and *Penicillum* species are believed to penetrate into the environment through dead plants materials and remains for long period of time. Similarly, Eze and Ogbaran (2010) cited *Penicillum* sp. as the most abundant fungi during his study on the micro-biological and physiochemical of fish pond water in Ughelli, Delta State Nigeria. In contrast to the present

Table 2. Distribution of isolates from the different ponds.

Isolates	Concrete pond1	Concretepond2	Earthen pond1	Earthen pond2	Frequency	Frequency (%)
E. coli	+	+	+	+	43	20.7
Staphylococcus	+	+	+	+	28	13.5
Salmonella	+	+	+	+	10	4.8
Aeromonas	+	+	+	+	33	15.7
Shigella	+	+	+	+	21	10.1
Pseudomonas	+	+	+	+	5	2.4
Proteus	+		+	_	8	3.8
Enterobacter	+	+	_	_	4	1.9
Vibrio	+		+	+	25	12
Serattia	-		+	_	2	0.96
Streptococcus	+	+	+	_	12	5.7
Klebsiella	+	+	+	+	17	8.2

Table 3. Occurrence of fungal isolates within the ponds.

Ponds	*Aspergillus*	*Penicillum*	*Cladosporium*	*Fusarium*	*Mucor*
Concrete pond 1	+	+	+	_	_
Concrete pond 2	+	+	+	_	_
Earthen pond 1	+	+	+	+	+
Earthen pond 2	+	+	+	+	+
Frequency	28	21	10	2	2
Frequency (%)	44.4	33.3	15.9	3.2	3.2

result, Fafioye (2011) reported *Cladosporium* sp. as the dominant fungi species. The occurrence of *Fusarium* sp. and *Mucor* sp. in earthen ponds could be attributed to the fact that the earthen ponds was a more conducive environment for their growth and proliferation due to the presence of soil and plants in the earthen ponds.

Total aerobic heterotrophic bacteria count, fungal counts, *Salmonella/Shigella* count, *Vibrio* count and *Coliform* count (Table 1) were high and varied within the ponds. The values were due to water temperature, which was within optimum for bacterial growth and also due to the organic matter load found within pond water resulting from the diet used in feeding the fish. Thus, the pond water becomes an ideal culture medium for the proliferation of bacterial pathogens causing bacterial infection in fish and an important cause of food poisoning (Eze and Ogbaran, 2010).

Among water and food borne pathogens in coastal ecosystems, *Vibrios* contribute the major part. *Vibrio* sp. has been found to play a vital role in fish and shrimp culture system (Amand et al., 2010). They affect water quality causing disease and mortality to the fish as secondary and primary pathogens. The presence of specific pathogenic human species of *Vibrio* can serve as an indicator of public health risk of water and food destined for human consumption (Ganesh et al., 2009).

The pH recorded in all the ponds were within range required for aquaculture (6.5-9.5), thus suitable for fish production. The measurement of pH helps to determine if the water is a proper environment for fish, although most fish can tolerate pH as low as 5.0. The pH obtained in this study was similar to that of Ehiagbonare and Ogunrinde (2010) who studied the physiochemical analysis of fish pond water in Okada in Edo State. Ntengwe and Edema (2008) observed that the appro-priate pH for increased fish production is 6-9. While Mohammed (2005) reported that rapid changes can cause extreme stress in the fish similar to shock in humans.

Temperature is a factor of great importance for aquatic ecosystem, as it affects the organisms as well as the chemical and physiochemical parameters of water. The optimum condition for increased fish productivity was found to be at 20-30°C (Ntegwu and Mojisola, 2008). The temperature obtained from this study ranged from 26-29°C and was within the limit that supports fish productivity. Thus, this corroborates with the report of Fafioye (2011), who observed a temperature of 27-28°C in the preliminary studies and water characteristic and bacterial population in Kojalo fish pond.

