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Production, microbial and physico-chemical evaluation of 'dawadawan botso' (a condiment) produced by the fermentation of *Hibiscus sabdariffa* seeds

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ABSTRACT

Production and physico-chemical characteristic of 'dawadawan botso' was evaluated. A decrease in pH was observed after the second fermentation from an initial pH of 8.10 after cooking to 7.63. The mean bacteria count was between 2.7×10^4 $CFUg^{-1}$ to 1.7×10^6 $CFUg^{-1}$. The organisms associated with fermented dawadawan botso were isolated and identified as *Bacillus Pumilus, Bacillus subtilis, Brevibacillus laterosporus, Paenibacillus polymyxa, Bacillus amyloliquefaciens, Bacillus licheniformis, Brevibacillus brevis, Leuconostoc mesenteriodes, Lactobacillus plantarum, Pediococcus pentasaceus and Staphylococcus species*. *Bacillus* species appear to be the dominant microflora involved in the fermentation. The proximate composition and mineral content revealed variations at ($P < 0.05$) level between unfermented and fermented seeds of *H. sabdariffa* with lipid having a value of 17.60 and 17.17%; protein value 15.94 and 25.19%; then carbohydrate was 37.96 and 15.98%. This suggests that 'dawadawan botso' is a good and cheap source of protein for the lower class, who cannot afford other expensive sources of proteins and its consumption may have health benefit due to the presence of probiotic bacteria.

Keywords: *Hibiscus sabdariffa*, alkaline fermentation, proximate composition, mineral content, *Bacillus sp*, Lactic acid bacteria.

INTRODUCTION

'Dawadawan botso' is a condiment produced by a traditional uncontrolled alkaline fermentation of the seeds of *Hibiscus sabdariffa* by the rural dwellers of Zuru who call it 'chwande'. This condiment is also produced by other northern states such as Plateau, where the Tarok people ferment roselle seeds to make a cake to be used as 'sorrel meat' or Iyu (Schippers, 2000) and Borno where the Babur/ Bura ethnic groups, call it Nwanza Ntuza (Ayodele and Musa, 2008) and African Countries such as Burkina Faso where it is called Bikalga, Mali where it is called Datou, Cameroon where it is called Mbuja, Sudan where it is called Furundu and Niger where it is called 'dawadawa botso' (Bengaly et al., 2006; Parkouda et al., 2008). 'Dawadawa botso' is mainly produced by women and constitutes an economical source for the producers. 'Dawadawa botso' is used during cooking as substitute for fish or meat. It is poured into a bowl of water (preferably warm-like) then crushed for few minutes,

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using fingers to extract the oil and other components of the condiment and then the steeping water is used to prepare soups, stews, sauces, and other foods as desired by the consumer. In Zuru, the condiment is sometimes pounded into powder with dry pepper or alone and poured into a particular kind of soup made using okro and the leaves of *Hibiscus cannabinus* called "Jejebi" or added to hot shea butter to be used to fry the cooked leaves of *Hibiscus cannabinus.* It is also pounded into powder and used to prepare dry pounded okro soup usually as soup thickener and without fish but during occasions such as during harvest of farm produce, fish or meat is added to the steeping water of this condiment make a particular kind of soup called "Torsim zaba".

The northern parts of Nigeria are rich mostly in carbohydrate foods which need to be supplemented to meet the nutritional requirements of the people. In most developing countries particularly Nigeria, where people are malnourished because of the high rate of poverty among the lower class, which has necessitated this class to come-up with alternative means of cubing their malnutrition problem since they cannot afford other sources of protein such as egg, fish and meat. Production of 'dawadawan botso' has been described by other workers in some African countries where the production method and uses of the condiment differs (Bengaly et al., 2006; Mohammadou et al., 2007; Mohammed and Yagoub, 2007; Ouaba et al., 2007; Parkouda et al., 2008). Much has not been documented about the production, uses and nutritional composition of 'dawadawa botso' in Kebbi and north western part of Nigeria. Therefore, this research work aimed at producing 'dawadawa botso' and ascertaining its nutritional composition.

MATERIALS AND METHODS

Sample collection and processing

Two "Mudu" of the seeds of *Hibiscus sabdariffa* were purchased in Zuru market, Zuru Local Government Area of Kebbi State all in Nigeria.

