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Nutritional Compounds and Anti-Nutritional Factors of Fruits of *Detarium microcarpum* Guill & Perr. and *Detarium senegalense J.F. Gmel* in Benin

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Abstract: *Detarium microcarpum* and *Detarium senegalense* are among the forest fruit plants found in several forests of Benin. Increasing studies are being carried out to date on the two species in Benin. However, the biochemical characterization of fruit remains incomplete. These fruits remain underexploited as a whole with significant postharvest losses despite interesting nutritional characteristics. The objective of this study was to compare the biometric, nutritional and antinutritional characteristics of fruits of the two species harvested in Benin. The analyzes focused on determining the mass and diameter of fruits, supplemented by biochemical analysis of pulp (water content, acidity, total sugars, lipids, proteins, ash, vitamin C, minerals and antinutritional compounds) by reference methods. For the parameters studied, statistical analysis showed significant differences at the level of several parameters (mass, diameter, dry matter, pH, titratable acidity, total sugars, vitamin C, flavonoids and tannins) of the fruits of these species. There are no significant differences for minerals (iron, phosphorus, magnesium and calcium), total polyphenols and phytates. It should be remembered that fruits of *D. senegalense* are larger (diameter: 46.16mm ± 0.025) and heavier (mass 35.3g ± 0.02) than those of *D. microcarpum* (diameter: 32.08mm ± 0.035 and mass: 16.075g ± 0.01). They are also richer in vitamin C (1977.23mg/100gFM ± 0.37) and more acidic (pH = 3.65 ± 0.01) than fruits of *D. microcarpum* (vitamin C: 1817.07mg/100gFM; pH = 6.288 ± 0.012). On the other hand, the fruits of *D. microcarpum* are smaller, sweeter (total sugars: 35.03% ± 0.085 against 23.70% ± 0.8) and less acidic. In view of the results obtained, a good orientation could be given to the field of efficient transformations of these fruits. Additional work on the in-depth characterization and processing of these fruits is therefore necessary for a sustainable development of these species.

Keywords: *Detarium microcarpum*, *Detarium senegalense*, Forest Fruit, Sweet Detar, Nutritional Composition

1. Introduction

In Africa, it is recognized that the rational exploitation of local resources, through improved processing technologies, would make a valid contribution to the sustainable development of the continent [1]. Non-timber forest products (NTFPs) would therefore represent a considerable food and economic stake for local populations. The FAO defines non-timber forest products (NTFPs) as goods of biological origin other than wood, derived from forests, other wooded land, and trees outside forests [1]. More recently, the role of

these biologically diverse species in maintaining human health and the environment has been highlighted, particularly regarding global food security, sustainable development and the Millennium Development Goals [2]. However, a large number of non-timber forest products (NTFPs) remain little scientifically studied and valued in West Africa. The fruits of *Detarium senegalense* and *Detarium microcarpum* belong to this category in Benin. The physicochemical and nutritional characterization and food valuation of the fruits of *D. senegalense* and *D. microcarpum* remains in a rudimentary condition [3]. And yet, in the countries of the subregion such as Nigeria, Burkina-Faso and Senegal, a strong valuation is made [4]. These fruits therefore remain in Benin, underexploited as a whole with postharvest losses despite interesting nutritional characteristics [3, 5]. Although some of the nutritional characteristics (total sugars 50.87 to 81.21g/100gDM, proteins 2.86 to 6.12 g/100g DM, lipids 0.70 to 2.23 g/100gDM, vitamins and minerals, [6, 7]) are known fruits from the sub-region, in Benin, little scientific work has been carried out on the nutritional characterization of the fruit pulp of the two Detarium species encountered. The nutritional characterization of these fruits could contribute to enriching knowledge and make it possible to clearly identify the agro-industrial fields in which the pulp of these fruits could be used as an ingredient. It is in this context that this work aimed to characterize the pulp of the fruits of *D. senegalense* and *D. microcarpum* from Benin, in terms of nutritional compounds and antinutritional factors.