The dissolved oxygen, obtained in this study was below

the 5 mg/L required for fish production. Generally, concentration below 5 mg/L may adversely affect the functions and survival of biological organisms while below 3 mg/L leads to death of most fish (Swan, 2006). The low dissolved oxygen recorded in ponds could be attributed to elevated temperature, increased microbial and organic load and resultant increase in metabolic activity may also account to low dissolved oxygen concentration (FAO, 2005), coupled with high phosphate that lead to eutrophication, hence reduces the amount of circulating oxygen in the water, especially in the concrete pond where the concentration of phosphate was above the recommended value. Similar results were also reported by Okpokwasili and Ubah (1991), who worked on water quality and bacteria disease in fish ponds but different from the reports of Onome and Ebinimi (2010) who recorded higher DO of 4.34 – 6.33 mg/l as a result industrial input from the surrounding industries near the fish farm.

Alkalinity of water is the amount of dissolved calcium, magnesium and other compounds in water. The optimum alkalinity for increased fish production is 20-300 mg/l. The alkalinity in this study was higher in the concrete ponds than the earthen ponds. This could be attributed to the leaching outs of these substances (lime) from the walls of the concrete ponds into the water (UNESCO/WHO/UNED, 1996). The values obtained in the earthen ponds were below 20 mg/l and therefore should be adjusted for maximum productivity by liming.

Ammonia occurs in fish ponds as a result of accumulation of left over rich protein feed, fish waste, and microbial decomposition of organic matter (Durbrow et al., 1997). The ammonia concentrations in all the ponds were above the permissible level of 0.2 mg/l recommended for aquaculture (Table 1), although no adverse effect was observed on the fish during the period of study because at higher pH and temperature, the more toxic the ammonia, thus the pH and temperature were within the recommend range. Normally, the concentration of ammonia in pond water should be zero. Studies have shown that ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and longtime gill damage (Durborow et al., 2000). Similarly, higher ammonia concentration was also obtained by Onome and Ebinimi (2010) who attributed the high ammonia concentration in new Calabar River to industrial nitrogenous waste.

Nitrite is termed the invisible fish killer as it is deadly to the smallest fish at a concentration as low as 0.25 ppm. Nitrite concentration obtained in this study was above the permissible limit of 0.1 mg/l (Figure 2). This could be as a result of decomposition of excess feed which polluted the pond environment or by the action of algae through nitrogen fixation and water plants. Nitrite is a skin irritant and will cause the fish to display symptoms of irritability such as rubbing themselves, jumping and skimming across the surface of the pond (Eze and Ogbaran, 2010). Nitrite prevents the blood cells from absorbing oxygen from water. This process turns their blood to a dull brown color and hence the popular name of nitrite poisoning “brown blood disease” (Durbrow et al., 1997).

Phosphate level in concrete ponds was above the 3 mg/l. This limit should be controlled to avoid eutrophication of the ponds as it fostered the growth of algae which also accounted to the low dissolved oxygen in the ponds (Eze and Okpokwasili, 2010). Phosphate may be introduced into the pond through fish feed or through surface run off, and could also be from the building materials used in the construction of the ponds. These fishes can also store phosphate in their organs and when they die, they release the previously absorbed into the water which triggers the growth of new algae (Durbrow et al., 1997).

Sulphate concentration in the ponds varied from 0.25-4.0 (mg/l) with the concrete ponds significantly higher than the earthen ponds. These values are similar to that of Ehiagbonare and Oguunrinde (2010) (0.66-1.09 m/l) and different from Utang and Akpan (2012) who reported 42.46-57.36 mg/l. He suggested the use of detergent and soaps by residents which got into the water body may be responsible for the high value of sulphate in New Calaber River.

The electrical conductivities of the water samples generally varied significantly ($P < 0.05$) and ranged from 9 to 400 µs/cm for both ponds throughout the period of study. Higher conductivities were observed in concrete ponds than in earthen ponds. Electrical conductivity is a useful indicator of mineralization and salinity or total salt in a water sample. The FAO acceptable limit for conductivity in aquaculture is 20-1500 µs/cm (DWAF, 1996). This limit was not exceeded in these ponds. Thus, the parameter is suitable for fish production.

Turbidity is the result of several factors including suspended soil particles, planktonic organisms and humic substances produced through decomposition of organic matter (FAO, 2002). It hinders the penetration of sunlight into the pond making it difficult for aquatic habitat to receive the positive effect of light (Ali et al., 2004). Turbidity values obtained from the concrete ponds and earthen ponds were within the range which allows light penetration and photosynthesis of plant and algae that supports aquatic life. Thus, at average turbidity of less than 25 NTU as recorded in these ponds coupled with phosphate concentration, favored the growth of planktons.

