

Original Research Article

Effect of Wood Ash In The Production Preparation of Soumbala From *Parkia biglobosa* Seeds ~~in Côte d'Ivoire~~

Formatted: Line spacing: 1.5 lines

ABSTRACT

Formatted: Line spacing: 1.5 lines

Aims: Evaluation of the biochemical and microbiological characterization of *Parkia biglobosa* seeds added with wood ash during the fermentation for the production preparation of "soumbala" (traditional condiment used across West Africa).

Formatted: English (United States)

Study design: Food safety.

Methodology: Five kilograms of *Parkia biglobosa* seeds divided into 14 samples were collected on Adjamé's market and 32 soumbala samples were collected on Dimbokro, Abobo, Adjamé and Cocody market's due to eight samples per commune. The loads of different microorganisms (mesophilic aerobic germs, lactic acid bacteria, *Bacillus*, yeasts and moulds) were determined by counting after culture in agar medium. The physico-chemical composition (pH, titratable acidity and soluble sugars) of Soumbala and *Parkia bigloboda* seeds has been determined according to standard methods.

Results: The results of physico-chemical analyses showed that the pH varied from 5.53 to 7.95 for the control and from 8.2 to 8.6 for the "soumbala" produced in the presence of ash during 72 hours of fermentation, while that of the commercial "soumbala" was between 6.4 and 7.7. The protein, reducing sugar and fat contents increase while the dry matter and total carbohydrate contents of the seeds decrease during the fermentation process. Microbiological analyses identified four microbial groups with *Bacillus* loads varying from 5.2 to 9.9 log (CFU/g) for the control and from 4.2 to 10.9 log (CFU/g) with the addition of wood ash with a respective optimum of 60 hours, those of lactic bacteria varied from 2 to 3.6 log (CFU/g) both in the control and in the seeds with wood ash added. Yeast and moulds loads varied from 2 to 5.2 log (CFU/g) for the control and 3 to 4.5 and 5.6 log (CFU/g) respectively

Comment [MAA1]: I understand that this is a traditional dish from Côte d'Ivoire

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Line spacing: 1.5 lines, Don't keep with next

Formatted: Font: 10 pt, Not Bold, Not All

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Comment [MAA2]: Could the authors mention the respective provinces of Côte d'Ivoire for these markets?

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt, Not Bold, Not All

Formatted: Font: 10 pt

Formatted: Line spacing: 1.5 lines

with the addition of wood ash.

Conclusion: Wood ash had a catalytic-enhancing effect on the fermentation of *Parkia biglobosa* seeds where *Bacillus* are in the majority. "Soubala" produced in the laboratory were free of lactic acid bacteria and fungi at the end of fermentation.

Keywords: African locust bean, Fermentation, Wood ashes, Microflora, "Soubala"

1. INTRODUCTION

Parkia biglobosa, commonly known as African locust bean, is a woody food species of the multiple-use tree savannas belonging to the genus *Parkia* and the legume family [1]. It is a food tree that can live for several decades. The fruits of *Parkia biglobosa*, in the form of pods, are consumed not only for their floury and sweet pulp but also and especially for their seeds used in the preparation of fermented condiments in West Africa. These dried seeds are among the hardest seeds [2]. The nutritional value of African locust bean seeds lies in its high protein content. It plays a remarkable role in regions where the consumption of meat and meat products is almost non-existent on a daily basis. The seed pulp is used to make fritters. It is also used to prepare porridge or couscous with millet flour [3]. The most prized part of the African locust bean is the seed. In West Africa, the processing of cowpea seeds into different types of condiments uses technologies that are mostly based on traditional production processes. In Côte d'Ivoire, the main product derived from African locust bean seeds is "soubala". It is a food additive or fermented condiment with flavours that is widely known by various names: "soubala" in Côte d'Ivoire, Burkina Faso and Mali [4-5], "soubara" in Guinea Konakry [1], "néétou" in Senegal [6], "dawadawa" in Nigeria [7], "afitin", "iru" and "sonru" in Benin [8-9]. "soubara" is an important source of nutrients. Indeed, it is rich in calcium, phosphorus and vitamin A. In addition, this condiment is said to regulate blood pressure through its therapeutic properties. It is a product obtained from a traditional spontaneous and therefore uncontrolled alkaline fermentation of néré seeds [10]. Its preparation is long (4 to 5 days) and includes three essential stages, namely a double cooking of the seeds, a fermentation of the cotyledons in the presence of either wood ash, millet flour or maize flour and finally a drying of the fermented product [6].

