Biochemical, Nutritional and Microbiological Quality of Baobab (*Adansonia digitata* L.) Fruit Pulp Marketed in Ouagadougou

ABSTRACT

Baobab pulp is consumed in Burkina Faso. The objective of this study was to evaluate biochemical, nutritional and microbiological quality of baobab pulp sold on the markets of Ouagadougou.

A total of five samples were collected in five markets. Total aerobic mesophilic flora, *Salmonella*, *Sighella*, yeasts and molds, water content, total sugars, protein content, dry matter, pH, Ash content and total phenolic content were determined by standard methods. Water content were ranged from 9.25 and 9.85% with an average of 9.63%; from 4.07 to 9.98 % with an average of 5.67% for Ash content; from 49.84 to 56.85 % with an average of 52.58 % for total sugars; from 90.14 to 90.74 with an average of 90.36 for dry matter ranged and a low protein content (2.95 to 5.30%) with an average of 3.95%. pH and total phenolic values are respectively from 4.78 to 5.06 with an average 4.96 and 1790.64 to 2385 with an average of 2029.31 mg of gallic acid equivalent. Total aerobic mesophilic flora (TMFA) revealed load of 7.73×10^{7} to 1.06×10^{8} CFU/mL and an absence of *Salmonella* and *Sighella* (SS) in 25 g. As for yeasts and molds, they varied from 1.00×10^{5} CFU/mL to 2.91×10^{5} CFU/mL.

The microbiological results revealed cases of non-compliance. However, cases of conformity were observed for *Salmonella*, *Sighella*, yeasts and molds. An interesting total sugars and total phenols and low proportions of protein were revealed. It is important to focus on microbiological quality to preserve consumer health.

1. INTRODUCTION

The baobab (*Adansonia digitata*) is a very old tree that can reach 1000 years [1] and Baobab fruit tree occurs naturally in dry areas of Africa [2]. It belongs to the *Bombacaceae* family. It is a 20 to 30 m high tree with a diameter of 3 to 9 m [3]. Hunger and malnutrition remain a global scourge and affect between 702 and 828 million people. 670 million people will still be suffering from hunger in 2030. The majority of whom are in developing countries where the notion of food security remains a luxury [4].

In order to contribute to the fight against food insecurity, it is important to increase production and valorize local products, especially fruits. Every part of baobab fruit is used where trees are found. Baobab is used as a source of food, traditional medicine, as well as sold (fresh fruits or processed) for household income [2]. Baobab fruits are processed into different products including juice, yoghurt, gruel, sour dough, oil, a coffee-like drink and dried as food reserves [5]. Baobab pulp is an example of this and is used by local populations to meet their food needs. The pulp is widely consumed traditionally in different forms [6]. In particular, it is used in the formulation of local drinks. In addition to meeting nutritional or organoleptic requirements, the baobab has a beneficial potential for health. Food is said to

be 'functional' if, in addition to its nutritional benefits, it has positive effects on one or more of the body's target functions, improving the state of health of individuals or reducing the risk of a disease [7]. Based on this definition and due to its specific composition of various nutrients, the baobab belongs today to this class of foods. It has long been recognized that baobab has a very interesting profile of biochemical compounds that are beneficial to human health. A baobab-based diet is low in saturated fatty acids and cholesterol, and provides few calories compared to a meat or dairy-based diet [8]. Baobab pulp and leaves are rich in nutrient compounds such as carbohydrates, proteins, polyphenols, carotenoids, acids, vitamins and mineral elements including calcium, iron, zinc, potassium and phosphorus. Baobab bioactive compounds may reduce the risk of chronic diseases such as cardiovascular disease and cancer [2]. Baobab pulp or monkey bread is rich in mineral compounds with a predominance of potassium and a low presence of sodium [3]. Baobab pulp contains very high levels of minerals, vitamin C, organic acids, essential amino acids, sugars, sterols, saponins, triterpenes, flavonoids, cellulose, fibers and tannins which may be responsible for its antioxidant, anti-inflammatory, antipyretic, analgesic, hepatoprotective, antimicrobial, antiviral, anti-trypanosoma, antidiarhoea properties and healing effects [9]. The pulp constitutes the most valuable Baobab product for the international market [10].