2. Materials and Methods

2.1. Raw Material

Raw material consists of the fruits of *D. microcarpum* and *D. senegalense* collected in 2020 in the municipality of Tchaourou in central Benin. After biometric characterization, these fruits were pretreated and the powder obtained was packaged in plastic bags and kept in the freezer for various analyzes.

2.2. Physical Characterization and Pretreatment of Fruits

2.2.1. Physical Characterization of Fruits

The analysis focused on a biometric characterization of fruits according to the method of Diop et al [5]. Per sample, 30 fruits were analyzed individually. The diameter was measured using a caliper while the mass of the fruit was determined by weighing.

2.2.2. Fruit Pretreatment (Figure 1)

Three main operations were carried out for the pretreatment of the harvested fruits:

1) Shelling: this is a manual operation that involves breaking and removing the pericarp with a knife;
2) Grating pulp: the fruit without its shell is rubbed on the mesh of a manual grater to collect the pulp;
3) Grinding of the grated pulp: the grated pulp is ground in a laboratory mill.

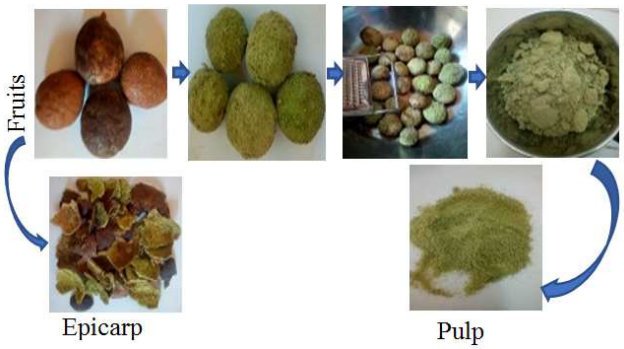

Figure 1. *Pretreatment of Detarium fruits (Original-Tchatcha D.).*

2.3. Determination of Nutritional Characteristics

Characteristics such as dry matter (DM), pH, titratable acidity (TA), total sugars, ash, proteins, lipids (TL), vitamin C, and some minerals were determined on the pulps of *D. microcarpum* and *D. senegalense*.

2.3.1. Water and Dry Matter Content

The water content were determined according to ISO standard 665-2000 [8]. It corresponds to the loss of mass undergone by the sample after heating in an oven at 103 ± 2°C until constant weight. The water content (Te) is expressed as a percentage by mass by the formula:

$$T_e = \frac{m_1 - m_2}{m_1 - m_0} x100$$

The dry matter content (DM) of the sample is deduced from the value of Te by the formula:

$$DM = 100\text{-}T_e$$

m_0 is the tare of the crucible (g);

m_1 is the mass of the crucible and the test portion before heating (g);

m_2 is the mass of the crucible and the residue after heating to a constant weight (g).

2.3.2. Determination of pH and Titratable Acidity (TA)

The pH and titratable acidity are determined according to the modified procedure of the AACC Method, 1984 [9]. The pH was measured using an Inolar 730 pH meter with glass electrodes. 5g of Detarium pulp powder are mixed with 45ml of distilled water and then homogenized. Ten milliliters (10 mL) of the resulting solution were taken and the pH value read directly on the display of the pH meter.

The acid assay consisted of determining the total natural acid content of the product. To the ten milliliters (10 mL) of the solution obtained above, were added 2 drops of a colored indicator (phenolphthalein). The mixture was assayed with 0.1 N sodium hydroxide solution until pale pink. The titratable acidity (TA) expressed in milliequivalents per 100 g of sample (meq / 100g) was calculated:

$$TA = N \times V_1 \times \frac{50 \times 100}{V_2 \times m}$$

N_1: Normality of standard NaOH solution used for titration;

V_1: Volume of standard NaOH solution used for titration in milliliters;

V_2: Volume of sample solution in milliliters;

50: coefficient of dilution;

m: Samples size in grams.