"soubala" provides a favourable environment for the development of a variety of micro-organisms, including *Bacillus*, lactic acid bacteria, yeasts, moulds, total coliforms, thermotolerant coliforms, staphylococci and clostridium [11]. However, *Bacillus*, lactic acid

Comment [MAA3]: It would necessary to add the threshold value of CFU. For example, reporting how many CFU were found in the commercial soubala, which could serve as reference.

Formatted: English (United States)

Formatted: Font: 10 pt

Comment [MAA4]: This conclusion does not agree with the results that you are reporting above: «lactic bacteria varied from 2 to 3.6 log (CFU/g) both in the control and in the seeds with wood ash added». Please, amend accordingly.

Formatted: English (United States)

Formatted: Line spacing: 1.5 lines

Formatted: Font: 10 pt

Formatted: Font: 11 pt

Formatted: Font: 10 pt

Comment [MAA5]: Try to link more the very short sentences to create slightly longer sentences.

Formatted: English (United States)

Formatted: Font: 10 pt

Comment [MAA6]: I would not call it product but use: «The main use for hte locust bean seeds is the preparation of the condiment widely eaten across West Africa»

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt

bacteria, yeasts and moulds are recognised as the main micro-organisms responsible for the spontaneous fermentation of many fermented foods, including African locust bean seed, guinea sorrel and cassava [11-12]. The fermentation of African locust bean seeds to make "soubala" varies from country to country and from ethnic group to ethnic group. It is the most important step and determines the quality of the finished product in terms of taste. Practices such as spreading cereal flour, adding wood ash and using plastic bags or tree leaves are used by women producers to increase the chances of a successful fermentation process. Because of its purely traditional character, "soubala" is not subject to any sanitary or biochemical quality control before being put on the market. Only the organoleptic quality and mainly the ~~flavouring~~flavoring quality of this product is important to those who are used to this food additive. These parameters are usually assessed subjectively and only through the use of the sensory organs of individuals. Yet the consumption of this food additive has spread quite rapidly from rural areas to urban areas where consumers are increasingly numerous among all social strata. This lack of sanitary quality control associated with "Soubala" does not prevent it from being marketed nationwide. In fact, factors such as the use of sand to hull the seeds, wood ash or cereal flour to accelerate the fermentation process and the very strong aromatic potential of the finished product, create legitimate suspicions among consumers. It is in the face of this situation that the present work was initiated with the general objective of contributing to the food valorization of néré seeds by standardizing the sanitary and nutritional quality of "soubala".

Comment [MAA7]: If the use of wood ash to prepare the soubala with locust seeds, the manuscript lacks of novelty and originality. It could be regarded as a confirmatory study.

Formatted: English (United States)

Formatted: Font: 10 pt

Comment [MAA8]: This is a good explanation for the originality of hte manuscript. However, in the results section, the authors compare the trends obtained with the data of similar studies available in the literature. Hence, I suggest to provide an clear statement on the contribution to the knowledge of the present study, and what is the intended advance with regard to the previous studies that can be found in the scientific literature.

Formatted: English (United States)

Formatted: Font: 11 pt

Formatted: Font: 10 pt

2. MATERIAL AND METHODS

2.1 Plant Material

The plant material used in this study consisted of seeds of African locust bean (*Parkia biglobosa*) (Figure 1) and "soubala" (Figure 2). The raw African locust bean seeds used for fermentation trials were purchased in the market of Adjamé (Abidjan, Côte d'Ivoire).

