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# An attempt towards standardization of the production process of dawadawan botso (a fermented condiment)

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**The production of 'dawadawan botso' a local condiment produced by traditional uncontrolled fermentation, in Nigeria and other parts of Africa is usually based on experience rather than standard measurements. This work was aimed at evaluating the effect of substrate (*Hibiscus sabdariffa* seeds) and ash leachate on the chemical composition, amino acid profile and the taste of 'dawadawan botso.' Locally prepared 'dawadawan botso' was collected from a local producer and its quality was compared to that prepared in the laboratory under optimized conditions. The ash leachate was standardized by dissolving known masses of ash in a fixed volume of water. The pH of the ash leachate was determined and then added to the substrate before the second fermentation. The pH of the unfermented, fermented *H. sabdariffa* and the 'dawadawan botso' were determined using a pH meter. Determination of the proximate composition, mineral and amino acid profile were done by AOAC method. The results were analyzed statistically using ANOVA and the post-hoc test using Duncan multiple range test (DMRT) for means that were significantly different from each other. Paired sample T- test was also done to test for the significance between groups at P<0.05 using SPSS. The results show that the volume of leachate decreased (385 ml/100 g ash to 144 ml/250 g ash) with increasing pH (13.60 to 13.70) as the mass of ash increased. Proximate analyses showed that carbohydrates were the highest followed by proteins and least was lipids. Potassium (K) and sodium (Na) were the major minerals; magnesium (Mg), phosphorus (P) and calcium (Ca) were insignificant compared to the later. The pH of the substrate decreased after second fermentation for all standards and also decreased as mass of substrate increased. The following amino acids; glutamic, aspartic and methionine were observed to decrease with increasing mass of substrate. Fermentation increased the value of total amino acids in substrate. Taste analyses indicated that monosodium glutamate-like taste was dominant, followed by bitter and sweet, respectively for all samples analyzed.**

**Key words:** Ash, *Hibiscus sabdariffa,* "dawadawan botso", amino acid profile, proximate composition.

## INTRODUCTION

Many African tribes have inherited at least one product of their tradition fermentation. These products are mostly produced from the fermentation of neglected and underutilized plants species. The process takes a long time and often it

lacks a standard protocol for its production. In Nigeria, this product includes soup condiments such as 'dawadawa' made by fermentation of soybean seeds (Popoola and Akueshi, 1984; Ogbadu and Okagbue, 1988), dawadawa-/iru by the Hausa and Yoruba people made by the fermentation of *Parkia bigloboso* seeds (Odunfa, 1981a), ogiri by Ibos made by fermentation of melon seeds (Odunfa, 1981b), ogiri-Igbo made from castor oil seeds (Odunfa, 1985), ogiri-ugu made from flutted pumpkin seeds (Barber and Achinewhu, 1992; Odibo and Umeh, 1989) owoh by the Urhobo and Tsekiri people of Niger Delta made by either African yam beans (Ogbonna et al., 2001) or cotton seeds (Sanni and Ogbonna, 1991), okpiye by the Igala and Idoma people produced from *Prosopis Africana* (Achi,1992). Dawadawan botso is a condiment produced by fermenting the seeds of *Hibiscus sabdariffa* by the rural dwellers of Zuru where it is call chwande (Ibrahim et al., 2011b). This condiment is also produced in other northern states of Nigeria 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 other African countries like Burkina Faso who called it 'Bikalga', Mali who called it 'Datou', Cameroon who called it 'Mbuja', Sudan who called it 'Furundu' and Niger who also called it Dawadawan botso (Parkouda et al., 2008; Bengaly et al., 2006).

There has been a major concern as to the required standard for the production of African food condiments as most workers are focusing on the issues of introducing starter culture or quality control. For industrial or large scale production of these condiments, there is a need for the standardization of the production process, since all industries are aiming at profit maximization. Standardizing the production process will go a long way in helping to work out the economics of its production. It was on the vein that this research work was conducted with the aim of determining the effect of substrate and ash leachate on the nutritional profile of dawadawan botso.

## MATERIALS AND METHODS

### Sample collection and processing

A mass of about 10 kg of the seeds of *H. sabdariffa* was 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 bagco sack and brought to laboratory.

### Testing for the effect of ash quantity on the pH and nutritional quality of dawadawan botso

The ash leachate (solution) production was standardized by weighing different amount of ash (solute) (100, 150, 200, 250 and 300 g) to fixed volume of water (solvent) (600 ml). Each of the ash leacheate collected was used to produce the dawadawan botso by adding 37.5 g/22.5 ml and 75 g/22.5 ml of the seeds of *H. sabdariffa* and ash leachate in milliliter. This was added after the first fermentation for two days and the seeds were pounded, mixed with the ash leachate and it was allowed to undergo second fermentation for one day. The second fermentation was altered by spreading the condiment on polyethene bags and dried under the sun.

### Testing the effect of substrate (seed) on the pH and nutritional quality of "dawadawan botso"

The effect of substrate on the pH and nutritional quality of dawadawan botso was done by weighing 150, 250, 300 and 400 g of the seeds of *H. sabdariffa* into separate pots, washed two to three times and cooked for 8-10 h. The cooked seed were allowed in the pot for the first fermentation for two days, pounded using local mortar and pestle, and a fixed volume of ash leacheate (90 ml) was added to all seed quantities and fixed using fingers and it was allowed to undergo second fermentation for 1 day. The fermentation was altered by spreading the condiment on polyethene bags and dried under the sun.