Locally prepared 'dawadawan botso' was collected from a local producer in Kwendo village of Zuru Local Government Area, Kebbi State.

Dried Sorghum Stalks were collected from harvested farms at Kwendo village in Zuru, Kebbi State Nigeria. The leaves of the dried sorghum stalks were removed and the stalks burnt to ashes. It was allowed to cool naturally and collected in bags or sacks and brought to the laboratory.

Production of 'dawadawan botso'

Figure 1 gives the flow chart for 'dawadawan botso' production in Zuru.

Preparation of raw material

Raw seeds were pre-processed before the real production step. The pre-processing consists of a selection by manually sorting. The seeds were winnowed to eliminate stones, part of calyx, and other impurities and were repeatedly washed with water (02 to 03 times). The water cleaning step is in fact a sorting by gravity in the sense that immature seeds and spoiled seeds as well as other light impurities floated while heavy impurities (stones, sand) deposited as sediment.

Cooking

After the initial cleaning process, the seeds were cooked for 8-12 hours according to intensity of fire. Seeds were considered well cooked when soft and easily crushed with fingers. The water is allowed to dry without allowing the cooked seeds to burn. This step is laborious, time and energy consuming.

Fermentation

Fermentation took place in two phases; in the first phase, the cooked seeds were allowed in the pot to ferment naturally for two days. The pot was closed tightly to ensure that air does not gain access. After the first fermentation, the cooked seeds were pounded nearly to paste in a mortar with the addition of ash leachate and mixed. This was returned back to the pot for second fermentation for 1 day. The pot was also tightly closed in the phase.

Processing of 'dawadawa botso'

At the end of the second fermentation, the ammonia-like flavour condiment was sun dried by repeated turning to form balls and enable a good drying for 2 to 3 days according to the intensity of sunshine and package in polyethene bags or sacks.

Microbiological analysis

Ten (10 g) gram of each of the samples was weighed and dissolved in 90 ml. of sterile distilled water and was serially diluted to 10^3, 10^4 and 10^5. 0.1ml. from each test tube was transferred using sterile pipette onto sterile molten nutrient agar plate, spread using a sterile bent glass rod and incubated at 37 ^{0}C for 24 hours.

Processing, maintenance and identification of isolates

The isolate that emerge after 24 hours incubation were continually subculture until a pure culture is obtained. The pure cultures were then subculture on nutrient agar slants, incubated for 24 hours and refrigerated. The isolates were maintained on the slant until when required. Following series of biochemical reactions the isolates were identified as described by Holt et al. (1994).

Determination of pH

The pH was determined as done for fermented seeds of African locust beans (Ouoba et al., 2005) and *H. sabdariffa* (Parkouda et al., 2008). The pH of unfermented grinded seeds, fermenting seeds of *H. sabdariffa* and 'dawadawa botso' was measured directly in a mixture prepared with 10 grams of sample and 30 ml of distilled water mixed. A glass electrode pH meter was used for the measurement (CLIDA instrument PHS-25C precision pH/mV meter).

Proximate composition

Samples were analyzed in triplicate for proximate composition in accordance with the Official Methods of the Association of Official Analytical Chemists (AOAC, 1995). Ash was determined by incinerating two grams (2 g) each of ground unfermented and fermented seeds of *H. sabdariffa* at 550 °C in lenton furnaces (England) over night. Fiber was determined by drying two gram (2 g) each of ground unfermented and fermented seeds of *H. sabdariffa* over night at 105 ^{0}C in the oven (Gallenhamp Oven BS) and incinerated at 550 ^{0}C for 90 minutes in lenton furnaces (England). Moisture Content was determined by drying two gram (2 g) each of ground unfermented and fermented seeds of *H. sabdariffa* over night at 105 ^{0}C in the oven (Gallenhamp Oven BS). Crude lipid was determined using saturated method. Two grams (2 g) of ground unfermented and fermented seeds of *H. sabdariffa* were weighed into 50 ml conical flask and N-hexane was added and allowed to stand at room temperature overnight. It was drained into an empty flask, earlier weighed and designated W_1. It was placed in an oven to allow the N-hexane to evaporate in the oven (Gallenhamp Oven BS). Protein (% N * 6.25) was determined by the Micro-kjeldahl Method. Soluble carbohydrate is not determined directly but obtained as a difference between the sum of ash, protein, crude lipid and crude fiber.

