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EFFECTS OF PROCESSING METHODS ON THE NUTRITIONAL QUALITY OF 'DAWADAWAN BOTSO' (A CONDIMENT) PRODUCED FROM CASSIA SIEBERIANA SEEDS

ABSTRACT

The effects of processing methods on the nutritional quality of dawadawan botso (DDB) produced from the seeds of Cassia sieberiana was undertaken. The dehulled fermented product had the highest microbial standard count of 47.0 ± 3.5 x 10^8 CFU/g while those from undehulled seeds had 23.0 ± 5.7 x 10^8 CFU/g. The result of microbial analysis revealed the presence of Bacillus subtilis, Pediococcus damnosus and Acinetobacter calcoaceticus as the predominant organisms associated with DDB. The proximate and mineral analysis revealed that DDB produced from dehulled seeds had the highest carbohydrate and crude protein contents of 69.81 and 12.69% respectively. Sodium and potassium are the major minerals in DDB with 1750.00 and 2000 mg/kg respectively. Result of amino acid analysis showed that fermentation increased the quantity of all amino acids and non essential amino acids particularly leucine and phenylalanine with 1.92 and 1.27 g/ 100 g protein while, aspartic and glutamic acids recorded the highest increase after fermentation with 1.34 g/ 100 g protein each. Glutamic, aspartic acids and leucine are the major amino acids in the unfermented seeds. Fermentation decreased the contents of tannin, cyanide and phytate significantly (P<0.05). The results of this study suggest that seeds of C. sieberiana could be exploited as a source of raw material for the production of DDB and dehulling and fermention improves the quality of the product.

KEYWORDS: Amino acids, antinutrient factors, C. sieberiana, dawadawan botso

INTRODUCTION

Dawadawan botso a traditional African fermented condiment produced from seeds of many leguminous plant species in Nigeria. A number of authors have established that fermentation of seeds improve the sensory and nutritional quality of the condiment[1, 2, 3, 4, 5]. Traditionally, Daddawan botso is produced from the seeds of Hibiscus sabdariffa mainly produced by women which constitutes an important source of income to them. 'Dawadawan botso' is used during cooking as substitute for fish or meat. The rural dwellers of Zuru area in Kebbi state, Nigeria believe that, consumption of dawadawan botso promotes health by making people to look younger and strong[6].

Cassia sieberiana D.C. also known as African laburnum belong to the family caesalpiniaceae (leguminosae-cae salpiniodeae) and was considered to be a very large genus of about 550-600 species. It is a very common tree with a wide distribution, growing on any kind of soil from rich loam to the driest and most barren sand or clay. The form varies with the locality, tall, slender trees growing on good soils and wide-spreading, the full-grown tree is some 30 feet height, branching from ground level and forming hemispherical growth with foliage to the ground[7]. C. sieberiana is distributed from Senegal and Gambia East to Dr. Congo and Uganda[8]. C. sieberiana is mainly propagated by seed; ripe, fresh seeds have nearly 100% viability. Leaves, roots, and pods of C. sieberiana are widely used in traditional medicine. Other uses of Cassia includes the entire plant is given against all children's disease such as bilharzias, bloody dysentery, eczema etc. The wood is used for making furniture, tools construction and as firewood and also as a food condiment[9].

The seed of Cassia sieberiana is a valuable food resource with high content of carbohydrate and crude protein content and 10% of fat on dry weight basis[10]. It also contains substantial amount of fiber and valuable micronutrients[11]. Despite the high nutritional content of C. sieberiana the potential of its seeds in the production of daddawan botso and the effects of processing method on the quality of the condiment has not been exploited. The aim of this work is evaluate the effects of processing methods on the nutritional quality of daddawan botso produced from the seeds of C. sieberiana with the view to understanding its potential as a source of condiment.

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MATERIALS AND METHODS

Sample collection and Preparation of Raw Material

Three hundred grams of Cassia sieberiana seeds were purchased from Sokoto central market, Sokoto State, Nigeria. Raw seeds were pre-processed before the production step which involved breaking the pod to remove the seeds. The seeds were washed with water (2-3 times) and cleaning step involved seed sorting by gravity and immature seeds and spoiled seeds as well as other light impurities floated while heavy impurities (stones, sand) deposited as sediment. After the initial cleaning process, the seeds were cooked for 8-12 hours according to intensity of fire. Seeds were considered cooked when soft and easily crushed with fingers and water is allowed to dry without allowing the cooked seeds to burn. Seeds fermentation was done in two phases; in the first phase, the cooked seeds were allowed in the pot to ferment naturally for two days by closing the pot tightly to prevent aeration. 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 which lasted for 1 day and the pot was tightly closed.

Processing of “dawadawan botso”

At the end of the second fermentation, the ammonia-like flavour condiment was sun dried by repeated turning to form balls and to enable good drying for 2 to 3 days according to the intensity of sunshine and packaged on polyethene bags. The procedures for the local production of dawadawan botso have been reported elsewhere[12].

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 10^4 and 10^5. Zero point one (0.1) ml. from each test tube was transferred using sterile pipette into 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 was obtained. The pure cultures were 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. [13].