### 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 ground seeds, fermented seeds of *H. sabdariffa* and dawadawan botso was measured directly in a mixture prepared with 10 g 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 (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 2 g each of ground unfermented and fermented seeds of *H. sabdariffa* overnight at 105°C in the oven (Gallenhamp Oven BS) and incinerated at 550°C for 90 min in lenton furnaces (England). Moisture content was determined by drying 2 g each of ground unfermented and fermented seeds of *H. sabdariffa* overnight at 105°C in the oven (Gallenhamp Oven BS). Crude lipid was determined by weighing a known weight of the dried sample into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006). The extraction lasted for

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15 h. It was drained into an empty flask. 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 and the weight was determined by difference. Soluble carbohydrate was determined indirectly as the difference between crude protein and 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 Hack (2000). The investigated minerals include phosphorus, potassium, sodium, calcium and magnesium. Phosphorus was determined using spectrophometer (JENWAY 6100) at 660 $\gamma$ (wavelength), potassium and 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.

**Determination of amino acid profile**

The amino acid profile in the known sample was determined using methods described by Spackman et al*.* (1958). The known sample was dried to constant weight. The sequential multi-sample amino acid analyzer (TSM) was used to analyze amino acids. Four samples with the highest characteristic dawadawan botso aroma as described by the consumers were subjected to amino acid analysis. The samples were defatted by weighing a known weight of the dried sample into extraction thimble and the fat was extracted with chloroform/methanol (2:1 mixture) using soxhlet extraction apparatus as described by AOAC (2006). The extraction lasted for 15 h. Nitrogen was determined by weighing a small amount (200 mg) of ground sample, wrapped in Whatman filter paper (No. 1) and put in a Kjedhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate ($Na_2SO_4$), copper sulphate ($CuSO_4$) and selenium oxide ($SeO_2$) in the ratio 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added. The flask was then put on Kjedhal digestion apparatus for 3 h until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. Aliquot (10 ml) of the diluted solution with 10 ml of 45% sodium hydroxide were put into the Markham distillation apparatus and distilled into 10 ml of 2% boric acid containing four drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected. The distillate was then titrated with standardized 0.01N hydrochloric acid to grey colour end point. The percentage nitrogen in the original sample was calculated using the formula:

$$\text{Percentage Nitrogen} = \frac{(a-b)\times 0.01\times 14\times V}{W\times C}\times 100$$

Where, a = titre value of the digested sample; b = titre value of blank sample; *V* = volume after dilution (100 ml); *W* = weight of dried sample (mg); *C* = aliquot of the sample used (10 ml); 14 mg = Nitrogen constant in mg; 100 = Conversion factor to percentage (Spackman et al*.,* 1958). Hydrolysis of the samples was done by weighing a known mass of the defatted sample into glass ampoule. Seven millilittres of 6 N HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis for example methinonine and cystine). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105°C for 22 h and the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator and the residue was dissolved with 5 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer. The hydrolysate was loaded into the TSM analyzer by loading 5 to 10 µl (5 for acidic/ neutral amino acid and 10 for basic amino acids). This was dispensed into the cartridge of the analyzer. The TSM analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of an analysis lasted for 76 min.

**Grouping of free amino acid**

This was done in accordance to the taste characteristics described by Tseng et al. (2005), amino acids were grouped as MSG-like (monosodium glutamate-like) (Asp+Glu), sweet (Ala+Gly+Ser+Thr), bitter (Arg+His+Ile+Leu+Met+Phe+Trp+Try+Val), and tasteless (Cys+Lys+Pro).

**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 AND DISCUSSION

Ash leachate preparation was standardized and the result is presented in Table 1. To the best of our knowledge, this is one of the few work describing in details the standardization of ash leachate preparation. More ash leachate (385 and 325 ml) was harvested when low amount (100 and 150 grams) of ash is used to fix amount of water. This is probable because low ash quantity tends to hold less amount of water thereby allowing the rest to pass through the perforated container to be collected as ash leachate and at 300 g it retained the 600 ml of water used. This was the reason for not harvesting any amount of ash leachate. There was a significant difference in the pH at $P<0.05$ of ash leachate harvested from low ash concentration as compared to those obtained at higher ash concentrations.

The effect of different ash leachate preparations on the pH during the production of dawadawan botso has been conducted (Table 2). The result shows various pH values for each of the ash leachate standards after first and second fermentations. There was significant effect of the prepared ash leachate standards on pH of 'dawadawan botso' at $P<0.05$ between the pH of each of the standards before and after second fermentation. An initial pH of 11.07 to 11.70 was recorded when 100 and 250 gash leachate was used as compared to pH of 10.93 to 10.97 recorded when 150 g was used. A fall in pH was observed for all the standards after second fermentation, an indication

**Table 1.** Standardization of ash leachate preparation.

| Quantity of ash (g) | Amount of water ($H_20$) (ml) | Leachate collected (ml) | pH |
|---|---|---|---|
| 100 | 600 | 385 $^{e}$ | 13.60 ± 0.000$^{b}$ |
| 150 | 600 | 325 $^{d}$ | 13.63 ± 0.058$^{b}$ |
| 200 | 600 | 223 $^{c}$ | 13.70 ± 0.000$^{c}$ |
| 250 | 600 | 144 $^{b}$ | 13.70 ± 0.000$^{c}$ |
| 300 | 600 | 0 $^{a}$ | 0$^{a}$ |

Each data point presented as mean±standard deviation (n=3).

