**[[1]](#footnote-1)Evalution of Biochemical, Nutritional and Microbiological parameters of dried néré (*Parkia biglobosa*) Pulp Sold in the Markets of Daloa (Central-West, Côte d’Ivoire)**

**ABSTRACT**

**Background:** Nutritional deficiencies affecting populations and the lack of hygienic quality of food constitute major public health issues that developing countries like Ivory Coast are facing, and against which we must necessarily fight.

**Aim:** The aim of this work was to determine the values of various biochemical and nutritional components of the powder made from the dried pulp sold in the markets of Daloa and, above all, to verify their safety.

**Methodology:** To obtain the values of these components, several methods were used. Total polyphenols were determined by spectrophotometry using the colorimetric method with the Folin-Ciocalteu reagent. The determination of condensed tannins was done using the vanillin method with hydrochloric acid. Dry matter, ash, proteins, lipids and all other nutrients were determined according to standard methods (AOAC).

**Results:** The results showed the presence of phenolic compounds in the powders and especially high levels of macronutrients and micronutrients. The microbiological analysis was conducted on potentially pathogenic germs found in the dried pulp of *Parkia biglobosa*. The samples notably contain enterobacteria as well as yeasts and molds. The loads obtained for the different germs are below the microbiological criteria. The loads are 33 CFU/g for enterobacteria, 920CFU/g for yeasts and molds, 280CFU/g for *Bacillus cereus*, 00 CFU/g for *Staphylococcus aureus*, and 00 CFU/g for *E. coli*. Thus, the analysed samples demonstrate satisfactory nutritional and microbiological quality.

**Conclusion:** the use of dried pulp powder may be recommended for the diets of the most vulnerable populations such as infants and the elderly. Thus, the daily consumption of this powder could help combat diseases caused by nutritional deficiencies observed in children.

***Keywords:*** *Germs, macronutrients, microorganisms, néré, phytochemical,*

# 1. INTRODUCTION

*Parkia biglobosa* commonly known as néré is a species of tree in the *Mimosaceae* family. It naturally grows in savana areas and gallery forests. Its fruits are an economic source and a staple food for the populations of northern Côte d’Ivoire and the sahelian regions (Tapsoba *et al*., 2014; Kokou *et al*., 2024). In West Africa, néré seeds are traditionally processed into fermented and ground condiments known and sold in the Ivorian market under the name of soumara. The production of dried néré powder is done from two sources. The powder comes from the seeds. It is obtained after cooking and fermentation and is often used as a flavor enhancer and thickener in preparations (Guissou et al., 2020). Some medicinal properties such as its ability to lower blood pressure and its tonic virtues are attributed to this néré powder (Coulibaly *et al.*, 2017). The powder obtained from the dried pulp is very rich in vitamins A and C. It is used in the preparation of refreshing drinks (Collen et al 2020). It is therefore generally consumed raw. However, it can be mixed with dough for making cakes and fries. The yellow powder of dried néré pulp can be stored and consumed during periods when food resources are limited while new harvests are not yet available (Thiombiano et al., 2014; Dao et al., 2021; Parkouda et al., 2021).

However, like the powder of fermented seeds and other foodstuffs, the dried pulps of néré are subjet to various microbial constraints leading to their degradation (Roukaya, 2020). The microorganisms capable of degrading these products are bacteria, molds, total and fecal coliforms. Once present in the powders, they release their toxins into them. Aflatoxin, which causes liver diseases, carcinogenic diseases, hemorrhages, and immune suppressions, is one such example (Ismail *et al*., 2010). A thorough knowledge of the different components of these dried pulp powders and possible contaminetions is crucial for understanding the consequences of these microbial contaminations on humain health. In case of presence of compounds of good nutritional values and absence or non-alarming presence of microbial contaminants, dried pulp powder could be recommended for households. It is in this context that the present study is situated, the objective of which is to determine the values of the various biochemical and nutritional components of the dried powder of *P. biglobosa* sold in the markets of Daloa, but also to verify their safety through the search for microbial germs that could degrade the quality of this ptroduct.

# 2. MATERIAL AND METHODS

**2.1. Material**

Dried pulp powder samples were obtained by purchase from the Lobia and Orly markets, which are neighborhoods in the city of Daloa. A total of 10 samples was collected per market at a rate of 100g. The powders were package after purchase in a container with a cold accumulator and then transported to the laboratory. The saleswomen from different markets who have the same supplier that sources from a single region, which is Worodougou in Côte d’Ivoire, a composite sample was obtained by taking 10 g of powder per sample and market. The whole is mixed to constitute the composite sample of 200 g that was used for the different analyses.

