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Original Research Article

Biochemical Characterization and Amino Acid Profile of some Local Resources Used in Food Supplementation

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Abstract: Nutritional studies make little reference to essential amino acids to explain undernutrition in protein-energy malnutrition. This study aimed to carry out biochemical characterization and amino acid profiling of *Detarium microcarpum* and *Adansonia digitata* fruit pulps and *Moringa oleifera* leaf powder to show their contribution to protein balance. Biochemical analyses and amino acid profiles were carried out using standard methods and statistically analyzed using XLstat 2016 software. Results are expressed on a dry matter basis. Moringa powder had the highest ash, lipid and protein contents, at 10.72%, 8.75% and 23.85% respectively, compared with 4.14%, 1.99% and 4.19% for Detarium. Adansonia pulp had the lowest lipid and protein contents, at 1.36% and 1.83% respectively. In terms of total amino acids, Moringa powder had the highest content at 15.59%, while baobab pulp had the lowest at 2.18%. Essential amino acids for Moringa powder were higher and more balanced than for fruit. Histidine was absent in *Adansonia digitata* pulp, while methionine was absent in both *Adansonia digitata* and *Detarium microcarpum* pulps. Thus, blending the three resources could result in products with high nutritional value.

Keyword: *Adansonia digitata*, Amino acids, *Detarium microcarpum*, Food supplementation, *Moringa oleifera*, Nutritional composition.

INTRODUCTION

Plants have always been an important source of nutrition for many living beings. In some parts of the world, due to health problems, dietary choices or economic reasons, plant products are the only way to obtain all the nutrients the body needs especially when resources are very limited (Burlandoa *et al.*, 2019). In the Sahelian landscape, the three plants *Detarium microcarpum* (sweet detar), *Adansonia digitata* (Baobab) and *Moringa oleifera* are of particular importance in terms of diet and therapeutic practices. In rural areas, women and children are responsible for harvesting the fruits and leaves of these plants (MEDD, 2012; Ricci *et al.*, 2020). Their importance in nutrition and traditional medicine is based on their mineral and vitamin content, their antioxidant power and the presence of numerous other biological molecules of therapeutic aptitude. Indeed, in 2019, a study presented the pharmacological and therapeutic properties of *Detarium microcarpum*, including antioxidant, anti-diabetic, antibacterial, anti-fungal, antiviral and anti-cancer activities, as well as its nutritional and functional properties (Burlandoa *et al.*, 2019). Monkey bread, the fruit of *Adansonia digtata*, has been shown to help maintain phospho-calcium balance, in addition to its acidic nature due to its organic acids (Parkouda *et al.*, 2007). *Moringa oleifera* powder has long been known for its qualities in the nutritional recovery of immunocompromised people, notably following infection by the Human Immunodeficiency Virus (HIV), in preventing lipid oxidation and other food-altering reactions (Hodas *et al.*, 2021) and in reducing aflatoxin B1 levels (Gard *et al.*, 2021). Its virtues are such that Moringa is called '*arzantiga*' or tree of paradise in the local Moore language. Unfortunately, the dietary benefits of Moringa are negatively influenced by its low consumption (Zongo *et al.*, 2013). In Benin, a 20% refusal rate by malnourished children

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aged 6 to 59 months was recorded by researchers following the administration of a Moringa-enriched diet due to taste and smell (Agossadou *et al.*, 2018).

On the other hand, protein requirements boil down to the amino acids needed to synthesize body proteins and other nitrogenous substances (Kenfack, 2010). In nutrition, the notion of protein quality is thus relative to its digestibility and amino acid composition. While researchers agree that digestibility depends on the presence of several factors in the feed, some of which are unfavorable (anti-nutritional factors such as tryptic inhibitors), amino acids in turn are assessed based on their essential amino acid (EAA) content. In the absence of essential amino acids, the body is unable to synthesize specific proteins (Darmaun, 2008). Yet (Müller and Krawinkel, 2005), proteins are irreplaceable by other nutrients. This results is a low protein mass following a negative nitrogen balance, with consequences such as undernutrition and its attendant morbidities (Hsairi *et al.*, 2021). Studies have extolled the virtues of Moringa, Detarium and monkey bread as supplements, particularly for vulnerable people, basing their approach on micronutrients (minerals, trace elements and vitamins). However, the investigation of amino acids as essential micronutrients for maintaining healthy body function has often remained a secondary priority. The present study is therefore a contribution to our knowledge of the essential amino acid composition of products from three Sahelian plants, to clarify their interest in supplementation. The study aimed to characterize the biochemistry and amino acid profile of *Detarium microcarpum* and *Adansonia digitata* fruit pulps and *Moringa oleifera* leaf powder used for food fortification in Africa.