The BOD and COD were low and within the permissible range of <10l and <20 mg/l, respectively. The result is different from that obtained by Odewumi and Zakari, (2010) who recorded a higher value of COD and BOD in the study of cat fish ponds in Jos suggesting that the fish ponds were grossly polluted. Similarly, Ehiagbonare and Ogurinde (2010) reported BOD of 2.2, 2.36mg/l in concrete ponds in Oluko and Igusa and 1.6 mg/l in earthen pond at Afugle during the study of physio-chemical analysis of pond water in Okada and its environs.

In conclusion, the microbial qualities of the two ponds were not significantly different. However, there were differences in the concentration of nutrients with the concrete pond showing concentrations above the recommended limit of the FAO in most of its parameter, while the earthen ponds were within the recommended limit. The high concentrations in the concrete ponds could be attributed to leaching of these substances from the walls of the concrete pond into the water. Thus, the earthen pond could be said to be a better pond for fish production in terms of physiochemical parameters. The study also revealed that both ponds were grossly contaminated with pathogenic bacteria that could affect fish cultivated, since the microbial quality of any fish pond water is a reflection of the microbial flora of the fish itself. These organisms could lower fish yield, causes diseases and economic loss and equally endanger the ultimate consumers (humans) particularly if the fish harvested from the ponds are under processed.

Conflict of interest

Authors did not declare any conflict of interest.

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Full Length Research Paper

Production and quality evaluation of flavoured yoghurts using carrot, pineapple, and spiced yoghurts using ginger and pepper fruit

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Production and quality evaluation of plain yoghurt, spiced yoghurt (pepper fruit, ginger) and flavoured yoghurt (carrot and pineapple) were carried out being proximate composition, mineral analysis, microbiological analysis, organoleptic evaluation and statistical analysis. Results show significant ($p<0.05$) nutritional enhancement of the plain yoghurt by the addition of spices and flavourings. The mineral content of the plain yoghurt were likewise increased. Organoleptically, the spiced and flavoured yoghurts were all acceptable by consumers but pepper fruit spiced yoghurt was the most preferred in terms of general acceptability.

Key words: Yoghurt, pepper fruit, carrot and pineapple.

INTRODUCTION

Yoghurt is a fermented dairy product obtained from the lactic acid fermentation of milk. It is one of the most popular fermented milk products in the world (Willey et al., 2008).

Bourlioux and Pochart (1988) defined yoghurt as a coagulated milk product that results from the fermentation of lactose in milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Other lactic acid bacteria (LAB) are also frequently used to produce yoghurt with a unique characteristic (Adolfsson et al., 2004). It is a nutritionally beneficial product generally considered safe, with taste liked by many people.

Yoghurt is produced commercially by pasteurising the milk mixture, cooling to 45°C before being inoculated with known cultures of microorganisms referred to as starter culture. The starter culture may be mixed *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in a ratio of 1:1. They act on lactose and result in the production of lactic acid which increases the acidity of the yoghurt, thereby forming gel.

The decrease in pH inhibits the growth of pathogenic bacteria. The lactic acid produced is also responsible for the characteristic flavour and aroma of yoghurt and helps to maintain the quality of the yoghurt during storage and packaging (Saint et al., 2006).

Yoghurt can boost immunity. The regular consumption of live cultured yoghurt produces a higher level of immunity boosting interferon as this bacteria cultures stimulate infection-fighting white cells in the blood stream with anti tumor effects (Maltock, 2007). Yoghurt is nutritionally rich in protein, carbohydrate, vitamins and minerals (for example calcium) which contributes to a

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healthy living including decreasing the risk of colon cancer, improved digestion and many other benefits (Gray, 2007).

Sensory appeal is one of the essential strategies associated with the market success of fermented products like yoghurt. The popularity of yoghurt as a food component has been linked to its sensory characteristics (Routray and Mishra, 2011). Furthermore, there is need to introduce other fruit and vegetable flavours like carrots, pineapple and spices such as ginger and pepper fruit so as to produce yoghurt with spicy taste with a characteristic aroma and fragrance. Also, to improve the nutritional composition of yoghurt produced.