**2.2. Methods**

**2.2.1. Determination of biochemical compounds**

The dry matter (DM), ash content, protein content and lipid content were determined according to the AOAC method (1990). The total carbohydrate content was calculated following the calculation method recommended by FAO (1998). The total sugar content was determined according to the method of Dubois *et al.* (1956). The quantification of reducing sugars was performed according to the method of Bernfeld (1955).

### **2.2.2. Determination of phytochemical compouds**

The determination of total polyphenol content was carried out by spectrophotometry using the colorimetric method with Folin-Ciocalteu reagent (Singleton & Ross, 1965). The quantification of flavonoids was performed using the method described by Zhishen *et al*., (1990). Furthermore, the determination of condensed tannins was carried out according to the Vanillin method with hydrochloric acid (Julkunen-Titto, 1985).

**2.2.3. Determination of vitamin C, A and beta-carotene levels**

The vitamin C (ascorbic acid) content was determined by the method of Barros et al. 2007). The extraction of carotenoids (Vitamin A, β-carotene) was performed using the following technique: 2 ml of distilled water were added to 500 mg of dried pulp powder. The retinyl acetate (standard) placed in 2 mil of ethanol was added to the sample. The mixture was extracted twice with 8 ml of hexane. After centrifugation (5oo ₓ g, 10 min at 4°C), 2 ml of Distilled water and methanol-dichloromethane (65/35, V/V) were added. A final volume of 150 µl for the samples was used for HPLC analysis. The carotenoids were separated as previously described (Gleize et al., 2012; Makambou, 2021). All molecules were identified by their retention time compared to pure standards

### **2.2.4. Determination of mineral content**

The minerals were determined according to the AOAC method (1990). An amount of 0,1 g of ash is dissolved in 10 ml of 36% hydrochloric acid. This volume is filled up to 100 ml with distilled water. Calcium and magnesium are measured using a 3% lanthanum chloride solution completed with distilled water to a volume of 100 ml. 5 ml of aliquot is taken and the mineral dosage is performed using atomic absorption spectrometry with standard solution of the different minerals.

**2.2.5. Microbiological analysis**

A 10 g analysis unit of pulp powder is dissolved in 90 ml of buffered peptone water (BPW) (BioRad, Paris, France). The suspension is left at laboratory temperature for 30 minutes. For the search for spores of the *Bacillus cereus* group,15 ml of the mother solution is taken in a test tube and heated to 80°C for 10 minutes in a water bath, then immediately cooled for 5 minutes in ice. As for the decimal dilution, one milliliter (1ml) of the treated and untreated mother solution was added to 9 ml of sterile distilled water. This operation was repeated until the desired dilution was achieved.

A quantity of 0,1 ml of each relevant decimal dilution is deposited in a petri dish containing 20 ml of previously prepared and poured agar. Then the 0,1 ml is spread over the surface of the agar using a sterile spreader. This type inoculation considered *E col, S. aureus*, and *Bacillus cereus*. As for the fungal flora and enterobacteria, 1 ml of each relevant decimal dilution is deposited in a petri dish. Subsequently, 20 ml of previously prepared agar is added to the dishes containing the inoculum. All the inoculated dishes are incubated. For the counting of *Eschrichia Coli*, the dishes containing the Rapid *E. coli* medium were incubated at 46°C for 24 hours. For the counting of *Staphylococcus aureus*, the dishes containing the Baird-Parker agar were incubated at 37°C for 24-28 hours. For the counting of Bacillus cereus spores on Mossel agar, the dishes were incubated at30+C for 24 hours. The fungal florawase seeded on Sabouraud medium with Chloramphenicol and the plates are incubated at 26°C for 5 days. Concerning the counting of enterobacteria, cultured on VRBG agar, the seeded plates were incubated at 37°C for 24 hours. The estimation of the microbial population is carried out using the following formula

 ∑Ci

N (CFU/g) =

 

N (CFU/g): Number of germs per gram of product;

∑Ci: Sum of the colonies counted on all the boxes retained from successive dilutions;

V: Volume of inoculum applied to each plate (ml);

n1: Number of boxes retained at the first dilution considered;

n2: Number of boxes retained in the second dilution considered;

d: Dilution factor corresponding to the first dilution retained

**2.2.6. Statistical analysis**

The results obtained in this study were carried out in triplicate. These results were expressed as means ± standard deviation.