MATERIAL AND METHODS

Obtaining plant material

The plant material consisted of *Detarium microcarpum* fruits, *Adansonia digitata* fruits and *Moringa oleifera* leaves. *Detarium microcarpum* and *Adansonia digitata* fruits were obtained from women growers in the villages surrounding the town of Gaoua. The fruits were packed in cartons and transported at room temperature to the food technology workshop. Moringa leaves were obtained from the vegetable garden in the town of Gaoua. The freshly picked leaves were packed in bags and transported to the food technology workshop for drying and grinding into powder.

Production of fruit pulp and dried leaf powder

Detarium microcarpum fruits, *Adansonia digitata* hulls (monkey bread) and *Moringa oleifera* leaves were processed according to the diagrams shown in Figure 1.

Pre-treatment of Detarium fruit involves sorting, washing and drying. These steps enabled us to eliminate fruit with defects, skins adhering intimately to the pulp, sand and surface dirt, and to make the skins drier. Processing consists of extracting the powder by crushing, followed by sieving to retain almond fibers and debris. The resulting pulp was then packaged in food-safe containers.

A series of operations, including brushing and breaking the shell, removed the yellowish-green layer covering the shell and extracted the contents, which consisted of the *Adansonia digitata* pulp surrounding a kernel, all entangled in a network of fibers. The dry pulp was obtained by crushing and sieving.

The moringa powder production protocol was developed by (Sauveur and Broin, 2010). The treatment applied to moringa petioles consisted in stripping the leaves, washing them in a 1% saline solution for around 3 minutes before draining them and then drying them at 50°C for at least 6 hours in an electric dryer. The dried leaves were ground and sieved to produce Moringa powder, which was packaged in food-safe containers.

Determination of physicochemical and nutritional characteristics of dried leaf pulp and powder

Physicochemical and nutritional characteristics of dried leaf pulp and powder were determined using standard methods.

Water content was determined by weight difference before and after oven drying at 105°C to a constant weight (AOAC, 2000).

Ash was determined using the ISO 2171 method by incinerating samples in a muffle furnace at 550°C. The ash was cooled in a desiccator and weighed. (ISO, 2007).

The Kjeldahl method (AFNOR, 1986) was used for nitrogen determination. The nitrogen determined after mineralization followed by distillation is multiplied by the conversion factor 6.25.

Lipids were extracted in hexane using the Soxhlet followed by oven drying according to (AOAC, 2000).

Fibers were determined after digestion with sulfuric acid and neutralization with sodium hydroxide under boiling conditions, washing with hot water, filtration, oven-drying at 105°C for 8 hours, incineration in a furnace at 550°C for 3 hours according to the method (AOAC, 1990).