Mineral content

Analysis of minerals in unfermented, locally fermented and laboratory fermented seeds of *H. sabdariffa* were done in triplicate according to methods described by Anhwange et al. (2006) and Walinga et al. (1989). The investigated minerals include Phosphorus, Potassium, Sodium, Calcium and Magnesium. Phosphorus was determined using Spectrophometer (JENWAY 6100) at 660 γ (wavelength), Potassium, Sodium was determined using flame photometer (Corning 400 Essex. England), determination of calcium and Magnesium was done by ethylene diamine tetra acetic acid (EDTA) Titration Method.

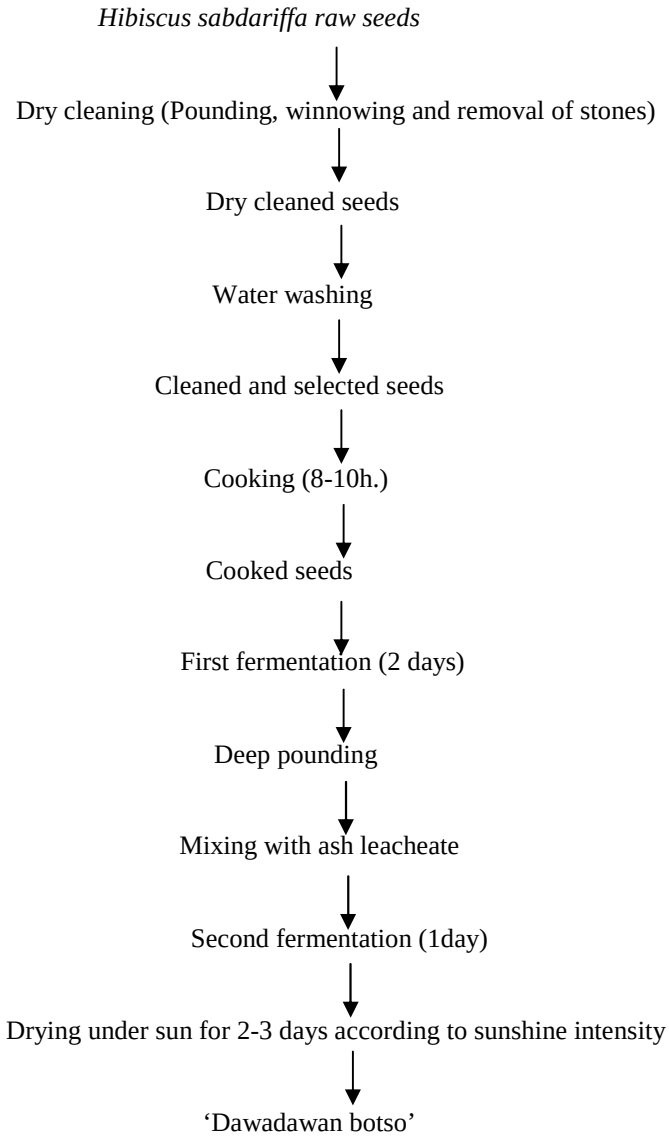

Figure 1: Flow chart for Dawadawan botso production in Zuru.

Statistical analysis

The data sets were expressed as mean ± standard deviation (n=3). Analysis of variance (ANOVA) was done using One-Way Analysis of Variance to test for the difference in means. Post-Hoc test using Duncan Multiple Range Test (DMRT) was carried out to test for the means that are significantly different from each other, which are presented by alphabets in superscripts. Paired Sample T-Test was used to test for the significance between samples at ($P<0.05$) level of significance using the SPSS for Windows, version 15.0. (Chicago IL, USA).

RESULTS

The biochemical identification of isolates from 'dawadawan botso', revealed the

following organisms namely *Bacillus Pumilus, Bacillus subtilis, Brevibacillus laterosporus, Paenibacillus polymyxa, Bacillus amyloliquefaciens, Bacillus licheniformis, Brevibacillus brevis*, some lactic acid bacteria such as *Leuconostoc mesenteriodes, Lactobacillus plantarum* and *Pediococcus pentasaceus and Staphylococcus species*. *Bacillus* species appear to be the dominant microflora involved in the fermentation.