2.3.3. Determination of Total Sugars

The determination of total sugars were carried out by the phenol-sulfuric colorimetric method developed by Dubois et al. [10]. To 2 ml of the aqueous extract (0.1% in water) were added 1 ml of a solution of phenol (5% in water). Then quickly added 5 ml of H_2SO_4 without letting it flow through the wall. The mixture were stirred immediately and then placed in a water bath at 25-30°C for 20 min. A stable yellow color develops. After cooling in water to 20°C, the absorbance were measured at 485 nm. The concentration of total sugars in the samples were determined from a calibration curve plotted beforehand with glucose as a reference (0; 0.25; 0.5; 1; and 2.5 mg / ml). Three concentration readings are taken per sample and the results are expressed in mg of sugars per 100 g.

2.3.4. Determination of Proteins

The protein content were determined by calculation from the determination of nitrogen according to the Kjeldhal method. This method consists of the transformation by mineralization of the organic nitrogen contained in the sample in the presence of sulfuric acid and a catalyst, the alkalinization of the reaction products, distillation and titration of the ammonia liberated. The mineralization of the test portion (between 0.5 to 2 g) of the sample depending on the estimated protein content) were carried out with concentrated sulfuric acid (25 mL at 95%) in the presence of two Cu catalyst pellets ($CuSO_4$). The whole is heated until the mass carbonizes, the foam disappears, and the liquid boils evenly, which has become clear. Carried out at 400°C using a digestion apparatus, it lasts about three hours. The reaction products are then basified with 33% sodium hydroxide solution. After cooling, the ammonia produced is entrained automatically by steam distillation using the Kjeldahl still. To determine the total nitrogen content of the organic matter, the ammonia is then titrated with a 0.1 N hydrochloric acid solution in the presence of a mixture of colored indicators in solution in 4% boric acid: Bromocresol green and methyl red. By convention, the protein content of the sample is then obtained by multiplying the total nitrogen content by a factor of 6.25. The percentage crude protein content (Tp) is given by the following formula:

$$Tp = \frac{6.25 * M_N * C * (V_1 - V_0)}{m}$$

MN is the atomic molar mass of nitrogen (MN = 14.007 g / mol);

C is the concentration of the hydrochloric acid solution (mol / L);

V0 is the volume of the hydrochloric acid solution used for the blank test (mL);

V1 is the volume of the hydrochloric acid solution used for the test portion (mL);

m is the mass of the test sample (mg).

2.3.5. Determination of Lipids

The fats are extracted using a continuous Soxhlet extraction apparatus with hexane as the solvent. The samples are first crushed and sieved and then 10 to 15g are weighed. The oil were extracted using a Soxhlet type continuous extraction apparatus with hexane. After eight hours of extraction at a rate of about 10 siphons per hour, the solvent is removed by distillation and is collected in the body of the extraction apparatus. The last traces of hexane are evaporated by placing the flask of the apparatus in an oven at 103 ± 2°C until constant weight. The extract obtained is weighed. The lipid content (TL) can be expressed relative to the dry matter as follows:

$$TL = \frac{m_2 - m_0}{m_1 * DM} * 100$$

m_0 is the mass of the test sample (g);

m_1 is the mass of the flask and the boiling regulator (g);

m_2 is the mass of the flask, the boiling regulator, and the extract after drying (g);

DM is the percentage of dry matter.

2.3.6. Determination of Ash

Crude ash was obtained by the AACC method [9]. The method consisted in placing the clean crucibles in the oven at 550°C for 1 hour. These crucibles were taken out and then cooled in a desiccator for approximately 30 minutes. They were then weighed under vacuum (Mo) and then 5g (PE) of sample were introduced into each crucible. The crucibles are placed in an oven at 550°C. for 24 h. They are taken out and cooled in a desiccator for 1 hour. Finally, weigh the crucibles.

Total ash (Ash) was calculated as follows:

$$Ash = \frac{M_1 - M_0}{PE} * 100$$

M_0: mass in grams of the empty crucible;

M_1: mass in grams of the crucible containing ashes;

PE: mass of the test sample (g).