Health benefits of yoghurt

Lactobacillus, a probiotic (friendly) bacteria found in yoghurt offers remarkable preventive and curative effects on arthritis (Gray, 2007). Yoghurt is easier to digest than milk and so many people including children who cannot tolerate milk, either because of a protein allergy or lactose intolerance can enjoy yoghurt more digestible than milk. Bacterial enzymes created by the culturing process partly digest the milk protein casein making it easier to absorb and less allergenic (Witton, 2004). Yoghurt contains intestine friendly bacteria culture (lactobacteria) that fosters a healthy colon and lowers the risk of colon cancer by promoting the growth of healthy bacteria and thereby deactivate harmful substances which can cause problem in the colon (Gray, 2007). It is also rich in calcium which contributes colon health and decreases the risk of colon cancer (Gray, 2007).

The regular consumption of live cultured yoghurt produces a higher level of immunity boosting interferon as these bacteria cultures stimulate infection fighting white cells in the blood stream with anti tumor effects (Maltock, 2007). Daily consumption of ounces (100 g) of yoghurt significantly improved the cholesterol while raising high density lipoprotein (HDL) (good cholesterol). This may be because of the ability of the live culture in yoghurt to assimilate cholesterol or because yoghurt binds the bile acids which lowers cholesterol (Maltock, 2007).

Carrot *(Daucus carita)*

Carrot *(Daucus carita)* is a root vegetable, horn like in shape, usually orange in colour, though purple, red, white, and yellowed varieties exist. It has a crisp texture when fresh.

The most commonly eaten part of the carrot is taproot, although the green are sometimes eaten as well. It is a domesticated form of the wild carrot *D. carota*, native to Europe and South Western Asia. The domestic carrot has been selectively bred for its greatly enlarged and more palatable, less woody textured edible taproot. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are traditional in many regional cuisines (Mabey, 1997; Rose, 2006).

Pineapple (*Ananas comosus)*

A. comosus is a tropical plant in the Bromeliaceae family. This delicious fruit is full of nutrients that promote good health. Raw pineapple are loaded with vitamins, enzymes and minerals including vitamin A, vitamin C, calcium, phosphorus, manganese and potassium, which are important to health, it is also rich in fibre and calories and low in fat and cholesterol (Marcela, 2012). Both the root and fruit may be eaten or applied topically as an anti-inflammatory. The anti-inflammatory properties can greatly alleviate the pain of arthritis (Marcela, 2012).

Pepper fruit (*Dennettia tripetala*)

Pepper fruit (*D. tripetala*) is a well-known forest fruit and indigenous spicy medicinal plant. It is a tropical rain forest plant widely domesticated in the Southern, Eastern and Western parts of Nigeria (Chandraseharen, 1994). It can be chewed in different forms. Okafor (1980) reported that pepper fruit (*D. tripetala*) contains minerals and vitamins. Pepper fruit is a spice medicinal plant for curing fever, cough, toothache, as well as stimulant and is used in the preparation of some special dishes for pregnant and postpartum women, during which it is claimed that the spices and herbs aid in uterine contraction (Oyemitan et al., 2006).

Ginger (*Zingiber officinale*)

Ginger (*Z. officinale*) is consumed as a delicacy, medicine or spice. Ginger produces a hot, fragrant kitchen spice. Young ginger rhizomes are juicy and fleshy with a very mild taste. They are often pickled in vinegar or sherry as a snack or just cooked as an ingredient in many dishes. They can also be steeped in boiling water to make ginger tea. Ginger is generally prized for its use as a herb, flavour and as a spice, not as a nutritional supplement. According to Catherine (2010), 2 g of ginger contains only 1.6 calories, 0.7 mg of omega-3 fatty acids and 2.4 mg of omega-6 fatty acids. It provides 0.1 mg of vitamin C and 0.2 mg of folate. Ginger also contains minute amounts of minerals: calcium (0.3 mg), magnesium (0.9 mg), phosphorus (0.l7 mg), potassium (8.3 mg) and sodium (0.3 mg).