The effect of fermentation on the pH of 'dawadawan botso' during production was determined (Figure 2). From the result a decrease in pH was observed after the second fermentation from an initial pH of 8.10 after cooking to 7.63.

The result of the mean bacteria count during 'dawadawan botso' production was determined (Table 2). An increase in mean plate count was observed after cooking, from the first fermentation to second fermentation. The mean bacteria count was between 2.7×10^4 $CFUg^{-1}$ to 1.7×10^6 $CFUg^{-1}$.

The proximate composition of the unfermented seeds of *Hibiscus sabdariffa* and the traditionally/ locally and laboratory based fermented seeds of *Hibiscus sabdariffa* was conducted (Table 3). The lipid value was 17.60, 17.17, and 21.00%; protein value was 15.94, 25.19 and 25.72%; then carbohydrate was 37.96, 15.98 and 36.90%.

The mineral content of the unfermented seeds of *Hibiscus sabdariffa* and the traditionally and laboratory based fermented seeds of *Hibiscus sabdariffa* was conducted (Table 4). Potassium was the highest mineral follow by sodium, then phosphorus, magnesium and calcium was the least mineral.

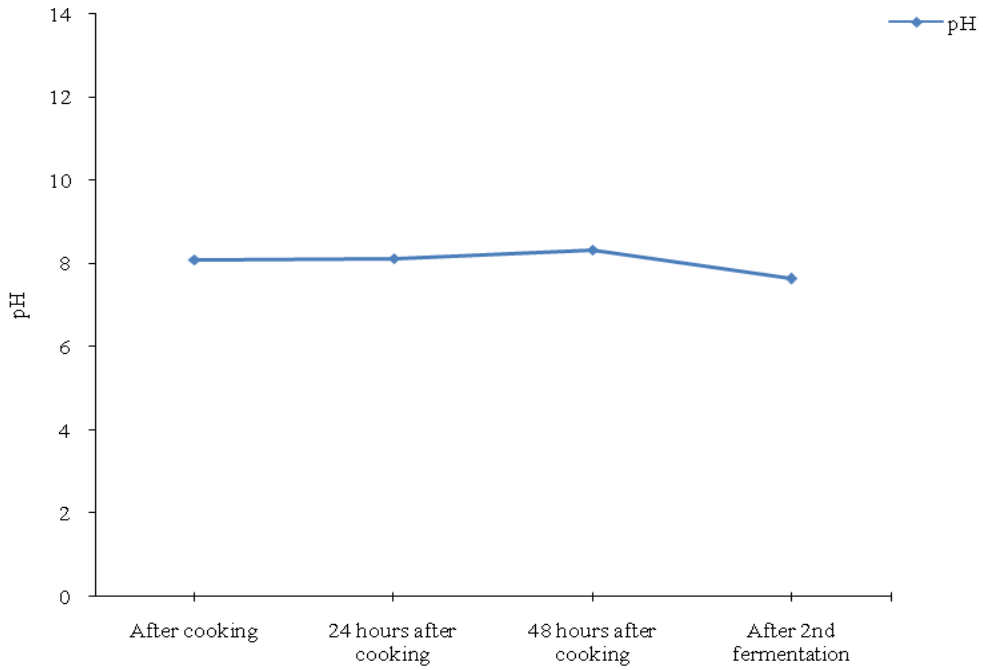

Figure 2: The effect of fermentation on the pH of 'dawadawan botso' during production.

Table 1: Result of mean heterotrophic bacteria count during 'dawadawan botso' production.

Sampling time	Mean bacterial Count (CFU/g)
After cooking seeds	2.7×10^4
24hours into 1st fermentation	4.0×10^4
48hours into 1st fermentation	4.3×10^5
After 2nd fermentation with ash leachate	1.7×10^6

Table 2: Proximate compositions of unfermented seeds of *H. sabdariffa*, locally and laboratory produced 'dawadawan botso'.