**Table 2.** Effects of 1$^{st}$, 2$^{nd}$, and 4$^{th}$ standards [(100 g/600 ml), (150 g/600 ml), (250 g/600 ml)] on pH.

| Quantity of seed | Effect of 1$^{st}$ standard (100 g/600 ml) on pH | | Effect of 2$^{nd}$ standard (150 g/600 ml) on pH | | Effect of 4$^{th}$ standard (250 g/600 ml) on pH | |
|---|---|---|---|---|---|---|
| | Before 2$^{nd}$ fermentation (E1) | After 2$^{nd}$ fermentation (E2) | Before 2$^{nd}$ fermentation (E3) | After 2$^{nd}$ fermentation (E4) | Before 2$^{nd}$ fermentation (E5) | After 2$^{nd}$ fermentation (E6) |
| 37.5 g/22.5 ml | 11.33±0.153$^{b}$ | 9.13±0.058$^{b}$ | 10.97±0.058$^{a}$ | 9.77±0.058$^{b}$ | 11.43±0.058$^{a}$ | 9.80±0.000$^{b}$ |
| 75 g/22.5 ml | 11.07±0.058$^{a}$ | 8.43±0.153$^{a}$ | 10.93±0.058$^{a}$ | 8.67±0.116$^{a}$ | 11.70±0.000$^{b}$ | 9.27±0.058$^{a}$ |

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.** Effect of ash leachate on the proximate composition (%) of fermented seeds of *Hibiscus sabdariffa.*

| **Proximate components** (%) | E1 | E2 | E3 | E4 | E5 | E6 |
|---|---|---|---|---|---|---|
| Moisture | 5.33±0.29$^{abc}$ | 6.00±0.50$^{c}$ | 4.67±0.29$^{a}$ | 5.00±0.50$^{ab}$ | 5.83±0.58$^{bc}$ | 5.67±0.58$^{bc}$ |
| Ash | 10.17±0.76$^{a}$ | 11.17±0.58$^{bc}$ | 10.67±0.29$^{abc}$ | 10.00±0.50$^{a}$ | 10.33±0.29$^{ab}$ | 11.50±0.50$^{c}$ |
| Lipid | 21.33±1.76$^{a}$ | 20.67±1.61$^{a}$ | 21.67±0.76$^{a}$ | 21.33±1.76$^{a}$ | 19.67±0.76$^{a}$ | 21.00±1.32$^{a}$ |
| Fibre | 5.50±0.50$^{bc}$ | 4.83±0.58$^{ab}$ | 4.50±0.50$^{a}$ | 5.67±0.29$^{c}$ | 4.17±0.29$^{a}$ | 6.17±0.29$^{c}$ |
| Crude protein | 30.56±0.33$^{c}$ | 30.57±0.29$^{c}$ | 27.13±0.06$^{b}$ | 27.14±0.18$^{b}$ | 26.20±0.15$^{a}$ | 26.20±0.29$^{a}$ |
| Carbohydrate | 27.11±0.10$^{a}$ | 26.75±0.68$^{a}$ | 31.36±2.42$^{bc}$ | 30.86±1.00$^{bc}$ | 33.80±2.90$^{c}$ | 29.47±0.66$^{ab}$ |

Each data point presented as mean±SEM (n=3). Means along the same row with different superscript are significantly different at P<0.05. 100 g Ash/600 ml water, before 2$^{nd}$ fermentation (E1). 100 g Ash/600 ml water, After 2$^{nd}$ fermentation (E2). 150 g Ash/600 ml water, before 2$^{nd}$ fermentation (E3). 150 g Ash/600 ml water, after 2$^{nd}$ fermentation (E4). 250 g Ash/600 ml water, Before 2$^{nd}$ fermentation (E5). 250 g Ash/600 ml water, After 2$^{nd}$ fermentation (E6).

strongly suggesting the degradation of carbohydrates and lipid to release acid compounds. This is particularly a common scenario for many fermented condi-ment (Ouoba et al., 2003; Harper and Collin, 1992).

The effect of different ash leachate concentration on the proximate composition of 'dawadawan botso' is shown on Table 3, the least and highest lipid values were; 19.67 and 21.67% respectively. The least and highest protein values were 26.20 and 30.57% respectively. The effect of substrate concentration on the proximate composition of dawadawan botso is shown on Table 4. The least and highest lipid value (20.00 and 22.17) was recorded when 250 and 150 g were used for the fermentation respectively. The least and highest protein value were; 25.63 and 28.06 for 150 g and 300 g respectively. The highest and least carbohydrate values were; 35.93 and 31.62 for 300 g and 400 g respectively. The highest and least carbohydrate value was 26.75 and 33.80% respectively. The amount and type of the alkalinizing leachate that is added as well as the precise step during the process where it should be added has been reported to have a significant effect on the organoleptic characteristics of Bikalga (Parkouda et al., 2008). The alkalinizing leachate had effect on the proximate composition of 'dawadawan botso'. There was significant difference at P<0.05 in crude protein within the various alkalinizing leachate standards used with that from 100 g having the highest protein content (30.56 and 30.57%). This variation in crude protein could be due to the pH of the alkalinizing leachate which also has effect on the