2.3.7. Vitamin C Content

The titrimetric method using 2,6-dichlorophenolindophenol (AOAC method, 1995 [11], with some modifications) was used. 10 g of the pulp sample were mixed with 20 ml of a 2% solution of oxalic acid. The mixture were homogenized, diluted to 100 ml with a 2% solution of oxalic acid, and filtered. Ten ml of filtered solution are titrated with 0.01% of 2,6-diclorophenol-indophenol solution. The end point is considered when the solution has a pink color for 15 seconds. The calibration of the 2,6-dichlorophenolindophenol solution were carried out with a 0.05% ascorbic acid solution. The results are expressed in mg of ascorbic acid per 100 g of fresh material (mg AA/100g of FM). The total duration of the dosages was between 10 and 15 min.

2.3.8. Determination of the Content of Certain Minerals

The determination of mineral ions is carried out in three stages: incineration, extraction, and actual dosage. The ash

obtained after incineration were dissolved in 100 ml of distilled water with magnetic stirring for 30 minutes. The solution obtained is filtered on Wathman No. 1 paper and the filtrate obtained is used for the determination of the various ions sought (Ca^{2+} , Mg^{2+} , PO_4^{3-} , Fe^{2+}) according to the procedural method DR 2800, 2007.

2.4. Determination of Antinutritional Compounds

2.4.1. Determination of Total Polyphenols

The Folin-Ciocalteu method [12] was used to determine the content of total polyphenols. Each pulp sample (5 g) was diluted in 50 ml of distilled water and filtered through Whatman No. 1 paper. 0.5 ml of the filtrate was then mixed with 2.5 ml of 0.2 N of the Folin-Ciocalteu reagent for 5 min and 2 ml of 75g/l sodium carbonate (Na_2CO_3) was added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm with a methanol white spectrophotometer. Gallic acid (0-200mg/l) was used as a standard and the total phenol content were expressed in mg of gallic acid / 100g of sample. The results are expressed in mg Gallic Acid Equivalents per g (dry basis).

2.4.2. Determination of Total Flavonoids Content

The quantification of the flavonoids was carried out by a method based on the formation of a very stable complex between the aluminum chloride and the oxygen atoms present on the 4 and 5 carbons of the flavonoids. The protocol used is based on that described by Zhishen et al. [13], and Kim et al. [14]. In a glass hemolysis tube, 400 µl of extract, or standard or distilled water for the control was added to 120 µl of 5% $NaNO_2$. After 5 minutes, 120 µl of 10% $AlCl_3$ has been added, and the medium is mixed vigorously. After 6 minutes, a volume of 800 µl of 1M of NaOH was added to the medium. The absorbance were read immediately at 510 nm against the control. Total flavonoids were determined using the calibration curve plotted with quercetin (0-50 mg/l) as a reference. The results are expressed in mg quercetin equivalent per g (dry basis).

2.4.3. Determination of Phytates Content

The determination of phytates was carried out according to the method of Reddy and Love [15]. Four (4) grams of sample mash were mixed with 100 ml of 2% hydrochloric acid for 5 hours. After filtration, 25 ml of the filtrate were added to 5 ml of 0.3% ammonium thiocyanate. The mixture were titrated with ferric chloride until the yellow-brown color persists for 5 minutes. The results are expressed in mg/100g.

2.4.4. Determination of the Tannin Content

Tannins were determined using the AOAC method [16]. About 2 g of the sample was degreased with petroleum ether for 2 hours using a Soxhlet extractor. The residue was oven dried for 3 hours at 80°C, boiled with 300 ml of distilled water, diluted to 500 ml in a standard volumetric flask, and filtered through non-absorbent cotton. A volume of 25 ml of the infusion was measured in an Erlenmeyer flask and titrated with 0.1 N potassium permanganate (0.1 N potassium permanganate was normalized against 0.1 N oxalic acid) until the blue solution turned green; the few drops of 0.1N potassium permanganate were added. The difference between the two titrations was multiplied by 0.006235 to obtain the amount of tannin in the sample. The results are expressed in g/100g.

2.5. Statistical Analysis

Means and standard deviations were calculated using the Excel 2013 spreadsheet. This was used for comparison of means and analysis of variance (ANOVA) by Newman and Keuls test at the threshold P <5% using SPSS 25 software.