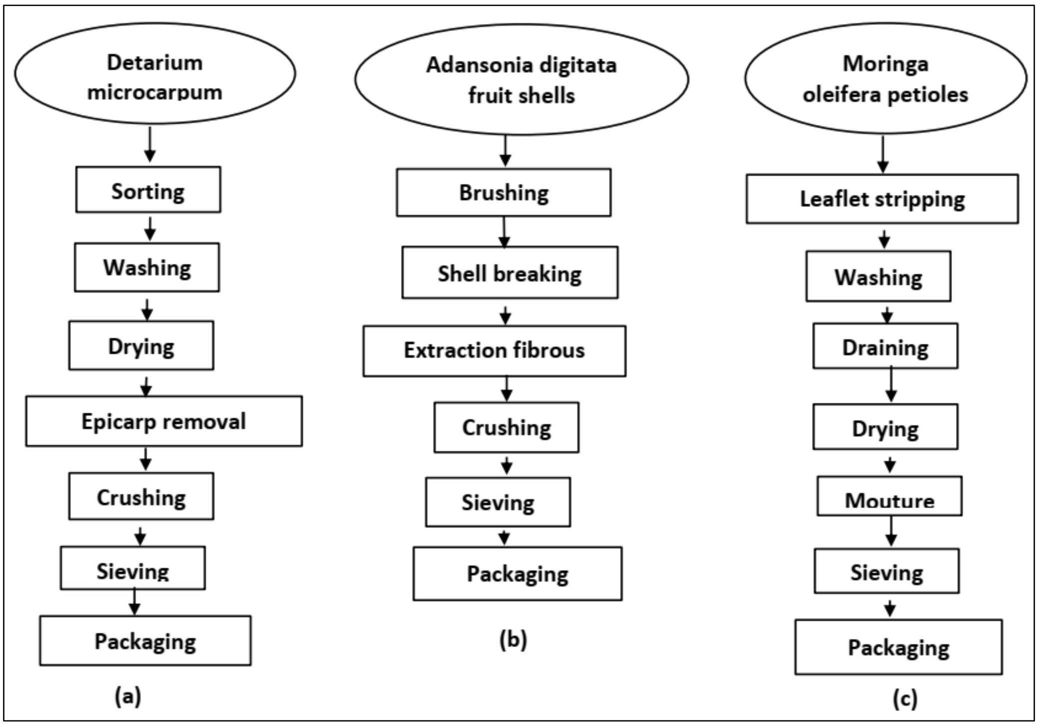

Figure 1: *Detarium microcarpum* pulp (a), *Adansonia digitata* pulp (b) and *Moringa oleifera* leaf powder (c)

Digestible carbohydrates were determined by the differential method according to the formula proposed by (Egan *et al*., 1981). Digestible carbohydrates content (%) = 100 - [water content (%) + protein content (%) + lipid content (%) + ash content (%) + fiber content (%)].

Energy value was obtained by multiplying macromolecule content by Atwater's coefficients: VA (kcal/100g) = [(% Carbohydrates x 4) + (% Protein x 4) + (% Fat x 9)] (Atwater and Benedict, 1899).

Amino acids were determined by HPLC using the PICO-TAG method developed by the DANIDA Project of the Danish Technological Institute (Kristoffersen, 2011).

Statistical analysis of data

Data were entered into Microsoft Excel 2016 and compared using ANOVA to assess differences between means. The Fisher test was used to compare variances at the 5% significance level. A Principal Component Analysis (PCA) using XLSTAT version 2016 was performed to show the correlation between essential amino acids and plant resources.

RESULTS

Biochemical characteristics and energy value of pulp and leaf powder

The physicochemical characteristics of *Detarium microcarpum* pulp, *Adansonia digitata* and *Moringa oleifera* leaf powder are summarized in Table 1.

Adansonia digitata pulp had a higher moisture content than *Detarium microcarpum* pulp and *Moringa oleifera* powder. *Moringa oleifera* is a good source of protein and lipids, while pulp is an excellent carbohydrate reserve. *Detarium microcarpum* fruit pulp showed a higher energy value, while Moringa powder revealed an ash content twice that of *Adansonia digiata* fruit pulp. For all physicochemical parameters, the difference was statistically significant at $p < 0.01$.

Table 1: Physicochemical and nutritional parameters and energy value of Detarium and baobab fruit pulps and Moringa powder expressed as dry matter.

Composition	Detarium Pulp	Adandonia Pulp	Moringa powder	P-value
Humidity (%)	6.59±0.17	9.15±0.09	6.75±0.17	< 0.01
Ashes (%)	4.14±0.12	5.87±0.33	10.72±0.00	< 0.01
Lipids (%)	1.99±0.05	1.36±0.08	8.75±0.4	< 0.01
Proteins (%)	4.19±0.13	1.83±0.13	23.85±0.63	< 0.01
Fibers (%)	8.44±0.07	9.98±0.11	15.40±1.40	< 0.01
Digestible carbohydrates (%)	74.64±0.00	71.81±0.16	34.51±0.37	< 0.01
Energy value (kcal/100g)	333.30±0.05	306.81±0.85	312.86±7.62	< 0.01

For moisture, protein, lipids, fiber and digestible carbohydrates, values are averages of two determinations ± standard deviation. For ash, values are averages of three determinations ± standard deviation.