Proximate Composition

The air dried samples of dawadawan botso were analyzed in triplicate for proximate composition in accordance with the Official Methods of the Association of Official Analytical Chemists[14]. Ash was determined by incinerating two grams (2g) of dried dawadawa at 550 ^{o}C in lenton furnaces (England) over night. Fibre was determined by drying two gram (2g) of grounded dried fermented and unfermented seeds of Cassia sieberiana 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 (2g) of ground dried fermented and unfermented seeds of Cassia sieberiana over night at 105 ^{0}C in the oven (Gallenhamp Oven BS). Crude lipid was determined using saturated method. Two grams (2g) of ground dried fermented and unfermented products 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 x 6.25) was determined by the Micro-Kjeldahl Method and soluble carbohydrate was determined as the difference between crude protein and the sum of ash, protein, crude lipid and crude fibre.

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Mineral and Antinutritional Factor analysis

Analysis of minerals were done in triplicate according to methods as described in [14,15]. The investigated minerals include phosphorus, potassium, sodium, calcium, and magnesium. Phosphorus was determined using Spectrophotometer (JENWAY 6100) at 660 γ (wavelength), Potassium and sodium was determined using flame photometer (Corning 400 Essex. England), while that of calcium and magnesium was done by ethylene diamine tetra acetic acid (EDTA) titration method. Oxalate was determined by the method of Krishna and Ranjhan[16], while, phytate and hydrocyanic acid were determined by the AOAC[17] method and nitrate was determined by IITA method[18].

Amino Acid Analysis

The amino acid content was determined using methods described by Spackman et al. [19]. The samples were dried to constant weight, defatted, hydrolysed and analysed using sequential Multi-sample Amino Acid Analyzer (TSM). 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 and nitrogen was determined using MicroKjedhal methods[20]. The samples were hydrolyzed by weighing the defatted sample into glass ampoule and seven millilitres (7 ml) of 6N HCl was added and oxygen was expelled by passing nitrogen into the ampoule. The glass ampoule was sealed with Bunsen burner flame and put in an oven preset at 105 ^{0}C for 22 hours and the content was filtered to remove the humins. The filtrate was evaporated to dryness at 40 ^{0}C under vacuum in a rotary evaporator and the residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic bottles and kept in freezer. The hydrolysate was loaded into the TSM Analyzer by loading 5 to 10 microlitres (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 which lasted for 76 minutes.

RESULTS AND DISCUSSION

Microbiological Evaluation

The result of viable plate count of bacteria isolated from dawadawan botso indicate that dehulled fermented product had the highest standard count of $47.0 \pm 3.5 \times 10^8$ CFU/g while those produced from undehulled seeds had $23.0 \pm 5.7 \times 10^8$ CFU/g. The high bacteria count observed in dehulled sample may be due to the fact that the organisms have to multiply enough to synthesize the required quantity of enzyme needed to degrade and hydrolyse the seed so that they could access the nutrients in the substrate. Spore count of 23.0 and 47.0 spores g^{-1} of bacteria and total counts of bacteria ranged between $23.0 \pm 5.7 \times 10^8$ and $47.0 \pm 3.5 \times 10^8$ in Hibiscus sabdariffa seeds[21]. However, the proportion of these bacteria did not show a significant difference ($P > 0.05$). The result of biochemical characterization revealed that Bacillus subtilis, Pediococcus damnosus, and Acinetobacter calcoaceticus were the dominant organisms associated with dawawan botso. The presence of Pediococcus damnosus and Acinetobacter calcoaceticus could contribute to the development of aroma compound to 'dawadawan botso'. Besides the production of aroma compounds, lactic acid bacteria in fermented foods have been reported to have some medicinal benefits which reinforce their use as probiotics[22, 23]. In the production of dawadawan botso from the seeds of C. sieberiana a longer cooking time of 12 hrs was observed which is comparatively higher than 3 hrs reported in Parkia biglobosa. Differences also appear in the fermentation process of dawadawan botso compared to the predicted similar products. For C. sieberiana seeds the fermentation takes place into two steps: 3-4 days for the first fermentation, the seeds are pounded, moulded and allowed to ferment again for 2 days. For Furundu, Hibiscus sabdariffa seeds fermentation is done at once and lasted for 7-10 days [24, 25]. 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 [26]. An initial observation was carried out after cooking the seeds for 12 h. There was no significant difference at this stage, and that obtained after 24 hour of fermenting seeds. A significant difference was observed after the first fermentation which lasted for 96 hours. This increase after the first

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fermentation might be due to the proteolytic activity of Bacillus isolated which may be responsible for the fermentation. The final observation was made after second fermentation and it was significantly different due to its high aroma when compared with the first fermentation and cooking when there are fewer aromas respectively.