3. Results and Discussion

3.1. Biometric Characteristics of Fruits

Table 1 shows the mass and diameter of *D. microcarpum* fruits and *D. senegalense* fruits collected in Benin. Regarding the mass, there is a significant difference between the mass of the fruits of *D. microcarpum* (16.075g ± 0.01) and the mass of the fruits of *D. senegalense* (35.3g ± 0.02). The fruits of D. microcarpum have an average diameter of 32.08mm ± 0.035. Statistical analysis shows that there is a significant difference between these values and that of the fruits of *D. senegalense* (46.16 mm ± 0.025). The values of the mean diameter of the fruits of *D. microcarpum* (32.08mm ± 0.035) are in the range of values (32.0 to 39.0 mm) found by Kouyaté et al. [17] for the fruits of *D. microcarpum* in Mali. Likewise, the mean values of mass and diameter of fruits of *D. senegalense* obtained in this study are in the range of values found by Diop et al. [5] in Senegal: mass (37.33 to 39.96 mm) and diameter (34.7 to 43.9 mm).

Table 1. *Mass and diameter of fruits of D. microcarpum and D. senegalense.*

Species	Mass (g)	Diameter (mm)
D. microcarpum	16.075± 0.01a	32.083±0.035a
D. senegalense	35.30±0.02b	46.16±0.025b

In the same column, the means followed by a different letter are significantly different at the threshold P <0.05.

3.2. Nutritional Characteristics of D. Microcarpum and D. Senegalense Pulps

Table 2 shows the average values of some nutritional parameters of the pulps of the fruits of *D. microcarpum* and of the fruits of *D. senegalense*. The dry matter of *D. microcarpum* pulps is 85.161% ± 0.024. These values are close to the range of values (87.83 to 92.85%) found by Makalao et al. [6]; Oibiokpa et al. [7]; Kini et al. [18]; Kouyaté et al. [17]. The dry matter content of *D. senegalense* pulp is 79.80% ± 1.02. Statistical analysis reveals a significant difference between the dry matter values of *D. microcarpum* pulp and those of *D. senegalense* pulp. The pH values of fruit pulp of *D. microcarpum* are 6.288 ± 0.012. A significant difference were observed between the pH values of fruit pulps of *D. microcarpum* (6.288 ± 0.012) and *D. senegalense* (3.65 ± 0.01). The pH of the fruits of *D. microcarpum* tends towards

a neutral pH, while the pH of the fruits of *D. senegalense* is an acidic pH. These pH results are confirmed by those of titratable acidity, which are 0.271 meq / 100g ± 0.08 for the fruits of *D. senegalense* and 0.072 meq / 100g ± 0.01 for the fruits of *D. microcarpum*. A significant difference is observed between the titratable acidity values of the fruits of *D. microcarpum* and fruits of *D. senegalense*. The total sugar content is 35.03% ± 0.085 and 23.70% ± 0.8 for the pulp of *D. microcarpum* and for the pulp of *D. senegalense*, respectively. A significant difference is observed between the values of total sugars of pulp of *D. microcarpum* and pulp of *D. senegalense*. The values of total sugars of pulp of *D. microcarpum* are higher than those of pulp of *D. senegalense*. These results confirm those of Cavin [19] who state that the pulp of *D. microcarpum* is sweeter than that of *D. senegalense*. However, the total sugar contents of the pulp of *D. microcarpum* studied (35.03% ± 0.085) are lower than the values observed by Makalao et al. [6] (50.87-52.27%) on fruits from Chad and Oibiokpa et al. [7] (65.38%) on fruits from Nigeria. All these differences are considered due to the characteristics of the soil, collection areas, and species. Indeed, Diop et al. [5] characterized *D. senegalense* fruits from six different zones of Senegal and observed significant variations in the results obtained, then they deduced that these variations would be due to the variability of the fruits. That is, habitat, maturity, storage and processing conditions, and species.