**3. RESULTS**

**3.1. Biochemical compounds**

The water, dry matter, and ash contents of the powder are respectively 13.1 ±0.002 % ; 86.9 ± 0.1 % and 4 ± 0.00 %. It also contains 3.75 ± 0.015 % proteins; 3.69 ± 0.05 % lipids and 88.55 ± 0.152 % total carbohydrates. The resulting energy value is 402.41 ± 1.5kcal per 100 g of dry matter. It has a reducing sugars content of 3.56±0.20 % and total sugars of 32.1 ± 0.01% (Fig. 1).

**Fig. 1. Macromolecule contents of dried pulp powder**

The dried pulp powder contains phenolic compounds which are secondary metabolites with respective value of 0.0015 ± 0.001(mg EAG/g) for polyphenols, 0.0401 ± 0.002 (mg EC/g) for tannins, and 0.0124 ± 0.02 (mg EQ/g for flavonoids. (Fig. 2).

**Fig. 2. Phenolic compound contents of dried pulp powder**

**3.2. Nutritional compounds**

The mineral composition of the dried pulp powder of P. biglobosa has been determined. Macroelements such as potassium (140.05 ± 0.2 mg/kg), phosphorus (123.22 ± 0.1 mg/kg), sodium (43.8 ± 0.1 mg/kg), magnesium (20.225 ± 0.105 mg/kg) and calcium (16 ± 0.2 mg/kg) have very high levels. In contrast, relatively low levels are observed for arsenic (0.0215 ± 0.0015 mg/kg), zinc (0.51 ± 0.01 mg/kg), cadmium (cadmium (0.12 ± 0.02 mg/kg), copper (0.085 ± 0.0052 mg/kg) and iron (0.8 ± 0.1 mg/kg) (Fig. 3).

**Fig. 3. Mineral composition of dried pulp powder**

The contents of vitamin A, C, and β-carotene were determined. These contents are respectively 0.1744 ± 0.01 mg/100g, 30 ± 0.3 mg/100g, and 0.0466 ± 0.005 mg/100g (Fig.4).

 **Fig.4. Vitamin content of dried pulp powder**

**3.3. Contaminating microorganisms**

Almost all of the pulp powder samples from the Lobia and Orly markets in the City of Daloa contain various microorganisms, namely enterobacteria (33 ± 0.02 CFU/g), yeasts and molds (920 ± 0.01 CFU/g), and *Bacillus cereus* (280 ± 0.01 CFU/g). However, a complete absence observed for *E. coli* and *Staphylococcus* *aureus*. All these average counts are well below the levels set by the standards (Table 1).

**Table 1. Average microbial loads (CFU) of the analyzed powder samples**

|  |  |  |
| --- | --- | --- |
| **Microorganisms** | **Powder dried pulp**  | **Microbiological criteria** |
| *E. coli* | 00 CFU/g | 10 CFU/g |
| *S. aureus* | 00 CFU/g | 102 CFU/g |
| *B. cereus* | 280 ± 0.01CFU/g  | 103 CFU/g  |
| Yeasts and molds  | 920 ± 0.01CFU/g | 104 CFU/g  |
| Enterobacteria  | 33 ± 0.02 CFU/g | 102 CFU/g  |

# 4. DISCUSSION

This study was conducted as part of the fight against nutrient deficiencies and the unsatisfactory sanitary quality of certain foods sold at public markets. The objective was to evaluate the impact of deficiencies, excesses, or insufficiencies of the constituents of these powders and the effect of microbial contamination on consumers health. The analysis of biochemical characteristics of the dried pulp powder of *P. biglobosa* showed a moisture content of 13.1%. This value is higher than that (5.1 to 10.2%) obtained by Oguniinka *et al*. (2017). This moisture content could be explained by insufficient drying. The high moisture levels observed in the dried pulp powder could promote the proliferation of microorganism and lead to its deterioration during packaging (Touré, 2020). This conducted study reports a protein content of 3.75%. This result may be due to the high moisture content present in the samples. The studies conducted by Fatoumata et al. (2016) on fermented seed powder showed a higher protein level ranging from 28.47% to 30.90%. Moreover, the exposure of the powder to open air during marketing could lead to high activity of proteases produced by microoganisms and significant protein degradation. However, this protein content (3.75%) is not significantly different from that (3.76%) obtained in the northern Sudanese region of Burkina Faso (Dao *et al.*, 2021). This plant-based protein could contribute to the prevention of cardiovascular diseases and certainly type 2 diabetes among populations consuming dried pulps (Kahleova *et al.*, 2017). The ash content was 4%. Similar results were abtained by Ogunyinka *et al.* (2017) who indicated that ash content depends on the mineral content of the powders. The ash content (4%) is lower than that (6.86%) of the dried pulp observed by Céline et al. (2022) during the analysis of 42 articles on the nutritional composition of *P. biglobosa*. The total carbohydrate content was 88.55%. This high value could suggest that the dried pulp powder contains mainly carbohydrates, hence its consumption by local population during lean periods. However, the rate (67.30%) given by Collen et al. (2020) remains lower than that found in this study. The presence of reducing sugars (3.56%) and total sugars (32.1%) only confirms the abundance of carbohydrates in the dried pulp powder. The total carbohydrate content of the dried pulp contrasts with those (15.41%-33.91%; 18.50%) of the powder from fermented seeds according to the respective results of Roukaya et al. (2020) and Cissé et al. (2021).