This work will introduce new varieties of flavoured and spiced yoghurt from carrot *(D. carita,* pineapple (*A. comosus*), pepper fruit (*D. tripetala*) and ginger (*Z. officinale*),

which will be of good nutritional quality and health benefits. This work will also reveal and get people informed of the nutritional significance and additional usefulness of these fruits and spices; thus increasing their importance to mankind.

The objectives of this research included production of flavoured and spiced yoghurt using ginger, pepper fruit, carrot and pineapple; evaluation of nutritional quality and the flavoured and spiced yoghurts; determination of microbial load of the flavoured and spiced yoghurt and determination of organoleptic qualities of the flavoured and spiced yoghurts.

Ingredients for yogurt production

Milk

Milk is the major product in the production of yogurt (Lopez, 1997). The type of milk used depends on the type of yogurt: whole cream milk for full fat yogurt, low fat milk for low fat yogurt. Milk in yogurt a rich flavour and smooth texture, contribute to energy and forms cream formation with water.

Starter culture

The main starter culture used in yogurt production is *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. They can grow independently but the rate of acid production is much higher when used together than when used individually.

S. thermophilus grows faster and produces both acid and carbon dioxide. The production of carbon dioxide stimulates the growth of *L. bulgaricus.* On the other hand, the proteolytic activity of *L. bulgaricus* produces stimulatory peptides and amino acids used by *S. thermophilus*. These micro-organisms are ultimately responsible for the formation of typical yogurt flavour and texture. The yogurt mixture coagulates during fermentation due to drop in pH. The streptococci are responsible for the initial pH drop of the yogurt mix to approximately 5.0. The Lactobacilli are responsible for the further decrease of pH to 4.5.

Gelatin

Gelatin is a protein produced by partial hydrolysis of collagen extracted from boiled bones, connective tissues, organs and some intestines of animals such as domesticated cattle, pigs, and horses. It is used as a stabilizer to increase the firmness and viscosity of yogurt. Tamime and Robinson (1999) reported that gelatin has the ability to bind water, reacts with the milk constituents (mainly proteins), and stabilize the protein network preventing free movement of water. Gelatin tends to degrade during process at high temperature.

Consequently, the yoghurt gel is considered weakened. Gelatin is used at a level of 0.3-0.5% to get a smooth shiny appearance and should be geared to the consistency standard for yoghurt. Alais and Linden (1999), disclosed that gelatin has a wide spread uses in foods without any limits imposed.

Water

Water plays an important role in yogurt making. The quantity of water must be carefully controlled such that the milk and the bacteria have correct available amount of water to make a yogurt of proper consistency.

Water provides the following functions in the making of yogurt: It creates an enabling environment for multiplication; it helps in the homogenization and forms the medium for enzyme activation and it helps to control yogurt temperature.

MATERIALS AND METHODS

Collection of samples

Skimmed powdered milk, carrot, pineapple, pepper fruit, ginger, gelatin, yoghurt culture and granulated sugar produced by Dangote Sugar Company were obtained from Eke-Ukwu Market in Owerri municipal council of Imo State, Nigeria.

The practical work which involved the production of flavoured and spiced yoghurt was conducted in the Food Processing Laboratory of the Department of Food Science and Technology, Imo State University, Owerri, Imo State. The laboratory analysis of the yoghurt samples was carried out at National Root Crop and Research Institute, Umudike in Abia State.

Sample processing

The samples of yoghurt were produced according to International Standard of yoghurt as described by Guler and Mutlu (2005). Here 400 g of skimmed powder milk was reconstituted with water and heated to 80°C for 15 min for pasteurization, and then allowed to cool to 42-45°C before inoculation with starter culture. The milk mixture was divided into five portions, raw yoghurt, carrot flavoured (sample C), pineapple flavoured (sample B), pepper fruit spiced (sample D), ginger spiced (sample E). They were incubated at 43°C for 10-12 h (overnight) until a pH of about 4.3-4.5 was attained. The yoghurts were cooled at 6□C in a refrigerator before they were analyzed.

Proximate analysis

The total solid of the yoghurt sample was determined using gravimetric method described by AOAC (1999).

The moisture and ash content of the yoghurt samples was determined using the indirect method employing drying oven and furnace incineration method described by Onwuka (2005). The protein content of the sample was determined by the semi-micro Kjeldahl, method reported by AOAC (1990).