Baobab products are achieved through local processing knowledge and often of low quality [2]. Also, local products such as baobab pulp, although rich, are little exploited. This study is part of a context of development and valorization of local products in Burkina Faso. The general objective of this study was to evaluate the microbiological, biochemical and nutritional quality of baobab pulp marketed in Ouagadougou.

2. MATERIAL AND METHODS

2.1. Sampling

The biological material to be studied was baobab pulp (Fig.1) and the samples were collected from female vendors in some markets in the city of Ouagadougou. A total of five samples batches of pulp were collected in aseptic plastic bags. The samples were stored in these plastic bags, protected from all factors that could influence their initial quality.

Fig.1. Whole fruit of the baobab tree

2.2 Microbiological analysis

2.2.1 Sample preparation and dilutions

A mass of 10 g of each sample was added sterilely to 90 mL of sterile NaCl solution (9%) and the well homogenized mixture corresponds to the 10^{-1} dilution. Dilution 10^{-2} is prepared from 1 mL of the previous dilution, added aseptically to 9 mL of sterile physiological water and so on to dilution.

2.2.1.1 Preparation of culture media

The culture media were prepared strictly according to the manufacturer's label recommendations. A quantity of the dehydrated media was dissolved in distilled water and the mixture was homogenized. The media were then sterilized using an autoclave at 121°C for 15 min except for the *Salmonella* and *Shigella* (SS) medium which was heated in a water bath and then cooled and poured into Petri dishes.

2.2.1.2 Enumeration of the TMFA and Yeasts and Molds

Total aerobic mesophilic flora was enumerated on Plate Count Agar (PCA) after 24 to 48 hours' incubation at 37°C [11]. Dilutions 10^{-5} and 10^{-6} were plated. Yeasts and molds were counted on Sabouraud chloramphenicol agar after 72 to 96 hours of incubation at 25 °C according to the French standard [12]. Dilutions 10^{-3} and 10^{-4} were plated.

2.2.1.3 Testing for Salmonella and Shigella

Salmonella and *Shigella* (SS) were tested according to ISO [13]. The research was carried out in three (3) steps (pre-enrichment, selective enrichment and isolation). Pre-enrichment: 25 g of each pulp sample was aseptically removed and placed in 225 mL of peptone water and incubated for 18 ± 2 hours at 37°C; Selective enrichment: It was done by transferring 1 mL of the pre-enriched liquid medium into 10 mL of Rappaport-Vassiliadis (RV) selective liquid medium using a sterile pipette. Incubation was done at 37°C for 24 hours; Isolation: It was done on *Salmonella* and *Shigella* agar, starting from the enrichment stock solution. Starting in streak, incubation was done at 37°C for 24 hours (Table 1).

Table 1. Summary table of the germs tested and their culture media used

Searched for germs	Culture media	Temperature and incubation time	Normative references
Total Mesophilic Aerobic Flora (TMFA)	PCA	37°C for 24-48h	NF/ISO 4833 (1991)
Yeasts and Molds	Sabouraud with chloramphenicol	25°C for 72-96h	ISO7954 (1988)
Salmonella and Shigella	SS	37°C for 24-48h	NF/ISO 6579 (2002)

PCA: Plate Count Agar; SS: Salmonella and Shigella

The results are based on the counting of plates containing between 15 and 300 colonies according to ISO [14]. The number N of microorganisms present in the sample was calculated as a weighted average of two successive decimal dilutions using the following formula:

$$N = \Sigma C/V*d*1.1$$

ΣC = Sum of the colonies counted on the two plates kept after two successive decimal dilutions; V = Volume of inoculum inoculated in each box; D = Dilution corresponding to the first dilution chosen; 1.1 corresponds to (n1 +0.1n2) where n1 is the number of boxes in the first dilution and n2 is the number in the second dilution. Assessment criteria used are recorded in Table 2.