**Table 4.** Effect of substrate (seeds of *H. sabdariffa*) concentration on the proximate composition of 'dawadawan botso'.

| Substrate (g)/90 ml of ash leachate | Moisture (%) | Ash (%) | Lipid (%) | Fibre (%) | Crude protein (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|
| 150 | 5.17±0.29$^{b}$ | 10.33±0.76$^{b}$ | 22.17±0.58$^{b}$ | 2.83±0.29$^{a}$ | 25.63±0.34$^{a}$ | 33.89±3.03$^{ab}$ |
| 250 | 6.00±0.50$^{c}$ | 8.33±0.58$^{a}$ | 20.00±0.50$^{a}$ | 3.17±0.29$^{a}$ | 27.44±0.46$^{b}$ | 35.06±0.06$^{ab}$ |
| 300 | 4.17±0.29$^{a}$ | 7.50±0.50$^{a}$ | 21.67±0.76$^{b}$ | 2.67±0.29$^{a}$ | 28.06±0.06$^{c}$ | 35.93±0.94$^{b}$ |
| 400 | 7.00±0.50$^{d}$ | 7.33±0.29$^{a}$ | 21.83±0.76$^{b}$ | 3.17±0.29$^{a}$ | 29.05±0.05$^{d}$ | 31.62±2.04$^{a}$ |

Each data point presented as mean±SEM (n=3) Means along the same row with different superscript are significantly different at P<0.05

**Table 5.** Effect of ash leachate on the mineral content of 'Dawadawan botso'.

| Mineral content | E1 | E2 | E3 | E4 | E5 | E6 |
|---|---|---|---|---|---|---|
| Magnesium (mg/kg) | 2.11±0.12$^{a}$ | 1.81±0.23$^{a}$ | 1.90±0.20$^{a}$ | 1.88±0.15$^{a}$ | 2.05±0.22$^{a}$ | 2.67±0.12$^{b}$ |
| Phosphorus (mg/kg) | 1.93±0.03$^{c}$ | 1.87±0.01$^{ab}$ | 1.85±0.02$^{a}$ | 1.90±0.02$^{bc}$ | 1.98±0.03$^{d}$ | 1.90±0.02$^{bc}$ |
| Sodium (mg/kg) | 136.67±8.04$^{ab}$ | 134.17±5.77$^{a}$ | 140.83±3.82$^{ab}$ | 135.83±7.64$^{a}$ | 141.67±3.82$^{ab}$ | 148.33±6.29$^{b}$ |
| Potassium (mg/kg) | 24000.00±1000.00$^{b}$ | 24000.00±1000.00$^{b}$ | 25166.67±1040.83$^{b}$ | 24166.67±1258.31$^{b}$ | 25500.0±1000.00$^{b}$ | 20666.67±1258.31$^{a}$ |
| Calcium (mg/kg) | 0 .54±0.08$^{a}$ | 0.63±0.13$^{a}$ | 0.50±0.13$^{a}$ | 0.59±0.08$^{a}$ | 0.50±0.25$^{a}$ | 0.47±0.03$^{a}$ |

Each data point presented as mean±SEM (n=3). Means along the same row with different superscript are significantly different at P<0.05. E1,100 g ash/600 ml water - before 2$^{nd}$ fermentation; E2,100 g ash/600 ml water - after 2$^{nd}$ fermentation; E3,150 g ash/600 ml water - before 2$^{nd}$ fermentation. E4, 150 g ash/600 ml water - after 2$^{nd}$ fermentation. E5, 250 g Ash/600 ml water- Before 2$^{nd}$ fermentation; E6, 250 g Ash/600 ml water- After 2$^{nd}$ fermentation.

fermenting organisms or their enzymes as microbial biomass also contributes to the crude protein content of fermented condiments. However, no significant difference at P<0.05 was observed in the lipid contents of the dawadawan botso. A significant difference at P<0.05 was observed in soluble carbohydrate. The constant low pH has been found to reduce protein degradation and increase non ammonia nitrogen (N) and dietary nitrogen (N) flow compared with constant high pH (Calsamiglia et al., 2002). The decrease observed in the lipid content may be due to microbial action during the fermentation process. The lipids will obviously contribute to short chain fatty acids and impart flavor on the condiment (Ibrahim et al., 2011a). Four hundred grams had the lowest soluble carbohydrate explaining that it may have supported the growth of more microbial cells which hydrolyzed the soluble carbohydrate to reducing sugars easily utilizable by the microorganisms as source of energy (Yagoub et al., 2004).

The effect of ash leachate standards on the mineral content of 'dawadawan Botso' is shown in Table 5. From the table, potassium and sodium were the major minerals which ranged from 20666.67 to 25166.67 and 134.17 to 148.33 mg/kg, respectively. In evaluating the effect of substrate concentration on the mineral content of dawadawan botso (Table 6), we also obtained similar result for potassium and sodium. The ash concentration used in the preparation of alkalinizing leachate has effect on some of mineral content of dawadawan botso. A significant difference at P<0.05 was observed in the phosphorus content of dawadawan botso with the alkalinizing leachate prepared from 250 g ash at low seed concentration having the highest value (1.98 mg/kg) compared to 100 g. Since the substrates contain different minerals apart from carbon which may serve as nutrient supplements, increasing substrate concentration has been found to increase the bioavailability of the mineral elements. No significant difference at P<0.05 between the potassium content of 250, 300 and 400 g was observed.