The fat content of the sample was determined on wet weight basis by Soxhlet's method as described by Suzanne (2003). The carbohydrate content of the sample was determined by estimation using the arithmetic difference method described by James (1995).

Mineral determination

The resulting ash was dissolved in 100 ml of dilute hydrochloric acid (HCL) and then diluted to 100 ml in volumetric flask using distilled water. The digest so obtained was used for the mineral analysis.

Phosphorus in the sample was determined by the vanado-mohybdate (yellow) spectrometry described by James (1995). Calcium and magnesium contents of the test samples was determined by the ethylenediamine tetraacetic acid (EDTA) complexiometric titration of AOAC (1990).

Potassium content of the sample was determined by flame photometry. The instrument was set up according to the manufacturer's instruction. The equipment was switched on and allowed to stay for about 10 min. The gas and air lets were opened and the start knob was turned on AOAC (1990).

Microbiological analysis of samples

Determination of microbial load (coliform, bacteria and fungi load)

The method of the international commission on microbiological specification for foods ICMSF (1978) was adopted and used.

Total viable microbial count

A suspension of the bacteria was serially diluted and aliquots of each dilution was placed in suitable culture media (Hausler, 2003).

Organoleptic evaluation

The product samples were evaluated using hedonic method for sensory characteristics and overall acceptability by a panel of 40 judges selected randomly. They were served coded samples of yoghurt and asked to compare it by testing for taste, aroma, texture, appearance, and overall acceptability. All tests were performed and rated on a 9-point hedonic scale described by Ihekoronye and Ngoddy (1985) : like extremely, 9; like very much, 8; like moderately, 7; like; slightly, 6; neither like or dislike, 5; dislike slightly, 4; dislike moderately, 3; dislike very much, 2; dislike extremely, 1.

Statistical analysis

The data obtained from sensory evaluation was analysed using analysis of variance (ANOVA), according to the method of Iwe (2002) to determine the variance ratio. Sample means were compared to determine treatment effects. The least significant difference was calculated at 95% level of significance using tukey test (T-test), (Ihekoronye and Ngoddy, 1985).

RESULTS AND DISCUSSION

Proximate composition of plain

Yoghurt

The proximate composition of plain yoghurt (neither flavoured nor spiced) shows protein (9.97%), moisture (84.67%), fat (1.80%), Ash (0.44%), and carbohydrate (1.70%) contain (Table 1).

Table 1. Nutritional composition of plain yoghurt (PY).

Parameter	Plain yoghurt
CHO	1.70
Protein	9.97
Fat	1.8
Ash	0.44
Fibre	0.32
Moisture	84.67

Table 2. Vitamin (mg) of yoghurt samples composition.

Vitamins	PY	PFY	GFY	CFY	PFY
Vitamins A	5.87	6.05	5.86	6.66	5.92
Vitamins C	3.90	4.01	3.91	4.25	4.48
Riboflavin	o.52	0.53	0.53	1.00	0.89
Thiamine	1.86	2.57	1.86	3.90	1.92
Niacin	2.01	2.02	2.01	2.02	2.25

The high moisture content of the product could be as a result of the dilution (reconstitution) of the milk prior to fermentation. The low fat content of the yoghurt could be attributed to the low oil content of the milk (Skimmed milk) which was the major substrate of the yoghurt produced.

This corresponds with the work of Amna et al. (2008) that non-fat (zero%) yoghurt can be produced but in general, the fat level of every yoghurt depends on oil content of the milk, whether skimmed or full cream milk. He stated categorically that yoghurt manufactured from skimmed milk will likely have very low fat content (within the range of 1-2%) while those produced from full cream milk will have fat content in the region of 4% (or slightly above).

Also the ash and fibre content were remarkably low and this result agrees with observation of Cheeseman and Lean (2000) that generally, yoghurts have poor fibre level because they are milk and water based products.

