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Evaluation of Nutritional Qualities of Dried Roselle flower (*Hibiscus sabdariffa* L) Calyx

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Abstract The proximate, functional properties, anti-nutritional factors and amino acids of dried Roselle flower (*Hibiscus sabdariffa* L) calyx were evaluated using standard analytical methods. All results were reported in duplicate. The mean values of ash, moisture, crude fat, crude fibre, crude protein and carbohydrate were 8.74%, 9.72%, 2.91%, 11.48%, 3.47% and 63.68% respectively. *Hibiscus sabdariffa L calyx* is a good source of carbohydrate, crude fibre and moderate levels of anti nutritional factors. The water and oil capacities were 496% and 301% respectively making *Hibiscus sabdariffa L* calyx to possess a high water retention capacity. Glutamic acid was the most concentrated amino acid (15.21g/100g) while tryptophan (1.11g/100g) was the least concentrated amino acid. Total amino acid was 97.20g/100g with TBAA, TAAA and TNAA each having the value of 16.1%, 25.2% and 58.7% respectively.

Keywords Evaluation, Nutritional, Qualities, *Hibiscus sabdariffa L*, Calyx

Introduction

Foods are sources of minerals, protein, fibre and vitamins which provide essential nutrients for the humans and animals.

Plant resources are nutritionally viable as foods and drugs for good health and general wellbeing of the body physiology. The survival of man on plants cannot be overemphasised. Unbalanced nutrition may result to poor health, mental and developmental malfunction tendencies. Therefore, there is need to consume adequate and well balanced diets which can be sourced from grains, legumes, vegetables e.t.c.

Plants are also known to possess anti nutritional factors that can undermine or interfere the bioavailability of nutrients, especially at high densities [1]. Nevertheless, it has been reported that these anti nutritional factors could help to prevent and treat several important diseases; however, anti-carcinogenic activity of phytic acid has been demonstrated by *in-vitro* and *in-vivo* assays [2].

The world market for protein is increasing on daily basis in most developing countries because of high demand of animal protein. Animal protein contains correct proportions of nutrients. The world demand for plant protein is growing upwardly because of increasing population and the improved living standard of human race across the globe.

The *Hibiscus sabdariffa L is* an annual or perennial herbaceous shrub, growing up to 1.5 – 3.0m tall which matures in about six months. Ethnomedicinal studies have valued Roselle calyx extract for its pharmacological properties [3] including antioxidant [4], antihypertensive [5], antianaemic [6], antidiabetic [7] and antidiuretic [3]. The

adequate information on the functional properties of any plant products precludes its usefulness and application in food and allied industries. It is pertinent to carry out quality assurance, routine and scientific analyses on the underutilized plants in order to know their nutrient densities, so as to be able to incorporate them into food system. Elowni et. al. [8] studied the application of *Hibiscus sabdariffa L* (Malvaceae) aqueous extract for assessment of viability of *Protoscolices* from hydatid cysts, Hence, this study was aimed at the evaluation of proximate, functional properties, antinuitrients and amino acids contents of dried Roselle flower (*Hibiscus sabdariffa L*) calyx.

Materials and Methods

Sample collection and preparation

Dried Roselle flower (*Hibiscus sabdariffa L*) calyxes were purchased from the central market in Ado-Ekiti, Ekiti State in South West Nigeria. The calyxes were thoroughly screened to remove the bad ones and the remaining good ones were dry-milled into powders using Marlex food blender, packaged in a polythene bag and stored in a freezer before analyses.

Determination of proximate composition

The moisture was determined using air-oven at temperature of 106^{o}C for 1 hour while the ash content was analyzed using a muffle furnace at 550^{o}C for 6 hours [9]. The sample was analyzed for crude fat and crude protein according to the methods described by AOAC [10]. The crude fibre was determined by adding 2g of the sample into 500cm^3 conical flask; 200 cm^3 of boiling 1.25% H_2SO_4 was added and boiled for 30 minutes. The mixture was filtered through muslin cloth and rinsed with hot distilled water. The sample was scrapped back into the flask and 200 cm^3 of boiling 1.25% NaOH was added and allowed to boil again for another 30 minutes; filtered and then rinsed with 10% HCl twice with industrial methylated spirit, drained and dried. The residue was scrapped into crucible, dried in the oven at 105^{o}C and then allowed to cool in the desiccator and weighed; later placed in the muffle furnace at 300^{o}C for 30 minutes and then finally allowed to cool at room temperature and re-weighed [9]. The total carbohydrate was obtained by method of difference. % Nitrogen Free Extract (NFE) = 100- [% Moisture+ % Ether Extract +%Ash + % Crude Fibre + % Crude Protein].

