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Determination of Proximate Composition, Ascorbic Acid and Heavy Metals Content in *Dialium Guineense*

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Abstract: *The pulp of Dialium guineense (Velvet tamarind) was analyzed and the proximate analysis and mineral composition evaluated. The pulp contained 30% moisture content, 7.09% crude protein, 0.75% crude lipid and below detection limit for crude fiber, 2% ash content, 57.04% available carbohydrate and energy value 263.27(KJ/100g). Mineral composition and Vitamin C content for the samples were investigated. Results compare well with those of other edible fruits. The accumulation of heavy metals and level of ascorbic acid seems to increase with age, while the fibre depreciates as the fruit Velvet tamarind has a very low oil yield. This shows that nutritional shown that the fruit and less likely to cause these metals poisoning.*

1. INTRODUCTION

Dialium guineense wild (English Name black *Velvet tamarind)*, tree is commonly called "Tsamiyarbiri" by Hausa, Awin" among the Yorubas, and Icheku by Igbos. The fruit pulp has a sweet-sour astringent flavor similar to baobab, but sweeter is eaten raw when dry by man and animal (Matsuda, 2006).Aroba*et.al.,* (1994) reported that edible part (pulp) of ripe *D. guineense* fruit is sweet but acidic (pH 3.3) relatively poor in protein and oil fairly low level of ascorbic acid. Ubbaonu*et al.,* (2005) investigated the pod weight pod diameter, percent fruit portion, sugar and acid composition of the *Velvet tamarind* pulp. Adepoju and Onasanya (2008), reported the nutritional composition and anti-nutritional factors of *D. guineense* wild fruit pulp. The pulp of Tamarindusindica can be soaked in water to prepare drink to heal dysentery and stomach upset. The pulp is a source of Vitamin C which is needed by both adult and children, Adepoju and Onasanya (2008).Its flower appear whitish and the branches are horizontally spread, Szolnok, (1985). Fruit are usually circular and flattened black in colour with stalk 6 mm long, a little collar is seen near the apex and a bristle shell encloses one or more seeds embedded in a dry brownish edible pulp.There is few data available in the literature on the mineral concentration of *Velvet tamarind.*

2. MATERIALS AND METHODS

The fruit of *Velvet tamarind* was collected from Yelwa market, area of Bauchi state. The fruit was dried under shade, the coat of fruit seed was removed by mechanical means.The proximate composition: moisture content, ash content, crude fat and crude fibre were determined as described by Fegbemi*et al*. (1991).The crude protein was determined using micro kjeldal technique, an auto analyzer with hydrogen peroxide as the solvent to crude protein. The carbohydrate was determined by different. Results were expressed as dry weight bases. AOAC (2003).2.0g of dried grinded sample was place in a crucible. It was then ignited in muffle furnace at 650^{o}C until a gray ash appear. The ash was allowed to cool in a desiccators and weighed. The percentage ash content was obtained from the expression below.

$$\% \, \mathbf{ash} = (\mathbf{weight\ of\ ash / weight\ of\ sample}) \times \mathbf{100}$$

Dry extraction method for fat determination was employed. It consist of extracting dry sample with some organic solvent, since all the fat material e.g. fat, lipids, phospholipids, sterols, fatty acid, carotenoids, pigment, chlorophyll etc. are extracted together therefore, the result are frequently referred to as crude fat. Fat was determined by intermittent soxhlet extraction apparatus. Crude fat was determined by petroleum ether extract method using soxhlet apparatus. Approximate amount of moisture free sample was wrapped in filter paper. Placed in fat free thimble and then introduced in the extraction tube. Weighed cleaned and dried receiving flask was filled with petroleum ether and fitted into the apparatus.

The percentage crude fat was determined using:

$$\%\ \boldsymbol{crude\ fat} = (\boldsymbol{weight\ of extract} \div \boldsymbol{weight\ of sample}) \times \mathbf{100}$$

A moisture free and ether extracted sample of crude fibre made of cellulose, was first digested with dilute H_2SO_4 and then with dilute NaOH solution. The undigested residue collected after digestion was ignited and loss in weight after ignition was registered as crude fibre. The residue of ether sample was first boiled with 2.5% sulphuric acid solution for 30 minute, washed with distilled water and then boiled in 2.5% sodium hydroxide solution and then washed with acetone and dry in a known weight crucible in an air circulating oven at 105^{o}C after which it is cooled and weighed. The residue was then ignited in a muffle furnace to gray ash cooled and weighed. Crude fibre was calculated below.

%crude fibre = weight of residure –weight of ash /weight of sample× 100

Protein in the sample was determined by kjeldahl method. 1.0g of dried sample was taken in digestion flask. 10-15 ml of concentrated H_2SO_4 was added and 8 g of digestion mixture i.e. K_2SO_4: $CuSO_4$ (8:1) in the flask was swirled in order to mix the contents thoroughly then placed on heater to start digestion till the mixture become clear (blue green in color) which takes 2 hrs to complete. The digest was cooled and transferred to 100ml volumetric flask and volume was made up to mark by the addition of distilled water.