The effect of substrate on pH of dawadawan botso

**Table 6.** Effect of substrate (seeds of *H. sabdariffa*) concentration on the mineral content of 'dawadawan botso'.

| Substrate (g)/90 ml of ash leachate | Magnesium (mg/kg) | Phosphorus (mg/kg) | Sodium (mg/kg) | Potassium (mg/kg) | Calcium (mg/kg) |
|---|---|---|---|---|---|
| 150 | 1.87±0.51$^{b}$ | 2.93±0.04$^{a}$ | 162.50±5.00$^{c}$ | 23333.33±763.76$^{b}$ | 0.92±0.19$^{a}$ |
| 250 | 1.37±0.22$^{b}$ | 2.99±0.03$^{ab}$ | 140.83±3.82$^{b}$ | 15833.33±288.68$^{a}$ | 0.79±0.08$^{a}$ |
| 300 | 1.75±0.33$^{b}$ | 3.06±0.03$^{b}$ | 127.50±5.00$^{a}$ | 16333.33±763.76$^{a}$ | 0.67±0.19$^{a}$ |
| 400 | 0.54±0.14$^{a}$ | 3.04±0.04$^{b}$ | 126.67±6.29$^{a}$ | 15000.00±1500.00$^{a}$ | 1.79±0.26$^{b}$ |

Each data point presented as mean±SEM (n=3). Means along the same row with different superscript are significantly different at P<0.05.

is shown in Figure 1. The highest pH before and after fermentation (11.4 and 9.03) was recorded when 150 g of *H. sabdariffa* seeds was fermented to produce dawadawan botso. The high pH may suggest the production of more acid product during fermentation of 150 g of substrate. In another study, we detected many acidic volatile flavor compounds in dawadawan botso (Ibrahim et al., 2011a).

The ash concentration used in the preparation of alkalinizing leachate had effect on some of amino acid profile of 'dawadawan botso' (Table 7). Alkalinizing leachate prepared from 150 g ash gave the highest value for the essential amino acid valine, methionine, isoleucine, and phenylalanine as well as non-essential amino acids such as arginine, serine, glutamic acid, and tyrosine while that from 100 g gave the highest value for non- essential amino acid proline, alanine and aspartic acid, in addition to some essential amino acids such as lysine, histidine and leucine. However, these values are not significantly different at P<0.05. When amino acids are incubated together, the degradation of free amino acid by lactic acid bacteria was found to be dependent on pH (Tavaria et al., 2002).

Effect of substrate concentration on amino acid profile of dawadawan botso was conducted (Table 8). The highest value of amino acid was recorded for glutamic acid with a value of 11.24, 10.82 and 9.98 g / 100 g protein) for 150, 250 and 300 g, then aspartic acid with 10.10, 9.41 and 8.54 g/100 g protein for 150, 250 and 300 g. Two hundred and fifty grams of seeds to 90 ml of alkalinizing leachate had the highest value of essential amino acid except for methionine and threonine and the non-essential amino acid Cystine. One hundred and fifty grams seeds to 90 ml of alkalinizing leachate had the highest value of non-essential amino acid and the highest value for methionine. The decrease in the essential amino acid may be suggestive of their probable role in stimulating microbial growth and improving the fermentation of *H. sabdariffa* seeds to produce the flavour of condiment. Yimiti et al. (2004) reported that amino acid fermentation byproduct additive improved the fermentation of silage. Also, the amino acid may have been used by the fermenting organisms to stimulate their growth (Argyle and Baldwin, 1989).

The total free amino acids were evaluated (Figure 2). Fermentation increased the value of total free amino acid as compared to the unfermented seeds. Two hundred and fifty grams (250 g) had the highest value (82.97%) for total free amino acids, then 150 g/90 ml with 76.93% and finally 300 g/90 ml with 73.37%. The free amino acids groupings, based on their taste characteristics as described by Tseng et al. (2005), are shown in Figure 3. One hundred and fifty grams (150 g) had the highest value (27.74%) for monosodium glutamate free amino acid, then 300 g/90 ml with 25.24% and the least was in sweet for two classes of free amino acid (sweet and bitter). The total free amino acid and the individual free amino acid classes' result show that 250 g substrate/90 ml ash leachate had the highest value for total free amino acid and bitter free amino acids groupings based on their taste characteristics (Figures 2 and 3). This may be explained by the fact that there may be increased microbial activity in the 250 g substrate samples through proteolysis resulting to high score for total free amino acid (Ibrahim et al., 2011a). The decrease in MSG-like and sweet amino acid class may be an indication that this class have further been utilized by the fermenting organisms to produce the characteristic flavor compounds that is associated with this condiment. Similar observation had been observed for the product and other fermented product such as cheese (Tavaria et al., 2002). Despite the increase in bitter amino acid class, it was not manifested in the overall taste of the product. This is because the other classes have the potential to exhibit a masking effect of the bitter taste class, a scenario that has been reported before for doenjang soybean paste (Kim and Lee, 2003).