Determination of Functional Properties

The water and oil absorption capacities of the samples were determined using the method of Beuchat [11]. 10 cm^3 of water was added to 1.0g sample in a centrifuge tube. The suspension was mixed vigorously using Vortex mixer. This was then centrifuged at 15,000 rpm for 15 minutes and the volume of the supernatant left after centrifuging was noted. Water bound was calculated from the difference in the initial volume of the solvent used and the final volume after centrifuging. The same procedure was used for oil absorption capacity by replacing water with oil in the above process.

Emulsion was prepared according to method of Lin et. al. [12], Sathe and Salunkhe [13]. A 2.0g sample flour was weighed with 100 cm^3 distilled water and blended for 30 seconds using Kenwood food mixer at high speed. After complete dispersion, vegetable oil of density 0.880g per cm^3 was added to 5 cm^3 portions from a burette with continuous blending until the emulsion break point (i.e. a separation into two layers) was observed. Emulsion capacity and stability determinations were carried out at 25^{o}C and the value obtained was expressed as gram of oil emulsified by 1 gram sample. The emulsion stability was determined as the amount of the water separated after 24 hours at room temperature.

The slight modified procedure of Sathe et. al. [14] was used to determine the least gelation concentration. Sample slurries range of 2 – 20% w/v was prepared in 5 cm^3 of distilled water. The test tubes containing these slurries were heated for one hour in boiling water followed by rapid cooling for 2 hours at 4^{o}C. The least gelation concentration was determined as the concentration which did not slip when the test tubes were heated.

The method of Coffman and Garcia [15] was employed to determine foaming capacity and stability. 1g of the sample was whipped with 50 cm^3 distilled water for 5 minutes in a Kenwood blender and later poured into a 100 cm^3 graduated flask to study the foaming capacity.

Determination of Anti Nutritional Factors

Oxalate was determined using the methods of Day and Underwood [16]. One gram of the sample was weighed into 100 cm^3 conical flask, 75 cm^3 3M H_2SO_4 was added and the mixture was stirred for 1 hour and filtered. The 25 cm^3 of filtrate was titrated with 0.1M $KMnO_4$, until faint pink colour persisted for 30 seconds. Alkaloid extraction was carried out using the modified method of Ngounou et. al. [17]. Determination of saponin was done by following the modified method of AOAC [10].

Phytate was determined on Spectronic 20 colorimeter (Gallenkamp) using the method described by Harland and Oberleas (18). The amount of phytate in the sample was calculated as hexaphosphate equivalent using the formula. Phytate (mg/g) = K x A x 20/0.282 x 1000; where A is the absorbance, K = Standard P, Phytate = 28.2% Tannin was determined using the method described by Ogungbenle [19] and absorbance measured at 725mm.

Determination of Amino Acid

The amino acid profile was determined using the method described by Spackman et. al. [20]. The sample was dried to constant weight and then defatted using Soxhlet extractor. After the defatting process, the defatted sample (2g) was weighed in a glass ampoule; 7 cm^3 of 6M HCl was added and oxygen was expelled by passing through nitrogen into the glass ampoule sealed with Bunsen burner flame and placed in an oven present at 105±5^oC for 22 hours. The ampoule was allowed to cool before broken at the tip and the content was filtered to remove the organic matters. The filtrate was then evaporated to dryness at 40^oC rotary evaporator. The residue was dissolved in 5mL of acetate buffer (pH 2.0) and stored in specimen bottles which were kept in the freezer. The hydrolysate (7.5µL) was dispensed into the cartridge of the Technicon Sequential Multi-Analyser (TSM) using a syringe. The TSM analyser is designed to separate and analyse neutral, acidic and basic amino acids of hydrolysate. The amount of amino acids was obtained from the chromatogram peaks. The whole analysis lasted for 76 hours and the gas flow rate was 0.50 cm^3 per minute at 60^oC with reproducibility consistent within ±3%.

Results and Discussion

Table 1 contained the result of the proximate analysis of dried *Hibiscus sabdariffa* L *calyx*. The ash content of any food substance is an indication of the mineral present in the food. *Hibiscus sabdariffa* L had a percentage ash that was lower than that of *Bidens pilosa* (12.31%) [21], but higher than those observed in *Afzelia Africana* (4.03%) [22], raw *I. gabonensis* (2.49%) [23] and common maize (1.62%) [24]. The moisture content of dried *Hibiscus sabdariffa* L was higher than those of sweet potato leaf meal (4.02%) [25] and *Terminalia catapa* (4.13%) [26] but lower than that of zero day cow (37.60%) and buffalo milk chedder cheeses reported by Murtaza et. al., [27]. The low moisture content reported in the present study indicates that dried *Hibiscus sabdariffa* L would not be susceptible to microbial attack during storage and would have a long shelf life [28].