Distillation of the digest was performed in markam still distillation apparatus. Ten milliliters of digest was introduced in the distillation tube then 10ml of 0.5N NaOH was gradually added through the same way. Distillation was continued for at least 10 min. and NH_3 produced was collected as NH_4OH in a conical flask containing 20ml of 4% boric acid solution with few drops of modified methyl red indicator. During distillation yellowish color due to NH_4OH. The distillate was then titrated against standard 0.1N HCl solution till the appearance of pink color. A blank was also run through all steps as above. Percentage crude protein content was calculated as;

% crude protein = 6.25 × % N × correlation factor

%N = (S-B) ×N×0.014×D× 100/WEIGHT OF SAMPLE × VOL×10

WHERE: S= sample titration reading B=blank titration reading N= normality of HCl D= dilution of sample after digestion V=volume taken for distillation 0.014 = mill equivalent weight of nitrogen

Carbohydrate also called nitrogen free extract (NFE) was calculated by difference after analysis of all the other items method in the proximate analysis. AOAC (2003)

NFE = {100 - (% Moisture + % crude protein + % crude fat + % crude fibre + % ash)}

The percent calories was calculated by multiplying the percentage of crude protein and carbohydrate with 4 and crude fat by 9. The value was then converted to calories per 100 grams of each sample AOAC (2003)

Energy= (carbohydrate×4) + (crudeprotein×4) + (crude fat×9)

The ascorbic acidcontent was determined titrimetrically using iodine. Anne Marie helmenstine, Olson and Hodges (1987). In both methods iodine which oxidizes the ascorbic acid is generated by the reaction of iodine and iodate.

$$IO^{-3} + 5I^{-} + 6H^{+} \longrightarrow 3I_2 + 3H_2O$$

$$C_6H_8O_6 + I_2 \longrightarrow 2H^{+} + 2I^{-} + C_6H_6O_6$$

5g of soluble starch was dissolved in 100ml near boiling distilled water mixed and allowed to cooled before used (5% starch solution)

1.14g of KIO_3 was dissolved in 500ml distilled water to make 0.01M Potassium iodate.

A burette was drained, rinsed with 0.01M potassium iodate (KIO_3) and then filled with the same solution. A bout 0.1g of standard L- ascorbic acid was weighed into a conical flask and about 150ml of distilled water was added to dissolve the solid. 5ml of 1.0 M HCl, 10ml of 0.6M of potassium iodide (KI) and 10-15 drops of starch indicator was added. Taken initial burette reading, the solution was titrated with the potassium iodate (KIO_3) solution by adding small portion until the solution in the flask assumes a permanent color. The final reading was recorded and the titre value calculated. The procedure was repeated twice so as to obtain triplicate determination.

For the unknown sample, 0.15g of the sample was weighed and placed into a conical flask, and about 150ml of distilled water was added to dissolve the solid. 5ml of 1.0 M HCl, 10ml of 0.6 M of potassium iodide (KI) and 10-15 drops of starch indicator was added. Taken initial burette reading, the solution was titrated with the potassium iodate (KIO_3) solution by adding small portion until the solution in the flask assumes a permanent color. The final reading was recorded and the titre value calculated as vitamin C. The procedure was repeated twice so as to obtain triplicate determination.

The heavy metal (cadmium, Cd, chromium, Cr, copper, Cu, lead, Pb and zinc, Zn), ware determined using atomic absorption spectrometry, AOAC (2003)

The bulk scientific atomic absorption spectrophotometer VGP (variable giant pulse) system model 210 was used. The VGP uses a time specific modulation of the hollow cathode lamp (HCL) to produced energy "pulse "that contains information on both the sample (analyte) absorbance and background (matrix) absorbance. Atomic absorption spectroscopy is based on the ability of an "excited atom of an element to absorb energy from wave length of light of the same frequency as the element. This creates a decrease in the initial signal energy and this difference is proportional to the concentration of the element. Each element has its own series of specific wavelength. This wavelength will have specific characteristics for sensitivity, noise and linearity. Sensitivity and noise will determined the limit of detection for that element, AOAC (2003)

The determination of trace metals in organic materials usually requires the sample to be ashed as a preliminary step. This removes interfering organic matrix and concentrates the organic constituents into readily volatile compounds either by burning in air (dry ashing) or by oxidizing in solution, (Wet digestion), Mnzewski, (1989).

The sample was dried in an oven and ground. 2g of dried sample was taken in dry crucible and ashed in a muffle furnace at 400^{o}C-540^{o}C for 3hrs, the temperature is chosen so that volatile metal such as Pb and Cd are not lost. The gray ashed which consist of mainly the oxide and salt was placed in a conical flask and 20ml of 2% nitric acid was added to dissolve it, the mixture was shaken and filtered into another flask and taken to AAS for analysis. Anon (1976) the digested sample was analysed for the Cd, Cr, Cu, Pb, and Zn. by atomic absorption spectrophotometer. Different electrode lamps were used for each element. The equipment was run for standard solution of each element before and during determination to check that it is working properly. The dilution factor for all element was 100.