## Conclusion

The chemical composition of 'dawadawan botso' was affected by the mass of the substrate (*H.*

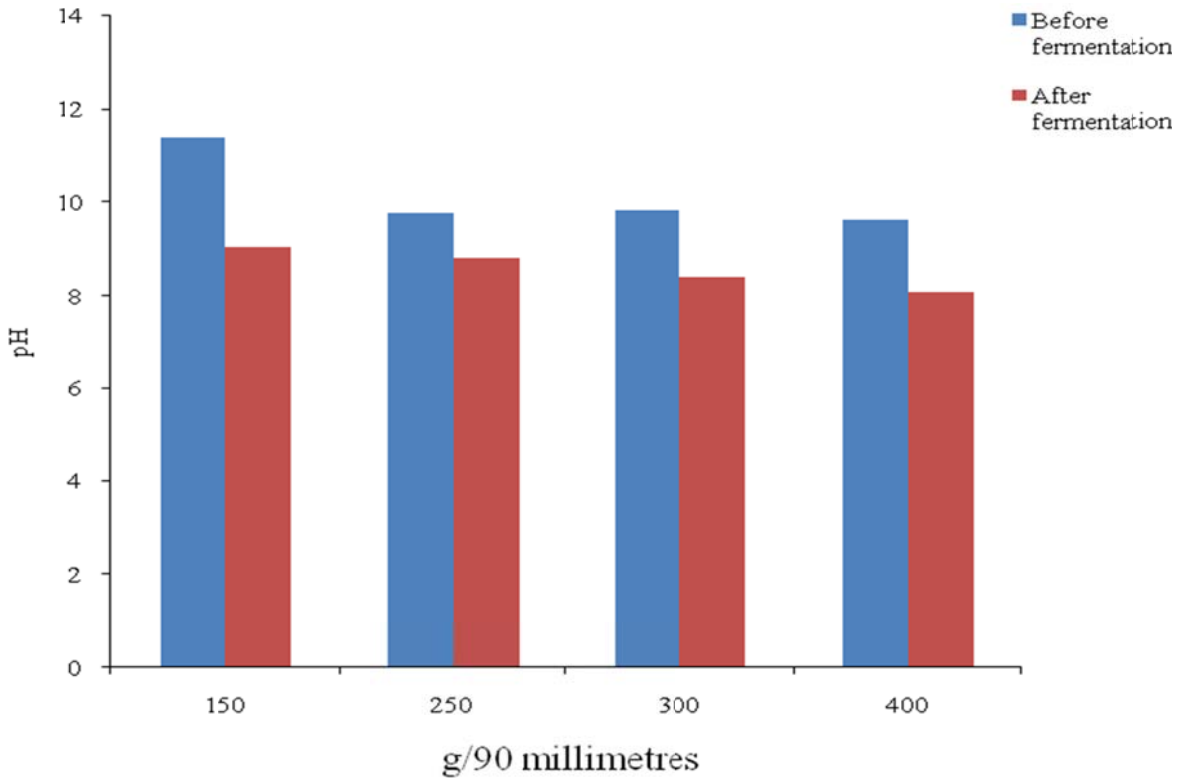


**Figure 1.** Effect of substrate (seeds of *H. sabdariffa*) concentration on the pH of 'Dawadawan botso'.

**Table 7.** Comparison of unfermented locally fermented and the effect of ash leachate size on the amino acid (g/100 g protein) profile of fermented seeds of *Hibiscus sabdariffa.*

| Amino acid | A | B | E1 | E4 | E6 |
|---|---|---|---|---|---|
| **Essential amino acids (g/100 g protein)** | | | | | |
| Lysine | 2.79 | 3.92 | 4.30 | 3.71 | 3.38 |
| Histidine | 2.26 | 3.07 | 2.88 | 2.07 | 1.82 |
| Threonine | 3.39 | 2.16 | 3.29 | 3.39 | 3.21 |
| Valine | 3.95 | 4.30 | 4.56 | 4.87 | 4.47 |
| Methionine | 1.02 | 1.25 | .83 | 1.44 | .73 |
| Isoleucine | 3.39 | 4.27 | 4.21 | 5.22 | 4.83 |
| Leucine | 5.38 | 6.92 | 8.10 | .83 | 5.82 |
| Phenylalanine | 4.57 | 5.58 | 5.07 | 6.37 | 4.90 |
| **Non-essential amino acids (g/100 g protein)** | | | | | |
| Arginine | 4.94 | 8.00 | 8.00 | 8.35 | 8.17 |
| Aspartic acid | 8.35 | 9.10 | 9.35 | 8.04 | 7.29 |
| Serine | 2.64 | 2.37 | 3.08 | 8.04 | 2.81 |
| Glutamic acid | 9.77 | 10.19 | 11.18 | 3.39 | 10.92 |
| Proline | 2.97 | 3.19 | 3.29 | 3.08 | 2.97 |
| Glycine | 4.96 | 4.52 | 4.89 | 12.29 | 3.79 |
| Alanine | 4.63 | 4.25 | 5.17 | 2.97 | 4.09 |
| Cystine | 1.70 | 1.44 | 1.44 | 4.67 | 1.31 |
| Tyrosine | 1.61 | 2.26 | 2.25 | 3.58 | 1.93 |

A = Unfermented; B = locally fermented; E1, 100 g Ash/600 ml water- Before 2nd fermentation; E4, 150 g Ash/600 ml water- After 2nd fermentation; E6, 250 g Ash/600 ml water- After 2nd fermentation.