Carbohydrate (lactose) is the major constituents of milk that is converted to lactic acid during yoghurt (fermen-tation) production task of the yoghurt. So the fermentation and conversion of lactose to lactic acid accounts for the low content of carbohydrate of yoghurt as observed in the result (Table 2).This corroborates with the works of Mistry and Hassan (1992) and Younus et al. (2002)

The observed protein content (9.97) of the plain yoghurt compares favourably with commercial standard stated by National yoghurt Association (2000), that commercial yoghurt should have 11-18% protein. Also, Adolfsson et al. (2004) reported protein content (1003) which is in agreement with the result in Table 1.

Table 3. Mineral analysis of yoghurt samples.

Parameter	PY (mg/g)	PFSY (mg/g)	GSY mg/g	C FY mg/g	PFY mg/g
Calcium	180	180.05	180.00	180.00	180.00
Phosphorus	158	158.12	158.33	158.04	158.10
Magnesium	170	170.08	170.001	170.14	170.00
Sodium	111	111.06	111.10	111.07	111.10
Potassium	121	121.50	121.02	121.11	121.12
Iron	108	110	108	108.1	108.1

Mineral analysis of raw plain, flavoured and spiced yoghurts

Mineral analysis of plain yoghurt (mg/g) revealed calcium (180), phosphorus (158), magnesium (170), sodium (111) and potassium (121) content. The result justifies the ascertion of Gray (2007) that yoghurt is a very good source of essential minerals needed for human metabolism or functionality of cells. Addition of pepper fruit and carrot respectively caused slight increase in calcium, phosphorus, magnesium, sodium and potassium (0.5, 0.11). A similar increase in minerals was also observed by Ihemeje et al. (2013) where pepper fruit was used in Zobo drink production.

D. tripetala (seed) has earlier been reported to contain minerals, vitamins, and oils (Okafor, 1980), protein, fibre, ash, and carbohydrate (Udoessian and Ifon, 1984; Okwu et al., (2004). Carrot according to Cohen et al. (2010) is rich in calcium, magnesium and potassium. This may have caused the observed increase in mineral value of the products. Carrot is well known for its B- carotene content (a precursor of vitamin A) and fruits containing B carotene can be used in the management of vibroacaustic disease (VAD) in adults especially in poor resource countries (Novotry et al., 1995)

The iron content was only remarkably improved in sample B (pepper fruit flavoured). This further justifies that pepper fruit contains appreciable quantity of iron as earlier reported by Ihemeje et al. (2013)

Ginger and pineapple respectively caused varied increase phosphorus (0.03,).11), sodium (0.1,0.1) and potassium (0.02, 0.12). Ginger is known for its pungent and stimulant effects and contains more oleoresins than minerals (Connell, 1970; Nwinuka et al., 2005). It has more of Cherepeutic effects than nutritional. On the other hand, pineapple contains more vitamins and minerals than ginger. (Nutrient data pineapple, 2012) especially water soluble Vitamins (Vit. C.) This explains why higher value of minerals was observed in pineapple favoured yoghurt than ginger spiced yoghurt.

Vitamin contents of plain flavoured and spiced yoghurt

The vitamin contents of plain flavoured and spiced yoghurts is shown in Table 4. The plain yoghurt contained vitamin A (5.87 mg), vitamin C (3.90 mg) riboflavin (0.52 mg), thiamine (1.86 mg), niacin (2.01). Addition of pepper fruit, carrot and pineapple respectively caused improvement in vitamin A (0.18, 0.79 and 0.05), vitamin C (0.11, 0.35 and 0.58), riboflavin (0.01, 0.48 and 0.37), thiamin (0.71, 2.04 and 0.06) and niacin (0.01, 0.01 and 0.24) contents above that the observed increased in the vitamin contents could be a justification of the report that carrot (Cohen et al., 2002) pepper fruit (Ihemeje et al., 2013) and pineapple(Hale et al; 2010) are all rich in vitamin .similar trend of increase in vitamin content of flavoured and spiced yoghurts was respec-tively recorded by Amna et al. (2008) and Mbaeyi and Anyanwu(2010)

Table 4. pH value of the yoghurt samples.

Yoghurt sample	pH
Plain	4.6
Pepper fruit spiced	4.7
Ginger spiced	4.7
Carrot flavoured	4.7
Pineapple flavoured	4.5

Value are means of triplicate determination.