Total amino acid content of Detarium pulp, *Adansonia digitata* and Moringa leaf powder

A total of 17 amino acids were used as standards. The proportion of total amino acids in Detarium and *Adansonia digitata* pulps and Moringa leaf powder is shown in the histogram in Figure 2. Moringa powder contained more amino acids than Detarium pulp, which contained more than *Adansonia digitata* pulp.

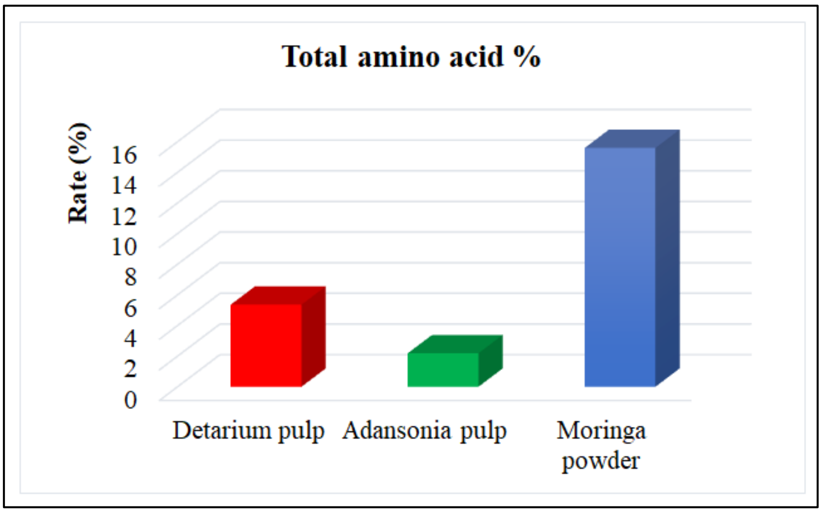

Figure 2: Total amino acid content of *Detarium microcarpum pulp*, *Adansonia digitata* pulp and *Moringa oleifera* powder

Principal component analysis of essential amino acids

Principal component analysis (Fig 3) was used to establish the correlation biplot between the essential amino acids quantified in the three local products: *Detarium microcarpum* and *Adansonia digitata* pulps and *Moringa oleifera* leaf powder. The first two factorial axes F1/F2 show 100% variability in eigenvalues; this means that the representation is of good quality. Threonine, alanine, valine, methionine, isoleucine, leucine, phenylalanine and histidine were collinear and significantly positively correlated with lysine. In addition, the F1 axis was more closely related to these variables, while both the F1 and F2 axes were equidistant from lysine. In terms of observations, Moringa powder was almost conflated with the F1 axis, while *Detarium microcarpum* and *Adansonia digitata* pulps had a more nuanced contribution. These two resources shared the subspaces formed by the intersection of the F1 and F2 dimensions.