**Table 8.** Comparison of unfermented seeds of *H. sabdariffa* and the effect of different seed concentration on the amino acid (g/100 g protein) profile of 'dawadawan botso' .

| Amino acid | Unfermented seeds | 150 g of *H. sabdariffa* seeds/90 ml ash leachate | 250 g of *H. sabdariffa* seeds/90 ml ash leachate | 300 g of *H. sabdariffa* seeds/90 ml ash leachate |
|---|---|---|---|---|
| **Essential amino acids (g/100 g protein)** | | | | |
| Lysine | 2.79 | 3.71 (0.92) | 4.88 (2.09) | 3.17 (0.38) |
| Histidine | 2.26 | 2.13 (-0.13) | 3.01 (0.75) | 2.13 (-0.13) |
| Threonine | 3.39 | 1.83 (-1.56) | 2.50 (-0.89) | 3.00 (-0.39) |
| Valine | 3.95 | 3.89 (-0.06) | 4.24 (0.29) | 3.25 (-0.70) |
| Methionine | 1.02 | 1.09 (0.07) | 0.99 (-0.03) | 1.04 (0.02) |
| Isoleucine | 3.39 | 3.83 (0.44) | 4.33 (0.94) | 4.08 (0.69) |
| Leucine | 5.38 | 6.32 (0.94) | 7.36 (1.98) | 6.04 (0.66) |
| Phenylalanine | 4.57 | 4.73 (0.16) | 5.66 (1.09) | 5.24 (0.67) |
| **Non-essential amino acids (g/100 g protein)** | | | | |
| Arginine | 4.94 | 7.49 (2.55) | 8.90 (3.96) | 8.17 (3.23) |
| Aspartic acid | 8.35 | 10.10 (1.75) | 9.41 (1.06) | 8.54 (0.19) |
| Serine | 2.64 | 3.24 (0.60) | 3.18 (0.54) | 2.43 (-0.21) |
| Glutamic acid | 9.77 | 11.24 (1.47) | 10.82 (1.05) | 9.98 (0.21) |
| Proline | 2.97 | 3.61 (0.64) | 3.19 (0.22) | 3.40 (0.43) |
| Glycine | 4.96 | 4.91 (-0.05) | 5.79 (0.83) | 4.38 (-0.58) |
| Alanine | 4.63 | 4.79 (0.16) | 5.02 (0.39) | 5.41 (0.78) |
| Cystine | 1.70 | 1.44 (-0.26) | 1.44 (-0.26) | 1.18 (-0.52) |
| Tyrosine | 1.61 | 2.58 (0.97) | 2.25 (0.64) | 1.93 (0.32) |

Values presented in parentheses are the differences between the unfermented seeds and 'dawadawan botso'.