Table 2. Assessment criteria used

Germs	Result (CFU/mL)		
	< m	m< x< M	>M
	Satisfactory	Acceptable	Unsatisfactory
FAMT	10^6 CFU/mL		10^7 CFU/mL
L M	10^4 CFU/mL		10^5 CFU/mL

Microbiological criteria for foodstuffs. Guidelines for interpretation, 2018 Edition. TMFA= Total aerobic mesophilic flora; LM= Yeasts and Molds

2.3 Biochemical analysis

2.3.1 Determination of water content

The moisture content was determined by difference in the weight of a sample before and after oven drying according to the AOAC method [15]. Approximately 5 g of ground sample (PE) are weighed into a sample pan (P0) and placed in an oven at 105°C for 24 hours. The sample pods are then cooled in a desiccator and weighed (PF). The percentage by mass of water is obtained according to the following formula:
%H= (PE-(PF-P0))/PE*100 with PE = test socket; P0n = Empty weight of nacelles; PF = final weight; %H = moisture content; Total Dry Matter (TDM) = 100 -%H.

2.3.1.1 Determination of ash content

The ash was determined according to AOAC [16]. About 5 g of ground sample (PE) was weighed into a crucible (P0). The crucible containing the sample will be placed in the oven at 550°C for 04 hours, cooled in the desiccator for 1 hour and then weighed again (PF). The ash to dry matter ratio is calculated according to the following formula:
% C =(PF-PO))/PE*100; PE = test socket; PF = final weight; P0 = empty weight of crucibles
%C = ash content; %CMS = %C*100/ (100 -%H).

2.3.1.2 Determination of the hydrogen potential

The pH determines the concentration of H+ ions in the sample. The potentiometric method of AOAC [17] was used. A volume of 50 mL of distilled water was added to 5 grams (g) of the pulp. After homogenization, the pH was determined using a HANNA PH meter.

2.3.1.3 Determination of protein content

The protein content was determined according to the French Standard [18] by the Kjedahl method. The organic nitrogen in the sample is transformed into mineral nitrogen in the form of ammonia $(NH4)_2S0_4$ by the oxidizing action of boiling concentrated sulfuric acid in the presence of a catalyst. After displacement by soda, the ammonia is distilled and then titrated in the presence of a colored reagent (boric acid) by acidimetric. The total protein content is calculated by multiplying the amount of nitrogen by a conversion factor (6.25) of nitrogen in protein. Weighing 0.2 gram (g) of ground sample (PE) will be put in a mineralization tube where a pinch of catalyst is added followed by 5 to 10 mL of concentrated H_2S0_4. Mineralization is carried out on a heating block at a progressive temperature (90, 120, 400°C) until the solution is completely decolored. The mineralization is then diluted with approximately 50 mL of distilled water. Distillation is then carried out. The distillate is collected in a beaker containing 5 mL of colored indicators consisting of bromocresol green,

methyl red and boric acid and then titrated with 0.1 N H2S04 until the indicator turns from green to red. The protein content is determined according to the formula:
%N(Nitrogen) = 0.014×0.1×(VE-VB)/PE*100; VE = drop of the burette (sample); VB = drop of the burette (white); PE = test socket; 0.1 = Sulfuric acid titer; 0.014 = conversion factor to sulfuric acid equivalent; %N: nitrogen rate; %P: protein content; %P(Protein) = %N*6.25
%PMS= %P*100/ (100-%H); %PMS: protein content in dry matter.

2.3.1.4 Determination of total phenolic

Extracts containing phenolic compounds, treated with the Folin-Ciocalteu reagent in alkaline medium, develop a bluish coloration (from green to violet) whose absorbance is measured at 765 nm with a spectrophotometer [19].