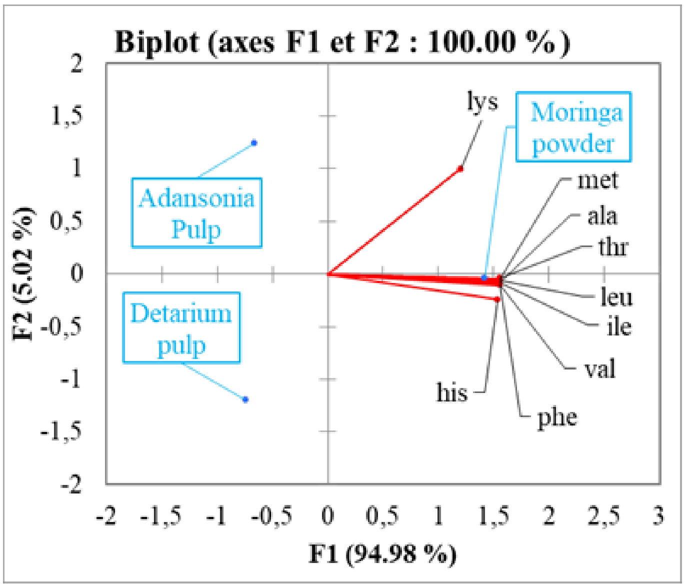

Figure 3: F1/F2 axis correlation biplot of essential amino acids

DISCUSSION

The moisture content of *Adansonia digitata* pulp was higher than that indicated in NBF 01-213, i.e. 8.7% (ABNORM, 2018a). This could be because the pulp extracted from the epicarp did not undergo drying. However, this content was close to that found by (Tapsoba *et al*., 2023), which was 9.63%. In contrast, *Moringa oleifera* powder had a moisture content in line with NBF 01-216, i.e. below 8% (ABNORM, 2018b). Detarium pulp moisture was higher than that reported by a team of researchers from the University of Ouagadougou, i.e. 4.93% (Thiombiano *et al*., 2014) but lower than the 7.15%, 7.12% and 7.26% respectively at the Bongor, Gaya and Laï sites (Makalao *et al*., 2015). The moisture content of Moringa leaf powder was lower than the 8.25% obtained in another study (Ouédraogo *et al*., 2018). The moisture contents obtained for the fruit pulps indicate that they should be dried further to ensure a long shelf life without spoilage for year-round consumption needs.

Moringa ash content was higher than that obtained from Adansonia fruit pulp and corroborates the results reported by other authors. The ash content of *Adansonia digitata* fruit pulp was higher than the average reported by a group of researchers, i.e. 4.9%. (Stadlmayr *et al*., 2013). Edaphic factors could explain this difference. In addition, for *Detarium microcarpum* pulp, ash contents obtained in the present study were similar to those reported by authors in Cameroon with rates of 4.14% (Bayang *et al*., 2021). Ash is a nutritional quality criterion as it is the inorganic residue of the mineral-containing feed (Nielsen, 2017). Thus, the present work confirms the nutritious nature of local products often used in improving the nutritional status of vulnerable people.

The fat content of Moringa powder was high compared with that of *Detarium microcarpum* and *Adansonia digitata* fruit pulps. A fat content of 7.85%, lower than in this study, was obtained in Senegal (Ndong *et al*., 2007). On the other hand, a group of researchers (Pamba *et al*., 2018) found for *Adansonia digitata* fruit pulp sold on the market in Côte d'Ivoire, fats contents of 0.42 g/100g dry matter, lower than those obtained in this study. However, the results of the present study are close to those of other authors, who found 2.23% on a dry basis (Oibiopka *et al*., 2014). More generally, Muthai and colleagues had shown that proximal compositions of plant resources differ according to geographical provenance (Muthai *et al*., 2017). Fats are necessary for the transport of fat-soluble vitamins and enter into the constitution of biological limbs.

The protein content of *Adansonia digitata* fruit pulp was lower than that indicated in the Burkinabe standard, i.e. 2.7% (ABNORM, 2018a), while that of Detarium fruit pulp was close to that (Thiombiano *et al*., 2014), i.e. 4.65%. The protein content of Moringa leaf powder was also close to that obtained in Mali by Coulibaly's team, i.e. 24.2% (Coulibaly *et al*., 2022). The age of the plants could be a determining factor in quantitative composition. According to some authors, proteins are more abundant in the leaves of young plants than in those of adult plants (Kane *et al*., 2010). In addition, dieticians (Pamplona-Roger, 2006) assert that it is easier to ingest large quantities of fruit than other foods such as meat. So, although overall protein levels in the fruits studied were lower, in the lean season when they are generally consumed a lot, their contribution to dietary balance is not negligible, given the inaccessibility of animal proteins to poor households (Kenfack, 2010).

The fiber content of Detarium fruit pulp was higher than the 7.87% on a dry basis (Jacob *et al*., 2016), while the content for baobab pulp was lower than the results of (Compaoré *et al*., 2011) i.e. 11.87%. These disparities could be linked to the maturity stage of the parties involved. Furthermore, (Cissé, 2012) finds an explanation for these differences in the diversity of methods used for the same biological material. The fiber value of Moringa powder was close to that found by (Yaméogo *et al*., 2011) i.e. 15.7%.