Proximate Components (%)	**Moisture**	**Ash**	**Lipid**	**Fibre**	**Crude protein**	**Carbohydrate**
Unfermented	4.17 ± 0.29^a	6.33 ± 0.29^a	17.16 ± 0.29^a	18.00 ± 0.50^b	15.94 ± 0.06^a	37.96 ± 1.04^b
Locally fermented	15.00 ± 0.50^b	9.33 ± 0.29^b	17.17 ± 0.29^a	17.33 ± 0.58^b	25.19 ± 0.31^b	15.98 ± 1.48^a
Laboratory fermented	4.17 ± 0.29^a	9.50 ± 0.50^b	21.00 ± 0.50^b	3.00 ± 0.50^a	25.72 ± 0.83^b	36.9 ± 0.65^b

Each data point presented as Mean ± Standard Deviation (n=3) Means along the same column with different superscript are significantly different at P<0.05

Table 3: Mineral content of unfermented seeds of *H. sabdariffa*, locally and laboratory produced 'dawadawan botso'.

Mineral Content (mg/kg)	**Magnesium**	**Phosphorus**	**Sodium**	**Potassium**	**Calcium**
Unfermented	0.14 ± 0.01^a	4.21 ± 0.02^c	78.33 ± 3.82^a	5600.00 ± 100.00^a	0.06 ± 0.00^a
Locally fermented	0.26 ± 0.03^a	3.50 ± 0.02^b	155.83 ± 2.89^b	13733.33 ± 115.47^b	0.04 ± 0.00^a
Laboratory fermented	2.71 ± 0.19^b	3.17 ± 0.03^a	206.67 ± 1.44^c	21500.00 ± 866.03^c	0.54 ± 0.08^b

Each data point presented as Mean ± Standard Deviation (n=3) Means along the same column with different superscript are significantly different at P<0.05.

DISCUSSION

The long cooking time observed during production of 'dawadawan botso' was also observed in *Parkia biglobosa* for Soumbala production (Diawara et al., 1998). A much lower time (about 3 h) as compared to 8-10 h of cooking for 'dawadawan botso' production has been reported during the production of Furundu and Mbuja (Yagoub et al., 2004; Mohamadou et al., 2007) making them less accessible for the action of degrading enzymes (Harper and Collin, 1992). Differences also appear in the fermentation process of 'dawadawan botso' compared to the precited similar products. For Bikalga, fermentation takes place in two steps: 2-3 days for the first fermentation, then the seeds are pounded, moulded into big balls and let to ferment again for 1-2 days (Parkouda et al., 2008). For Furundu, fermentation is done at once and lasts longer (7-10 days) (Harper and Collin, 1992; Yagoub et al., 2004). For Mbuja, fermentation takes place in two steps: seven days for the first fermentation, then the seeds are pounded and let to ferment again for three days (Mohamadou et al., 2007). In both cases, no steaming step occurred during the process. The long cooking time actually constitutes a first step of selection for heat- and alkali-resistance bacteria as *Bacillus species* (Ouoba et al., 2007).

An initial pH of 8.10 was recorded after cooking the seeds for about 8 – 10 h. There was no significant difference at P<0.05 for this value and that obtained after 24 hour of fermenting the seeds. A significant difference at P<0.05 was observed after the first fermentation which lasted for 48 hours. This

increase after the first fermentation might be due to the proteolytic activity of the *Bacillus* isolate responsible for the fermentation. Degraded proteins into amino acids, a part of which was further being degraded to ammonia may be responsible for the pH increase and the ammonia like-flavour. A final pH of 7. 63 was recorded after second fermentation and it was significantly different at P<0.05 when compared to that recorded after first fermentation and cooking respectively. This slight decrease could probably be linked to an increase in degradation of carbohydrates and lipids leading to a high production of acidic compounds responsible for the pH decrease during the final fermentation (Ouoba et al., 2003b). Harper and Collin (1992) reported an increased production of acids such as lactic acid and especially acetic acid accompanied with a decrease of pH during the production of Furundu. Volatile fatty acids as well as traces of propionic acids were also detected. Moreover, Ouoba et al. (2003a) reported that *Bacillus* proteolytic activity decreased after 36-48h of fermentation for Soumbala production.