2.3.1.4.1 Preparation of the standard range

The standard range is prepared for each assay run by performing a series of cascade dilutions from a stock solution of Gallic acid (GA) which can be stored in a refrigerator for 2-3 weeks. The stock solution was prepared by weighing 25 mg of GA and diluting it with 25 mL of methanol, giving a stock solution with a concentration of l mg/mL. The daughter solutions are obtained by dilutions of the mother solution.

2.3.1.4.2 Extraction of phenolic compounds

In 10 mL tubes, 200 mg of each sample were weighed and 4 mL of methanol were added. The mixture was vortexed for 30 s and then placed in an ultrasonic bath for 10 minutes, revortexed for 30 s, placed back in the ultrasonic bath for 10 minutes. The tubes containing the mixture are then centrifuged for 10 min at 6000 rpm at 4°C. After centrifugation, 3 mL of the supernatant is taken and poured into a light-protected tube, and 2 mL of the supernatant is put into a light-protected Ependorf tube, which can be stored in the refrigerator for further analysis.

2.3.1.4.3 Determination of total phenolic

In a 2 mL Ependorf tube, 100 µL of methanol, 100 µL of methanolic extract (supernatant), 100 µL of Folin-Ciocalteu reagent, 700 µL of 20% sodium carbonate, 1000 µL of distilled water are mixed. The whole is quickly vortexed and incubated at room temperature in the dark for 1 hour. After incubation, the tubes are centrifuged for 5 min at 6000 rpm at 4°C if there is a deposit. The absorbance is then read at 765 nm against methanol as a blank. Using the absorbance value, the total phenolic content is determined by the average of the calibration curve and expressed in mg Gallic acid (GA).

2.3.1.5 Determination of total sugars (DNS method)

In a strong basic medium and at high temperature, the reducing sugars, thanks to their reducing function, reduce sodium 3,5-dinitrosalycilate to a colored compound which presents an absorption maximum at 546 nm. 2.5 g of the sample were weighed into a beaker and 25 mL of hot distilled water (60°C) was added. The suspension was placed under magnetic stirring for 1 h 30 min, then the mixture was filtered and the residue washed twice with 25 mL of distilled water under stirring for 30 min. The filtrates were collected in a 100 mL volumetric flask and the volume was made up with distilled water. 1 mL of the filtrate was taken into a test tube, 2 mL of the DNS reagent was added and the mixture was homogenized and placed in a water bath for 15 min (two trials). After cooling under tap, the mixture was homogenized again and the optical densities (OD) were read against a control. A calibration

curve is established with solutions in glucose concentration. Total sugar levels are determined with reference to a concentration range of 0 to 100 µg/mL from a glucose stock solution.

2.3.1.6 Data analysis

The statistical analysis was performed using Excel 2013. Data were replicated three times by samples. The results of the statistical analyses are presented as mean ± standard deviation.

3. RESULTS AND DISCUSSION

3.1 Microbiological quality

The results found for TMFA range from 5.64×10^{7} to 1.58×10^{8} CFU/mL. These results are higher than the results found by Cissé *et al*. [20] which were 1.2 to 3.7×10^{3} CFU/mL. This would be due to the fact that the pulps were spread out in the open in the markets where dust could play an important role in the contamination of the pulps. As for yeasts and molds, they ranged from 1.00×10^{5} to 2.91×10^{5} CFU/mL. These results are higher than those reported by Tapsoba [21], who found 1.3×10^{3} CFU/mL. These results can be explained by contamination due to the storage of the pulp in the open air. Concerning *Salmonella* and *Sighella*, the results indicated an absence in 25 g of pulp analyzed. In sum, in accordance with the assessment criterion used, the results obtained in Table 3 show that the total aerobic mesophilic flora load was unsatisfactory. With regard to fungal flora, most of the samples analyzed were also unsatisfactory. On the other hand, all samples analyzed for *Salmonella* and *Sighella* were satisfactory. In fact, worldwide regulations require a total absence of *Salmonella* in food. It should be noted that the lack of respect for hygiene rules during the baobab pulp extraction process could account for the high load of microorganisms.