3. RESULTS AND DISCUSSION

Velvet tamarind (Dialiumguineese) obtained in Bauchi was analyzed for proximate composition, ascorbic acid, and some heavy metals content. Table1 shows the proximate composition of fruit of *Velvet tamarind* which is richer in moisture, ash, crude fat, crude fibre, crude protein, with values of 30.0%, 2.0%, 0.75%, 3.12%, 7.09%, respectively. Also the fruit is richer in carbohydrate as 57.04%. The fibre content (3.12 %) is appreciably high and also higher than that determined from ripped fruit (14.75%) by (Pugalenthi*et al.,* 2004) and the value of 2.9% as reported (Coronel, 1991) and (Feungchan*et al.,* 1996). the fibre content is higher than the amount of 7.4 – 8.8 as reported by (Siddig*et al.,* 2006) . The fruit can serve as good source of fibre for dietary need and in nutrition.

The ash content 2% to that determined by (Pugalenthi*et al.,* 2004). The (4.58_ 0.48%), and also range of (1.60 – 4.2%) from the work (Anon *et al.,* 2007) and (morad*et al.,* 2002). The carbohydrate determine by (pugalenthi*et al.,* 2004) (57.04%) is almost in agreement and also comparable to that reported by with in the work of (Anon *et al.,* 2007) and (morad*et al.,* 2002). This shows that *Velvet tamarind* is richer in carbohydrate. From the result obtain, the fruit can serve as good source of dietary need.

Table 1. *Result of proximate composition (%) of fruit of Velvet tamarind.*

Constituent	***Velvet tamarind***
Moisture	30.0
Ash	2.0
Crude fat	0.75
Crude fibre	3.12
Crude protein	7.09
Carohydrate	57.04
Energy (KJ/100g)	263.27

Table 2 present the ascorbic acid content of the fruit of *Velvet tamarind* which gives the ascorbic amount of 150mg/100g, which prove higher in ascorbic acid content.Ascorbic acid content range between (0.7-3.0) mg/100g was determined unriped fruit by (Morton and Miami 1987). Which can be compared to the amount recorded from the method of determination in which the amount (150mg/100g) fall within his recorded range? The ascorbic acid of *Velvet tamarind* is much lower than the amount in African walnut 53.50g/100mg (*Tetracarpidiumconophorum*) as reported by (Edem*et al.*, 2009).

Table 2. *Result of ascorbic acid content (mg/100g) of velvet tamarind*

Sample	*Velvet tamarind*
1	150

The result of elemental compositions (heavy metals) mg/kg shows that there is a lower concentration of cadmium, chromium and lead (4.5, 4.0, and 4.05 respectively).The level of copper and zinc in *Velvet tamarind* shows (65.0and 36.0).The lead content of fruit is low and may have no significant toxic effect, so also the amount of cadmium and chromium, based on the standard daily intake as given in the literature. The amount of copper in the fruit (65.0mg/kg) may be compared to amount of (0.8-1.2mg/100 of ripped fruit), (Parvez*et al.*,2003). The amount of zinc (36.0mg/kg) compared to (0.8-0.9mg/100 of ripped fruit), (Parvez*et al.*, 2003). The amount of copper and zinc in the fruit lower but almost agreement with the work of (Parvez*et al.*, 2003).

Table 3. *Result of heavy metals Velvet tamarind content of*

Element	**Conc. (mg/kg) *Velvet tamarind***
Cadmium (Cd)	4.5
Chromium (Cr)	4.0
Copper (Cu)	65.0
Lead (Pb)	4.05
Zinc (Zn)	36.0

4. CONCLUSION

Man's quest for a balanced diet demands the search for local food materials that could be genetically mass produced to meet up with human nutritional needs. From the result of this analysis, it can be concluded that the fruit of the proximate and mineral composition of the *Velvet tamarind* fruit indicates that they could be alternative source of human food. The pulp can also be a source of Vitamin C. (ascorbic acid) which could prevent scurvy in both children and adult.

The fruit contains % amount of 30% moisture, 2% ash, 0.75% crude fat, 3.12% crude fibre, 7.09% crude protein, 57.04% carbohydrate and energy 263.27(KJ/100g) . Ascorbic acid contains 150mg/100g. Heavy metals amount in (mg/kg) of 4.05 Pb, 36.0 Zn, Cr 4.0, Cd 4.5 and Cu 65.0.

The observed nutritional content of the fruit shows that it is the source of carbohydrate, with the fruit contains high amount of fibre and moisture content. Ascorbic acid content of the fruit shows the high amount of ascorbic acid of 150mg/100g.

The level of Cd, Cr, Cu, Pb, and Zn were obtained. Thus, the accumulation of heavy metals and level of ascorbic acid seems to increase with age, while the fibre depreciates as the fruit *Velvet tamarind* has a very low oil yield. This shows that nutritional shown that the fruit and less likely to cause these metals poison.

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