The mean heterotrophic bacteria count of fermenting organisms during the production of 'dawadawan botso' shows an increase from an initial count of 2.7×10^4 to1.7×10^6 $CFUg^{-1}$ after cooking and after the second fermentation (Table 1). This is probably due to the fact that the organisms have to multiply enough to synthesize the required amount of enzyme needed to degrade and hydrolyse the seed so that they could access the nutrients in the substrate. Mohamadou et al. (2007) also reported a count of spores of *Bacillus* to be 2.6×10^5 and 6.2×10^7 spores g^{-1} and total counts of lactic acid bacteria which range between 5.8×10^5 UFC g^{-1} and 6.6×10^7 UFC g^{-1} and that the proportion of these bacteria did not vary significantly. Mohammed and Yagoub (2007), reported that fermentation of sprouted karkade seed increased the initial number of viable bacteria from 7.0×10^4 $CFUg^{-1}$ to numbers ranging from 1.2×10^5 to 2.8×10^6 $CFUg^{-1}$ for a period that extends to 9 days.

The proximate composition of unfermented locally fermented and laboratory- based fermented seeds of *H. sabdariffa* (Table 2) shows variation. A significant difference at P<0.05 was observed in crude protein between the unfermented and the fermented seeds. However, no significant difference at P<0.05 was observed between the locally fermented and the laboratory-based fermented seeds. No significant difference at P<0.05 was observed in the soluble carbohydrate content of unfermented and laboratory-based fermented seeds. However, they differ significantly at P<0.05 with the locally fermented 'dawadawan botso'. No significant difference at P<0.05 was observed in the lipid content of unfermented and locally fermented seeds of *H. sabdariffa.* However, there was a significant difference at P<0.05 between their values and that obtained for laboratory based fermented condiment. No significant difference at P<0.05 was observed in the fiber content of unfermented and locally fermented seeds of *H. sabdariffa.* However, there was a significant difference at P<0.05 between their values and that obtained for laboratory based fermented condiment.

A significant difference at P<0.05 was observed in ash content between the unfermented and the fermented seeds. However no significant difference at P<0.05 was observed between the locally fermented and the laboratory fermented seeds. No significant difference at P<0.05 was observed in the moisture content of unfermented and laboratory fermented seeds. However, they differ significantly at P<0.05 with the locally fermented.

Recently, many workers have reported different proximate composition values for the unfermented and fermented seeds of *H. sabdariffa* for different cultivars. Yagoub et al. (2004) reported that cooking followed by fermentation resulted in deviation of nutrients from the raw seed. These workers reported a total protein value of 28.59%, 28.90% and 28.66% for whole raw 'Karkade' seed, cooked 'Karkade' seed and nine days fermented seeds 'Furundu'. Yagoub et al. (2004) reported a total protein of (29.79%), true protein (28.44%), non protein nitrogen (1.35%) and water soluble protein (6.81%) for raw karkade seeds and that they were changed during 'furundu' preparation to varied extents. The changes in nitrogenous constituents has been reported by workers to be a result of leaching out effect during working (Saikia et al., 1999)

and as a consequence of proteolytic activities of enzymes during fermentation (El-Faki et al., 1991). Harper and Collin (1992) reported that the decrease could be as a result of little loss of nitrogen. However, Bengaly et al. (2006) reported an increase of 5% of crude protein during fermentation of *H. sabdariffa* to produce 'Bikalga'. The reported range of crude protein by most workers is between 25.79 to 30.00% for raw seeds of *H. sabdariffa* (Harper and Collin, 1992; Abu-Tarboush et al., 1997; Omobuwajo et al., 2000; Yagoub et al., 2004; Bengaly et al., 2006; Parkouda et al., 2008). Anhwange et al. (2006) reported a crude protein value of 19.84% for Benue State of Nigeria cultivars. However, this value was within the range of 17-30% reported for protein representation in most legumes (Sagarika et al., 1999). Bengaly et al. (2006) reported a slight increase in a particular cultivar. Rao (1999) also reported lower protein levels (18.8-22.3%) for Indian roselle seed. However, these differences may be due to cultivars varieties differences and/or variations in the agro-climatic conditions (Yogoub et al., 2004).