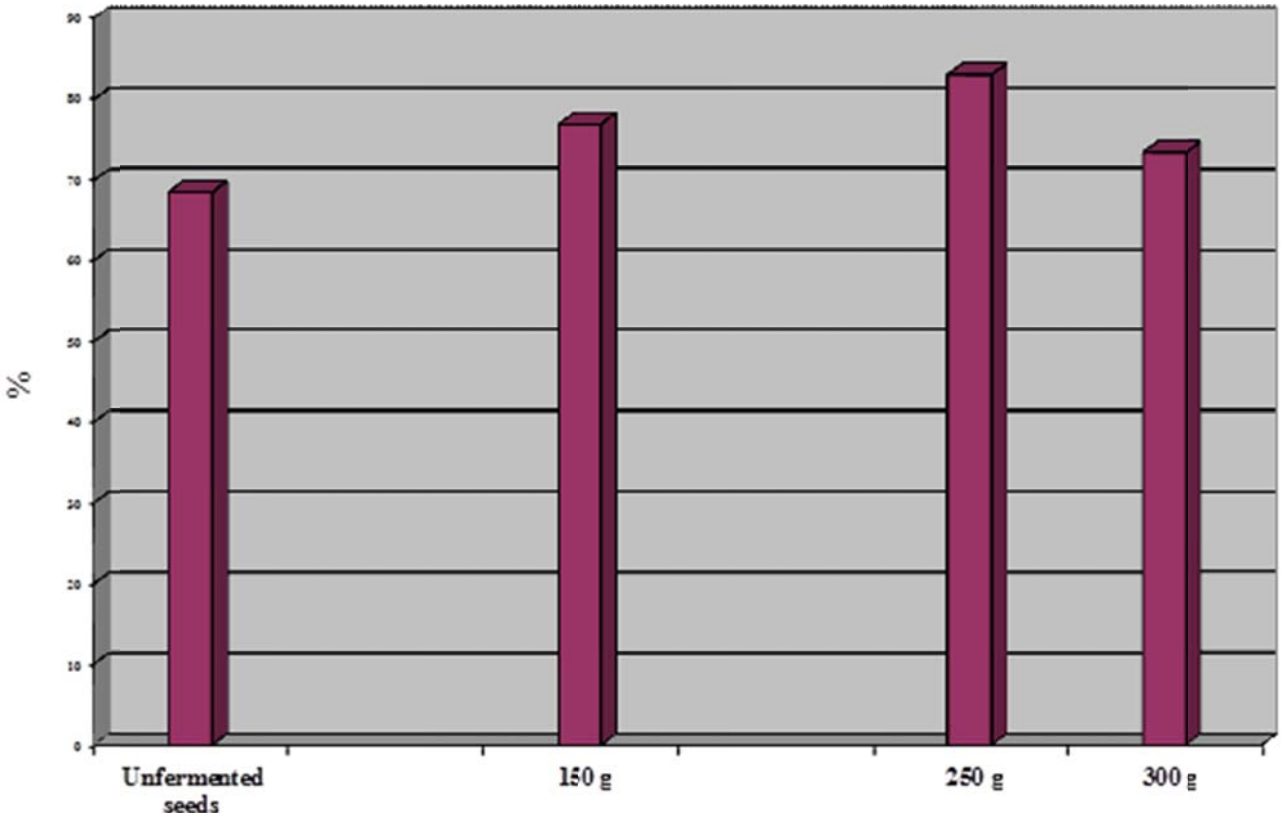


**Figure 2.** Content evolutions of the total free amino acid (%) in unfermented seeds and "dawadawan botso" produced from varying quantities of *H. sabdariffa* seeds per 90 mL ash leachate.

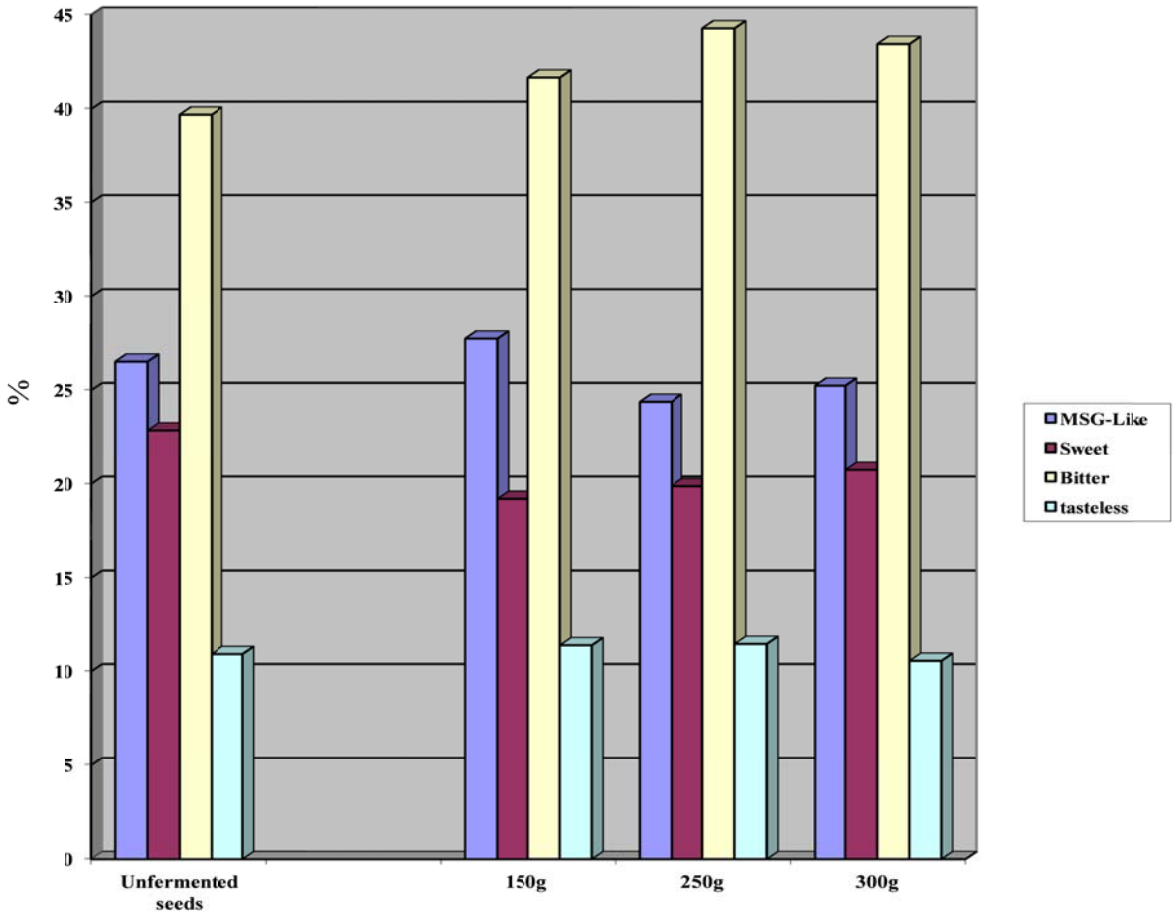


**Figure 3.** Content evolutions of the individual free amino acid classes (content in percent) that imparted the different taste in unfermented seeds and "dawadawan botso" produced from varying *H. sabdariffa* seeds' quantities per 90 mL ash leachate.

*sabdariffa*). The pH of the leachate increased with increasing mass of substrate. However, fermentation caused a reduction in pH as the mass of substrate increased. Fermentation also caused an increase in total amino acid profile in the samples.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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