Table 3. Microorganisms loads

Samples	E1	E2	E3	E4	E5
TMFA	7.73×10^{7}	1.06×10^{8}	1.58×10^{8}	5.64×10^{7}	7.64×10^{7}
L M	3.64×10^{4}	2.18×10^{5}	2.91×10^{5}	1.00×10^{5}	1.18×10^{5}
SS	Absence	Absence	Absence	Absence	Absence

TMFA= Total aerobic mesophilic flora; LM= Yeasts and Molds; SS= Salmonella and Sighella; E= Sample.

3.2 Biochemical quality and nutritional quality

The results of the analyzed samples gave moisture contents of 9.25 to 9.85% and dry matter of 90.14 to 90.74. Protein contents ranged from 2.95 to 5.30%; total sugars from 49.84 to 56.85%. Total phenols ranged from 1712.83 to 2385.88 mg GAE and the pH ranged from 4.78 to 5.06. The results are presented in Table 4.

Table 4. Physico-chemical characteristics of the analyzed samples

E	%H	%C	%P	%ST	MS	PT	p^{H}

E1	9.64±0.08	4.98±0.57	3.80±0.00	49.84±9.52	90.35±0.08	1790.64±10.18	5.06±0.08
E2	9.85±0.09	9.98±0.09	4.43±1.37	51.51±1.78	90.14±0.09	2385.88±162.68	4.91±0.07
E3	9.85±0.12	4.77±0.27	5.30±0.34	52.11±4.94	90.14±0.12	1943.27±53.25	5.03±0.02
E4	9.59±0.00	4.57±0.31	3.31±0.00	52.59±1.83	90.40±0.01	2133.94±313.21	4.78±0.26
E5	9.25±0.15	4.07±0.14	2.95±0.12	56.85±6.93	90.74±0.15	1712.83±150.10	5.02±0.02
M	9.63±0.38	5.67±0.27	3.95±0.61	52.58±4.98	90.36±0.09	2029.31±137.88	4.96±0.09

E=Sample; M=Mean ±Standard deviation; %H= Moisture; %C = Ash; %P = Protein; %ST = Sugars; PT = Total Phenols; MS = Dry matter.

3.2.1 Dry matter and moisture content

Moisture levels ranged from 9.25 to 9.85% with an average of 9.63%. The moisture content is higher than the FAO [22] value of 7.4%. Our results are also higher than the values reported by Cissé et al. (2005) who found values ranging from 6.2 to 7.5%. However, our moisture content is lower than the 10.94% reported by Kouamé *et al*. [23] and the 11.6% found by Soloviev *et al*. [24]. The dry matter was deduced through the moisture content, it varied from 90.14 to 90.74 with an average of 90.36, which is higher than the value of Cissé [8] which was found 88.33. The high water content of product such as baobab pulp and may negatively influence its shelf life [10] and is responsible for the proliferation of microorganisms. Other factors such as sunlight and wind exposure have been reported to contribute to fruit pulp dryness [25]. The moisture content can be attributed to the low altitude, low annual precipitation, and moderately high temperatures [26].

3.2.2 Ash content

The ash levels ranged from 4.07 to 9.98% with an average of 5.87%. The ash rate is slightly higher than the ash rate of the 5.61% found by Kouamé *et al*. [23] and the 5.21% reported by Cissé [8]. Also, our value is higher than the 4.9% found in others studies [27,28,29]) and the very low value of 1.9 % reported by Obizoba and Amaechi [30]. The methods used by the authors vary considerably with respect to the time temperature combinations [10]. These methods can influence the results. In addition, pulp origins at warmer temperatures may have influenced the biomass of the pulp [26].