Analysis of the phytochemical composition of extracts from samples of dried pulp powder of *P. biglobosa* allowed the identification of a number of secondary metabolites such as polyphenols (0.0015 mg EAG/g), flavonoids (0.0124 mg EQ/g), and tannins (0.0401 mg EC/g). These compounds are powerful antioxidants that could help populations by protecting them against oxidative damage from free radicals (Haddouchi *et al.,* 2016). These levels of polyphenols and flavonoids remain low compared to those (389.01 mg EAG/g; 975.0 mg EQ/g) of *Adansonia digitate,* a tree found in the same production areas (Kouamé *et al.*, 2018). These low concentrations could be due to certain factors such as the harvest period, storage conditions, as well as the extraction method used.

Furthermore, the analysis of the mineral composition of the dried pulp of *P. biglobosa* revealed the presence of several minerals such as magnesium (20.225 mg/kg) and calcium (16 mg/kg). These levels of calcium and magnesium are lowers than those reported by Omar *et al*. (2024). However, the phosphorus content (123.22 mg/kg) is significantly above those (0.70 mg/kg; 0.70 mg/kg; 0.60 mg/kg) from different regions of Senegal described by the same author. The potassium content (140.05 mg/kg), although high, remains much lower than that (2255.86 mg/kg) obtained by Dao et al. (2021). The presence of minor elements such as iron, zinc, and copper is evidence that the fruit pulp of the *P. biglobosa* is rich in mineral elements and could be used, to some extent, in the diet of children and the elderly. Additionally, phosphorus, potassium, calcium, and magnesium offer many health benefits. Indeed, phosphorus and calcium play a role in maintaining and growing bones. Potassium is used in nerve transmission and muscle function (Kouadio *et al.*, 2021).

The contents of vitamins C, A, and β-carotene were respectively 30 ± 0.3 mg/100g, 0.1744 ± 0.01mg/100g, and 0.00466 ± 0.005 mg/100g. The vitamin C content (30 ± 0.3 mg/100g) of our sample is higher than those (1.11 ± 0.01- 27.85 ± 0.25 mg/100g) found in various regions of Burkina Faso (Parkouda *et al*., 2021). However, it remains lower than the value (32± 0.01) found by Ahodegnon *et al*. (2018). The dried pulp therefore represents a good source of vitamin C, which is essential for the formation of blood vessels, tissue repair, and the absorption of minerals such as iron. Vitamin A and β-carotene are of paramount importance in human nutrition. The former enhances the immune system, maintains night vision, plays a role on reproduction, and strengthens teeth and bones… The latter is highly bioactive and a precursor to vitamin A. It is involved in embryonic development and protects skin lesions against oxidation (Zhang et al., 2016; Ludmila and Joanna, 2018).

According to this study, the average loads of Enterobacteria, Fungi and *Bacillus cereus* were 33 CFU/g, 920 CFU/g, and 280 CFU /g respectively. These different loads are lower than the microbiological criteria, which are 102 CFU/g, 104 CFU/g, and 1O3 CFU/g respectively. These low loads could be explained by the use of good manufacturing techniques for the different powders. Indeed, the prior drying of the pulps could be the basis for these results. *C. perfringens, E. coli,* and *S. aureus* were not present in our sample. The results may also reflect adherence to good hygiene practices during the processing of dried pulp into powder.

**5. CONCLUSION**

This study has made it possible to determine the biochemical, nutritional and microbiological composition of the powder obtained from the dried pulp of *P. biglobosa*. The results showed the presence of phenolic compounds such as total polyphenol, total flavonoids, and tannins. There is a high content of nutrients in the powder, including proteins, lipids, carbohydrates, and micronutrients such as potassium, magnesium, phosphorus, calcium, and sodium. These advantages could justify the use of dried pulp powder in food as well as in medicine. Thus, the regular consumption of pulp powder as a food ingredient would be beneficial for the Ivorian population in the fight against malnutrition and the prevention of oxidative stress.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**DATA AVAILABILITY STATEMENT**

Datais available on request from the corresponding author

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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