The digestible carbohydrate values of baobab fruit pulp were close to those of (Pamba *et al*., 2018), i.e. 71.39% for the Yopougon market. In contrast, the content of Detarium pulp obtained in this study was higher than that obtained in Nigeria, i.e. 65.38% digestible carbohydrates (Oibiopka *et al*., 2014). These disparities could be explained by climatic conditions or the degree of ripeness of the fruit, among other factors. In fact, according to some authors, fruit sugar content and acidity evolve inversely: at maturity, acids tend to decrease while sugar content increases (Tyl and Sadler, 2017). Given the high digestible carbohydrate content of Detarium microcarpum fruit pulp, it can be used as a sugar substitute in Sudan (Cavin, 2007). This ability of *Detarium microcarpum* fruit pulp could be used in conjunction with its other attributes to enrich infant flours.

The energy value of baobab pulp was much higher than those reported by (Makalao *et al*., 2015) i.e. from 65.46 kcal/100g to 68.1 kcal/100g but similar to those (Pamba *et al*., 2018) i.e. 306.08 kcal/100g for the Yopougon market. Compared with samples from other authors (Thiombiano *et al*., 2014), the values obtained in this study are close for Detarium fruit pulp (i.e. 335.5 kcal/100g). Moringa powder presented a lower energy value than that found in Mali, i.e. 396 kcal for 100g of dry Moringa leaf powder (Coulibaly *et al*., 2022). This difference would be due to the quantitative difference in calorific molecules (carbohydrates, lipids, proteins). Thus, each of these resources can contribute to improving energy density as part of a supplemental diet. In addition, the combination of *Detarium microcarpum* and *Adansonia digitata* fruit pulps and Moringa powder constitutes a concentrate of protein-energy nutrients. Such a compound would be effective in the fight against recurrent protein-infant malnutrition in Sub-Saharan Africa, resulting from a diet whose energy density is often deemed low about energy needs of young children.

In terms of overall amino acid composition, Moringa powder showed the highest content compared to Detarium and Adansonia fruit pulps. Total amino acids, 26.032%, had been identified by Moyo and colleagues in dried Moringa leaves (Moyo *et al.*, 2011). These levels were higher than in this study. This could be explained by edapho-climatic factors, the difference in variety or the number of amino acids. Indeed, Moyo's team had determined nineteen (19 amino acids), whereas we had only looked for seventeen (17). Furthermore, in terms of quality, Moringa powder contains the eight essential amino acids in a more balanced proportion. These results shed further light on Moringa powder as a resource for supplementing protein undernutrition in Africa. When comparing Malagasy and Ivorian baobab pulps, (Cissé, 2012) found six (6) free amino acids in the Ivory Coast species *Adansonia digitata*, whereas we found fifteen (15). Among others, we found lysine and leucine, which were only detected in certain Madagascar species by the same author. Furthermore, (Cissé, 2012) had reported the lowest values for the sulphur amino acids cysteine and methionine, whereas the present study revealed an absence of methionine but a cysteine content that exceeds that of valine, tyrosine, alanine, glycine, serine and glutamic acid. Methionine was not detected in Detarium pulp either. Thus, the protein contribution of pulps in essential amino acids is only partial. In his book on food science, Pamplona-Georges (Pamplona-Roger, 2006) revealed the absence of methionine among fruit amino acids, which corroborates the results of this study. Consequently, this author asserted that the amino acids in fruit are incomplete compared to those in vegetables. So, for a balanced diet, you need both fruit and vegetables. Hence the need to combine Detarium and Adansonia fruit pulps with Moringa powder for effective supplementation of essential amino acids. We plan to supplement infant flours with a combination of these three products to assess their essential amino acid profile.

CONCLUSION

Moringa oleifera leaves and *Detarium microcarpum* and *Adansonia digitata* pulps are nutrient reserves in terms of their macromolecular and micronutrient composition. Moringa powder has a higher nutritional value than pulp, particularly in terms of total and essential amino acids. The combination of Detarium and Adansonia pulps increases nutritional composition. However, the absence of methionine in each of the pulps makes the quality of their proteins low. For supplementation needs, we recommend combining *Moringa oleifera* powder with *Adansonia digitata* pulp and/or *Detarium microcarpum* pulp.

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Consent to publication

All the authors confirm the manuscript have not been published elsewhere, have not been considered by any other publisher. The all consent to the publication of the manuscript in EAS Journal of Nutrition and Food Sciences.

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