Table 3 shows the average values of vitamin C and some minerals such as iron, phosphorus, magnesium and calcium. As for vitamin C, there is a significant difference between the values of samples of *D. microcarpum* and samples of *D. senegalense*. The values are 1977.23mg/100gFM ± 0.37 for *D. senegalense* and 1817.07 mg/100gFM ± 0.001 for *D. microcarpum*. Compared to minerals, there is no significant difference between the values of iron, phosphorus, magnesium and calcium in samples of *D. microcarpum* and *D. senegalense*.

3.3. Antinutritional Compounds from the Pulp of D. microcarpum and D. senegalense

Table 4 shows the average values of the antinutritional compounds of the pulp of D. *microcarpum and D. senegalense*. There is no significant difference between the values of total polyphenols and phytates of samples of *D. microcarpum and D. senegalense* (0.863 mgEAG / gDM ± 0.047; 1.208 mgEAG / gDM ± 0.002 and 0.408 mg/100g ± 0.012; 0.654mg/100g ± 0.047 respectively). However, a significant difference was observed in the values of flavonoids and tannins between samples of *D. microcarpum and D. senegalense* (0.448mgEQ / gDM ± 0.044; 1.216mgEQ / gDM ± 0.021 and 0.078g/100g ± 0, 0114; 0.028g/100g ± 0.004, respectively). This table also shows that samples of *D. senegalense* have the highest values of antinutritional compounds.

Table 2. *Average values of some nutritional parameters of the pulps of D. microcarpum and D. senegalense.*

Species	Dry matter (%)	pH	TA*	Total sugar (%)	TP (%)	TL (%)	Ash (%)
D. microcarpum	85.161±0.024a	6.288±0.012a	0.057±0.01b	35.03±0.085a	4.211±0.040a	0.891±0.023a	4.868±0.090a
D. senegalense	79.80±1.02b	3.650±0.01b	0.271±0.08c	23.70±0.8b	1.770±0.06b	2.381±0.07b	4.400±0.2a

AT * (meq / 100 g); in the same column, the means followed by a different letter are significantly different at the threshold P < 0.05.

Table 3. *Composition of pulps of D. microcarpum and D. senegalense in vitamin C and certain minerals.*

Species	Vitamin C (mg/100gFM)	Iron (mg/100g)	Phosphorus (mg/100)	Magnesium (mg/100g)	Calcium (mg/100g)
D. microcarpum	1817.07±0.001b	1.904±0.031b	0.408±0.04b	61.826±0.006b	152.175±0.005b
D. senegalense	1977.23±0.37c	2.032±0.001b	0.600±0.04b	95.030±0.03b	152.560±0.02b

In the same column, the means followed by a different letter are significantly different at the threshold P < 0.05.

Table 4. *Antinutritional compounds in the pulp of D. microcarpum and D. senegalense.*

Species	Total polyphenols (mg EAG / g DM)	Flavonoids (mg EQ / g DM)	Phytates (mg/100g)	Tannins (g/100g)
D. microcarpum	0.863±0.047a	0.448±0.044a	0.408±0.012a	0.078±0.0114a
D. senegalense	1.208±0.002a	1.216±0.021b	0.654±0.047a	0.028±0.004b

In the same column, the means followed by a different letter are significantly different at the threshold P < 0.054.

4. Conclusion

Analysis of the fruits of *Detarium microcarpum* and *Detarium senegalense* collected in the municipality of Tchaourou in Benin, revealed significant differences between certain biometric and nutritional characteristics of the fruits studied. The results obtained showed that the fruit of *D. senegalense* is larger and weighs more than the fruit of *D. microcarpum*. However, the fruit of *D. microcarpum* is sweeter and less acidic than the fruit of *D. senegalense*. Compared to minerals, the difference is not statistically significant. This study also showed that the fruit of *D. senegalense* contains more antinutritional compounds such as total polyphenols, flavonoids, and phytates than the fruits of *D. microcarpum*. In view of the results obtained, a good orientation could be given to the fields of efficient transformations of these fruits. Additional work on the in-depth characterization and processing of these fruits is therefore necessary for a sustainable development of these species.

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