The pH of the various yoghurt sample are presented in Table 3 result indicates that pineapple flavoured by the plain yoghurt (4.6) pepper fruit spiced yoghurt, ginger spiced yoghurt and carrot flavoured yoghurt had the same pH (4.7). Addition of pineapple as a flavourant caused the pH to drop from 4.6 to 4.5. This could be attributed to two reasons; the pineapple juice contains appreciable quantity of ascorbic acid, also it was not diluted with water.

Unlike ginger, carrot and pepper fruit that were extracted with water before adding them into the yoghurt. A corresponding alteration of pH of plain yoghurt was observed by Gabriel et al. (2013) in their work on the production of probiotic yoghurt flavoured with the spice Aframomum danielli, strawbeing and vanilla. The result also corroborates earlier research by Mbaeyi and Anyanwu (2010) on production and evaluation of yoghurt flavoured with solar dried bush mango.

Table 5. Microbial count of plain, flavoured and spiced yoghurt.

Simple	Dilution factor	Total bacterial count (cfu/ml)	Total coliform (MPN/100 ml)
PLY	10^2	0.8×10^3	0
PFSY	10^2	1.0×10^3	1
GSY	10^2	0.9×10^3	0
CFY	10^2	1.4×10^3	1
PFY	10^2	1.3×10^3	1

PLY, plain yoghurt; PFSY, pepper fruit spiced yoghurt; GSY, ginger spiced yoghurt; CFY, carrot flavoured yoghurt; PFY, pineapple flavoured yoghurt.

Microbiological assessment of plain yoghurt

Coliform group of bacteria

The Table 5 shows the results obtained after microbial examination of the samples. There was no evidence of coliform in samples PLY and GSY while PFSY, CFY and PFY had some evidence of coliform after production. That could be because of the extraction of pineapple, pepper fruit, ginger and carrot during processing.

Turkish standard institute, (1989), state that a maximum count of 10 cfu/ml of coliform group bacteria was allowed in yoghurt. So, the samples with values less than 10 cfu/ml are therefore justified suitable and safe for consumption. But absence of coliform will help extend the shelf-life of the products.

Different studies showed higher yoghurt counts of the coliform group bacteria in yoghurt samples. Dun and Ozgunes (1981) reported that 30 cfu/ml of coliform group bacteria was found in the yoghurts sold commercially in Ankara, Turkey.

Total bacteria count

The total bacterial count of the microbiological analysis showed sample PFY had ($0.8X10^3$) PFSY had ($1.0X10^3$) GSY had ($0.9X10^3$) CFYhad (1.410^3) PFY had ($1.3X10^3$).

There was no much difference in the total bacterial count in the spiced and flavoured yoghurts.

Micro- organisms used as starter culture have contributed to the yoghurt total bacterial count of the yoghurt samples.

Also the source (farm to market) of flavourants (pineapple and carrot) and spice (pepper fruit and ginger) may have contributed to the total bacterial count which is above that of plain yoghurts.

The bacterial count levels are very much within or below the acceptable range (8.7cfu) according to National Yoghurt Association (NYA, 2006).

Conclusion

From the results obtained, the nutritional quality of the plain yoghurt was improved by the addition of pepper fruit, ginger, carrot and pineapple especially in terms of vitamins and minerals. This implies that the therapeutic potency of yoghurt could be improved because consumption of foods with high vitamins A and C can aid in combating deficiency diseases like scurvy and night blindness. Adequate supply of minerals improve functionality of cells and also supports immunity of the body. The sensory attributes (colour, texture, aroma, taste, general acceptability) evaluated revealed appreciable degrees of acceptance by consumers thereby increasing varieties of yoghurts, in the market. This also justifies additional economic importance /use of pepper fruit, ginger, carrot and pineapple. Pepper fruit spiced yoghurt was most preferred in terms of general acceptability.

Recommendation

It is recommended that information on the production of spiced and flavoured yoghurts using pepper fruit, ginger, carrot and pineapple should be disseminated to domestic and commercial manufacturers of yoghurts and also it very necessary that further work should be done where pepper fruit, ginger, carrot and pineapple may be incorporated in yoghurt formulation before fermentation rather than being added as mere flavourants or spices. This research if carried out would reveal nutritional implication and organoleptic attributes of such products.

Conflict of interests

The authors did not declare any conflict of interest.

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