Table 1: Proximate composition of dried *Hibiscus sabdariffa* L *calyx*

Parameter	Value (%)
Ash	8.74
Moisture	9.72
Crude protein	3.47
Crude fibre	11.48
Crude fat	2.91
Carbohydrate	63.68

The value of crude protein in dried *Hibiscus sabdariffa* L was relatively lower than those of *Afzelia africana* (16.52%) [22], water leaf (28.22%) [29] and *Cucumis sativus* peel (26.5%) [30]. The dried *Hibiscus sabdariffa L* calyx contained 2.91% of crude fat. This value was higher than those of *Moringa olifera* leaf protein concentrate (2.43%) [31] and velvet pulp (5.80%) [32]. The percentage of crude fibre of dried *Hibiscus sabdariffa* L was higher than those of *quinoa* (1.2%), benniseed (7.9%), 3.1% (pearl millet) [33], dried *Abelmoschus esculentus* (8.41%) [34], raw cashew nut (4.22%) [35], African nutmeg (3.04%) [36] and raw *Irvingia gabonensis* (8.32%) [23]. It is worth noting that due to the increasing awareness of the beneficial effects of dietary fibre towards health optimization [37], vegetable like dried *Hibiscus sabdariffa* L is a potential source of dietary fibre that provides

roughages that aid digestion process. Dietary fibre has the capacity to reduce the risk of colon cancer and various digestion disorders in small intestine.

Table 2: Functional Properties of dried *Hibiscus sabdariffa* L *calyx*

Functional properties	%
Water absorption capacity	496
Oil absorption capacity	301
Foaming capacity	16
Emulsion capacity	5.0
Emulsion stability	3.1
Least gelation concentration (w/v)	4.0

The functional properties of dried *Hibiscus sabdariffa L calyx are* presented in Table 2. The water absorption capacity was higher than those of quinoa flour (147.0%) [38], raw *Irvingia gabonensis* (241%) [23] and water leaf (137.5%) [29]. The high water absorption capacity of the sample suggests the presence of more hydrophilic groups in the matrix. The oil absorption capacity was higher than those of dehulled African nutmeg (256.0%) [36], *velvet tamarind* pulp (162%) [39] but lower than those of *Celosia spicata* leaves (303%) [40] and *Afzelia africana* (588.19%) [22]. Oil absorption capacity is important as oil act as taste retainer and improves the mouth feel of foods [41]. Therefore, dried *Hibiscus sabdariffa* L may be good for same purpose. The emulsion capacity of dried *Hibiscus sabdariffa* L was relatively low compared to those of dried Okra seeds (45.5%) as reported by Ogungbenle and Arekemase [34], sesame flour protein concentrate (27.43%) [42] and *Afzelia africana* (35.25%) [22]. This indicates that dried *Hibiscus sabdariffa* L may not be useful in the production of sausages and cakes [41]. The foaming capacity of dried *Hibiscus sabdariffa* L was higher than those of raw (8.5%), defatted (13.0%) cashew nut kernel [35] and *Celosia spicata* leaves (12.5%) [40]. The least gelation concentration of dried *Hibiscus sabdariffa* L was lower than cashew nut kernel (18.0% w/v) [35], raw African mango (12.0%) [23] and Okra seeds (8.0% w/v) [34] but comparable with that of nicker bean (4.0% w/v) [43] and dehulled African nutmeg (4.0% w/v) [36].

Table 3: Anti nutritional factors of dried *Hibiscus sabdariffa* L *calyx*

Anti nutritional factor	mg/g
Alkaloid	3.09
Phytate	1.55
Oxalate	0.97
Saponin	1.13
Tannin	1.30

Table 4: Amino acid profile of dried *Hibiscus sabdariffa* L *calyx*

Amino acid	g/100g
Glycine	6.32
Alanine	5.97
Serine	4.86
Praline	8.45
Valine*	5.06
Threonine*	4.60
Isoleucine*	4.43
Leucine*	5.94
Aspartic acid	9.27
Lysine*	6.42
Methionine	1.50
Glutamic acid	15.21
Phenylalanine	5.18
Histidine*	2.27
Arginine*	6.96
Tyrosine	3.38
Tryptophan*	1.11
Crystine	3.26

* represent Essential Amino Acids

Table 5: Classification of Amino Acid Composition

Amino acid classification	
Total Amino Acid	97.20
Total essential amino acid	41.97
With Histidine	39.70
Without Histidine	55.30
Total non essential amino acid (TNEAA)	55.89
%TNEAA	45.50
%TNEAA with Histidine	43.10
%TNEAA without Histidine	57.06
Total neutral amino acid (TNAA) g/100g	24.48
Total acidic amino acid (TAAA) g/100g	15.65
Total basic amino acid (TBAA) g/100g	58.70
%TNAA	25.20
%TAAA	16.10
%TBAA	4.76
Total sulphur amino acid (TSAA)	
%TSAA	4.90
% crystine	68.50