Proximate and Mineral Analysis

Result of proximate and mineral analysis of dawadawan botso produced from dehulled and undehulled fermented seeds of C. sieberiana is presented in Table 1. The result indicate that undehulled fermented had higher lipid and fiber content while, dehulled seeds had the highest carbohydrate and crude protein content with 69.81 and 12.69 % respectively. From the result of proximate analysis of dawadawan botso produced from undehulled and dehulled seeds of C. sieberiana shows a significant variation. A significant difference was observed in lipid content between undehulled and dehulled product seeds. No significant difference was observed in ash and moisture content between undehulled and dehulled fermented seeds. A significant difference was observed in carbohydrate, crude protein and fiber between undehulled and dehulled fermented product. Sodium and potassium are the major minerals observed in the fermented seeds with 2250.00 and 2000 mg/kg for dehulled and undehulled seeds while, the content of potassium was 1750.00 and 2275 mg/kg respectively. The result of minerals analysis shows reasonable concentration of the minerals in undehulled samples with high potassium and calcium content. The high K and low Ca contents of the varieties may make them good potassium – calcium balance in the human body and which are useful against heart disease. High potassium rich foods are not recommended in the diets of people with renal failure and low calcium or deficiency on calcium may cause osteoporosis diseases. However, the various proportions of minerals in fermented product could be of nutritional benefits.

Amino Acid and Antinutritient Analysis

The content of individual free amino acid in dawadawan botso produced from unfermented and fermented seeds of C. sieberiana is shown in Table 2. Fermentation increased the quantity of all essential and non essential amino acids. Among the essential amino acids, leucine had the highest increase with a value of 1.92 g/ 100 g followed by phenylalanine with 1.27 g/ 100 g protein. Aspartic and glutamic acids are among the non essential amino acid that recorded the highest content after fermentation with a value of 1.34 g/100 g protein each. Glutamic, aspartic acids and leucine are to be the major amino acids in the unfermented seeds. The results of antinutritional composition of dawadawan botso produced from Cassia sieberiana seed is presented in Table 3. From the result, fermentation decreased the content of tannins, cyanide and phytate significantly ($P<0.05$) and contents of nitrate and oxalate did not differ significantly ($P>0.05$). Amino acid profiles of dawadawan botso shows a slight difference in amino acid content due to processing method. The major amino acids are glutamic acid, aspartic acid and arginine. Similar observations were reported on Egyptians roselle[27], Burkina Faso roselle[28] and Nigerian roselle[12]. The observed increase in the amino acids content after fermentation may be due to the proteolytic activities of the bacteria during fermentation. Similar observation was made during the production of this condiment using roselle[6] and other leguminous seeds[29] which implies that dawadawan botso contain substantial amount of nutrient.

CONCLUSION

From the results obtained in this study, dehulling and fermentation increased proximate, minerals and amino acid contents of the seeds with a remarkable decrease in the antinutritional factors in dawadawan botso produced from C. sieberiana seeds. This may be due to the fermenting flora's ability to degrade these antinutrients into their less toxic forms thereby making the fermented seeds safe for consumption. This study suggests that fermentation of C. sieberiana seeds could lead to the production of daddawan botso with high nutritional quality and reinforce its use as a source of condiment to meet some nutritional requirements of humans particularly the less privileged in Nigeria.

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Parameters	Dehulled	Undehulled
Moisture	5.0	5. 0
Ash	10.0	10.0
Lipid	5.0	10.0
Crude protein	12.69	10.15
Carbohydrate	69.81	66.35
Fiber	2.50	3.50
Sodium (mg/kg)	2250.00	2000.00
Potassium (mg/kg)	1750.00	2275.00
Phosphorus (mg/kg)	2.66	2.93
Magnesium (%)	1.20	0.09
Calcium (%)	0.30	0.02

Table 1. Proximate And Mineral Composition Of Dawadawa Botso Produced From Dehulled And Undehulled Seeds Of C. Sieberiana

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Amino acid	Unfermented	Fermented
Essential amino acid		
Lysine	3.20	3.76(0.56)
Histidine	2.04	2.32(0.28)
Theonine	2.36	3.00(0.64)
Valine	3.05	3.66(0.61)
Methionine	0.76	0.91(0.15)
Isolaucine	2.35	3.11(0.76)
Leucine	5.63	7.55(1.92)
Phenylalanine	3.13	4.40(1.27)
Non essential amino acid		
Arginine	3.57	4.17(0.6)
Aspartic acid	7.26	8.60(1.34)
Serine	3.02	3.67(0.65)
Glutanic acid	7.38	8.72(1.34)
Proline	2.34	3.29(0.95)
Glycine	3.01	3.55(0.54)
Alanine	3.51	3.71(0.2)
Cystine	0.60	1.06(0.46)

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

Table 2. Amino Acid Content Of Dawadawa Produced From Fermented And Unfermented Seeds Of C. Sieberiana (G/100 Protein Dw)

Anti-nutritional factors	Unfermented	Fermented
Tannin (mg/ml)	0.076	0.030**
Cyanide (mg/%)	0.117	0.072**
Nitrate (mg/%)	0.087	0.079
Oxalate (g/%)	0.012	0.010
Phytate (mg/%)	21.5	17.3**

Values are means of three replicates and ** significant at 5%

Table 3. Anti-Nutritional Contents Of Fermented And Unfermented Condiment From Cassia Sieberiana Seeds

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