Bengaly et al. (2006) also reported that cooking and fermentation yielded a significant increase in protein content by 5% and soluble carbohydrate by 3%. These workers attributed this increment to the bacteria metabolism involving polysaccharides hydrolysis and proteogenesis during fermentation (Dauner et al., 2001). Similar protein increment was also reported during the African Locust bean (*Parkia biglobosa*) fermentation (Ibrahim and Antai, 1986). Anhwange et al. (2006) reported a value of 33.0% for soluble carbohydrate for raw seeds of *H. sabdariffa* and described this value as being relatively low as compared to those of *Diospyrous mespiliformis, Daneia Ogea* and *Afzellia* which were given as 77, 74 and 54% respectively (Herog *et al.*, 1994).

Recently, workers have also reported increase in the lipid content of *H. sabdariffa seed* during fermentation for the production 'Bikalga' (Bengaly et al., 2006; Parkouda et al., 2008) and for furundu production (Yagoub et al., 2004). However, Anhwange et al. (2006) reported a higher value (28.10%) for raw seeds of Benue State cultivars as compared to those (20-24%) reported by Duke and Atchley (1984) and other workers (Ibrahim and Antai, 1986; Yagoub et al., 2004; Bengaly et al., 2006; Parkouda et al., 2008), reported an increase of lipid content during the fermentation of African locust beans for soumbala production, this is due to a selective utilization of carbohydrate by the microflora during the fermentation. Anhwange et al. (2006) reported that the seeds of *H. sabdariffa* contain an appreciable fibre content which falls within reported values (6-7%) for most legumes (Saddhuraju et al., 1998). However, these values have been observed to increase after fermentation (Yagoub et al., 2004; Bengaly et al., 2006).

Parkouda et al. (2008) reported a substantial increase in ash content in fermented seeds of *H. sabdariffa* which reflects mainly the mineral contribution made by liberal addition of ash-leacheate. Earlier studies have shown that moisture content increased in fermented seeds probably due to the cooking period (Parkouda et al., 2008).

A significant difference at $P<0.05$ was observed following analysis of the mineral content of the seeds of *H. sabdariffa* (Table 3). The major mineral element is potassium which ranges from 5600 – 21500 mg/kg and sodium which ranges from 78.33 – 206.67 mg/kg from unfermented to fermented seeds respectively. The unfermented seeds had the least value of 5600 mg/kg for potassium. This value increased in the fermented seeds with locally fermented having 13733.33 mg/kg which differ significantly at $P<0.05$ with the laboratory-based fermented (21500 mg/kg). A significant difference at $P<0.05$ variation was observed in sodium, phosphorus, magnesium, calcium. Calcium had the least value (0.04 – 0.54 mg/kg) which significantly differ at $P<0.05$ between the unfermented to fermented seeds.

The significant variation in mineral content can be related to the type of soil from which the seed were harvested in the case of raw seeds and mainly to the addition of ash leachate for the fermented seeds (Parkouda et al., 2008). Infact, the amount and type of the alkalinizing leachate to be added as well as the precise step during the process where it should be added varied significantly from one producer to another according to the organoleptic characteristics expected (Parkouda et al., 2008). Harper and Collin

(1992) reported that dried leachate of ash from sorghum is largely composed of potassium bicarbonate with smaller quantities of potassium chloride, silicate and sulphate, explaining then the reason for the increase and the large variation for some minerals especially potassium. Leachate from other plants might be composed differently by large amounts of other alkalinizing compounds as sodium, calcium, iron derivates leading then to different mineral content and balance. Another origin of minerals could be attributed to the fermentation recipients (Harper and Collin, 1992).

Conclusion

The fermentation of *H. sabdariffa* for the production of 'dawadawan botso' is a less laborious and time consuming. 'Dawadawan botso' production is a process where lipolysis, proteolysis as well as degradation of carbohydrates seem to be equally important. *H. sabdariffa* is a good and cheap source of protein for human nutrition. 'Dawadawan botso' could be considered as an affordable fish or meat substitute particularly for low income earners in developing countries such as Nigeria. 'Dawadawan botso' provides some beneficial attributes to its consumers by providing bacteria with probiotic potential.

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