Table 3 presents the anti-nutrients of dried *Hibiscus sabdariffa* L calyx. The phytate value of *Hibiscus sabdariffa* L was relatively low compared to those of *Bridelia ferruginea* stem (7.83mg/g) [44], *D. oleveri* (25.5mg/g) [45], raw *Irvingia gabonensis* seed (26.6mg/g) [23] and Kidney bean (40.8mg/g) [46]. Phytate can affect digestibility by chelating with calcium or by binding with substrate or proteolytic enzyme. Phytate is also associated with increasing cooking time in legumes. Oxalate has negative influence on mineral availability [40]. The oxalate value of dried *Hibiscus sabdariffa* L was also lower than those of cooked walnut (1.13mg/100g) [19] and *Celosia spicata* (16.53mg/100g) [40] but higher than those of *Vigna unguculenta* (0.40 mg/100g) Udensi et. al. [47] and millet (0.50mg/100g) and sorghum (0.48mg/100g) [48]. The percentage of tannin in dried *Hibiscus sabdariffa* L was higher than that of *Afzelia africana* (0.43%) [22] and Kidney bean (0.77%) [46]. Tannin is well known for its antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, anti-inflammatory and diuretic [49]. The saponin value of dried *Hibiscus sabdariffa* L was lower than those of *Alchornea cordifolia* leaf meal (2.04 mg/g) [50] and *D. oliveri* (21.0mg/g) [45] but higher than that of *Ocimum sanctum* leaf (0.52mg/g) [51]. Saponin has a carbohydrate moiety attached to triterpenoid with the property of precipitating and coagulating red blood cells. Some of the characteristics include formation of foams- aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [52]. The presence of anti-nutritional factors in the sample is of significance importance since they have some dangerous effects on both human and animal [40]. Zheng et al [53] reported that the epidemiological studies suggest that the consumption of fruits and vegetables is effective in lowering the risk of cardiovascular diseases. The amino acid composition of dried *Hibiscus sabdariffa* L in mg/100g is depicted in Table 4. Glutamic acid was discovered to be the most concentrated amino acid in dried *Hibiscus sabdariffa* L with value of 15.21g/100g. Glutamic acid is essential for brain metabolism and metabolism of other amino acids. Aspartic acid was the next with a value of 9.27g/100g. Tryptophan is the concentrated amino acid in dried *Hibiscus sabdariffa* L with the value of 1.1g/100g. The result followed the trend of fortified pearl millet with soybean flour as reported by Oluwagbenle (54) where glutamic and aspartic acids were the most concentrated amino acids. The two amino acids are mono-acid and dicarboxylic are acidic.

Many parameters are presented in Table 5. The Total amino acid (TAA) of dried *Hibiscus sabdariffa* L was 97.20g/100g. The value obtained was higher than that of unfermented cocoa ribs (64.10g/100g) [55] but lower than those obtained for Faba bean *(Vicia faba L)* [56] and fortified pearl millet with soybean flour [54]. *Hibiscus sabdariffa* L can be considerably rich source of amino acids. Total non-essential amino acid was 55.89%. Total essential amino acid (with histidine) had a value of 41.91g/100g and 38.70g/100g without histidine with percentage

TEAA value of 42.18% and 40.84% respectively. TNEAA in *Hibiscus sabdariffa* L (55.30g/100g) was considerably higher than that obtained in dehulled Africana yam flour 32.72 – 45.38g/100g [57]. Table 6 also depicted that TAAA was found to be greater than TBAA indicating that the protein is probably acidic in nature [58]. The total amino acid was 4.76g/100g which is lower than the 5.8g/100g recommended value for infant [59], % Cysteine in TSAA was discovered to be 68.50%, while it is known that cysteine can spare part of the requirement for methionine, FAO (60) does not give any indication of the proportion of TSAA that can be met by cysteine. Most animal protein are low in cysteine, in contrast many vegetable proteins contain substantially more of cysteine than methionine. Thus for animal protein, cysteine is unlikely to contribute more than 50% of the TSAA [61].

Conclusion

It can be concluded that dried Roselle flower (*Hibiscus sabdariffa L) calyx has* high nutrient density, appreciable amounts of functional food properties, low levels of anti-nutrients with appropriate amounts of amino acids and the extract highly medicinal. Though the pharmacological activity was not studied, however, it is highly safe and recommended for the consumption as food by human, invalids and livestock.

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