3.2.3 Proteins content

The protein levels ranged from 2.95 to 5.30% with an average of 3.95%. Again comparatively, the protein rate found is higher than the rate of Cissé [8] which was 3.03%, the 2.88% reported found by Kouamé *et al*. [23] and the values reported in six countries (Zimbabwe, Tanzania, Malawi and Mali) which were 1.99%, 2.24%, 2.20%, 1.91% and 1.86%, respectively. However, protein average of this study is lower than the FAO rate [22] which is 6.6%, the value of 5.3% [28,29]. Thus, variation in pulp nutritive characteristics can be inferred from a combination of environmentally induced and genetically fixed differences among populations. This may reflect local adaptation or genetic drift leading to differentiation in population characteristics [26].

3.2.4 Total sugars content

Total sugars ranged from 49.84 to 56.85% with an average of 52.58%. The total sugar content is lower than that found by Tapsoba [21] (2011) which was 80.22 to 80.63% and also lower than the FAO [22] (1970) value of 81.5% and the 74.9% [29]. The sweetness may vary for different types of pulp. Simple sugars in baobab pulp account for about 35.6% of the total carbohydrate content [28]. This explains the noticeable sweet taste of the pulp.

3.2.5 Total phenolic content

Total phenolic ranged from 1790.64 to 2385 mg EAG with an average of 2029.31 mg EAG. Our results are much higher than the total phenolic reported by Cissé [8] which were 1084 mg EAG and those reported by Kouamé *et al*. [23] which were 683.10 mg EAG. Ibrahima *et al*. [31] reported lower value (1085 mg GAE /100 g) for baobab fruit pulp from Madagascar and much higher values (3518 - 4058 mg GAE/100 g) for baobab pulp from Burkina Faso [32]. The different values may be attributed to transformation of phenolic compounds to dimers or monomeric phenolic compounds or the formation of compounds over storage life which could also react with Folin-Ciocalteu reagent but which were not necessarily phenolic compounds [33]. Scalbert *et al*. [34] note that phenols are abundant in fruits such as grapes and cherries, and their contents can reach 500 mg / 100 g/MS. This content is lower than that of baobab pulp. These compounds may have significant benefit to human health and nutrition by acting as antioxidants required by the immune system for the prevention of chronic diseases associated with oxidative stress [35] such as cancer and cardiovascular diseases [36]. Baobab pulp could be recommended to populations to reduce the risk of cardiovascular disease.

3.2.6 pH values

The values of pH ranged from 4.78 to 5.06 with an average of 4.96. The pH is higher than the pH found by Tapsoba [21] which were values ranging from 3.09 to 3.13, the 3.29 found by Kouamé *et al*. [23] and the 3.3 of Nour *et al*. [37]. Soloviev *et al*. [24] show that baobab pulp contains 6.5-11.2 g equivalent malic acid per 100 g proving the acidic character of the pulp. pH is part of the conditions that favor the absence of yeasts, molds, pathogenic germs or fecal contamination, such as fecal streptococci, sulfite-reducing anaerobes, *salmonella* and *enterobacteria*. Very low pH can lead to the absence of yeasts and other microorganisms in the Baobab pulp [23].

4. CONCLUSION

All samples analyzed were unsatisfactory in terms of TMFA enumeration (5.64×10^7 to 1.58×10^8 CFU/mL). For yeasts and molds, we noted satisfaction for samples one and four (1.00×10^5 to 2.91×10^5 CFU/mL). This non-compliance could be justified by the fact that baobab pulps are exposed to the open air in the markets where dust could contaminate the pulps. Also, poor pulp extraction practices may be responsible for the high load of micro-organisms. However, as these products are generally intended to undergo pasteurization, this would reduce the microbial load. Biochemical and nutritional analysis of the samples revealed interesting levels of total sugars and total phenols and low proportions of protein with averages 52.58%, 2029.31 mg EAG and 3.95%, respectively. It is important to focus on microbiological quality to preserve consumer health.

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