Formatted: English (United States)



Fig. 1. *Parkia biglobosa* seeds



Fig. 2. "Soubala"

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt

Formatted: English (United States)

2.2 Sampling of African locust bean seeds and commercial "soubala"

African locust bean seeds weighing about five kilograms, were purchased from the market in the commune of Adjamé (Abidjan, Côte d'Ivoire). The seeds were cooked in tap water, dehulled and divided into two batches of seven samples, for a total of 14 samples. Each sample of cowpea seeds consisted of 235 g. For the commercial "soubala", two cities in Cote d'Ivoire, namely Abidjan and Dimbokro were selected for sample collection. The sampling of the city of Abidjan involved the communes of Abobo, Adjamé and Cocody with eight samples each, i.e. a total of 24 samples of commercial "soubala". As for the town of Dimbokro, eight samples were also collected. This makes a total of 32 commercial samples for the two cities (Abidjan and Dimbokro). These commercial "soubala" samples were stored in stomachers, labelled, sealed and kept in a cooler containing dry ice where the temperature was maintained at 4°C by cold accumulators before being transported to the laboratory, then stored in a domestic freezer (-18-20°C) for microbiological analysis.

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt

2.3 Preparation of "soubala" from African locust bean seeds in the laboratory

Seeds collected from the market in the commune of Adjamé were sorted and washed with tap water. The cleaned seeds were cooked in boiling water for 12 hours. After the cooking and cooling time, the cooked seeds were dehulled by manual pounding with a wooden mortar in the presence of coarse sand grains. The cotyledons were then washed to remove husks and shells by sieving through a perforated calabash. The cotyledons were then boiled

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Comment [MAA9]: Is this correct? Were the seeds in boiling water for 12 hours? What a expensive dish!

Formatted: English (United States)

a second time for 4 hours before being drained in the open air in a sieve for 10 minutes. After cooling, the cotyledons were weighed using an analytical balance and divided into two batches of seven samples of 235 g each. Subsequently, one sample from each batch was fermented without the addition of ash as a control. The remaining six samples from each batch were added 20 g of wood ash per sample. Each sample was manually mixed with the 20 g of wood ash by hand using sterile gloves before undergoing spontaneous fermentation in baskets woven with plant fibres and lined with banana leaves. Each sample of cowpea seeds was placed in a labelled basket and covered with a clean cotton cloth. All samples were placed at room temperature in a closed chamber to undergo fermentation for 72 hours. Every 12 hours from the initial fermentation time T_0 , a basket containing one sample from each batch was collected for biochemical and microbiological analysis.

Formatted: Font: 10 pt

2.4 Physical and biochemical analysis of African locust bean seeds and "*soumbala*"

Formatted: Font: 10 pt

Biochemical characteristics of African locust bean and "*soumbala*" seeds such as pH, dry matter, ash, protein and lipid contents were determined according to [13] methods, total carbohydrates were determined from [14] formula and reducing sugars according to the method described by [15]. The pH was determined by grinding 10 g of sample and homogenising it in 90 mL of boiling distilled water. After cooling the mixture, the resulting solution was filtered through Whatman paper. The electrode of the pH meter (Hanna, Spain) after being calibrated at pH 4.0 and 7.0 was introduced into the filtrate for the reading of the displayed pH. The dry matter content was determined by drying 5 g of sample in a capsule placed in an oven (Mettler, Germany) at 105 °C for 24 h. At the end of the drying time, the capsule containing the sample was removed from the oven and placed in a desiccator. After cooling, the whole (sample + capsule) was weighed and the mass was noted. The operation is repeated until a constant mass is obtained. The ash content was determined by incinerating 5 g sample in a muffle furnace (PYROLABO, France) at 550 °C for 12 h. The mass percentage of the residue was expressed as ash content. The total protein content was determined by the Kjeldhal method and the lipid content was determined by Soxhlet extraction using hexane as solvent. Reducing sugars were determined after extraction of ethanolsoluble sugars according to the method described by Martínez-Herrera et al. [15] using 3,5 - dinitrosalicylic acid (DNS). The total carbohydrate content was determined by the following formula [14] : $TC = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ ash})$ (1)

Formatted: Font: 10 pt

Comment [MAA10]: This form of citation should be applied for the remaining of the manuscript.

Formatted: English (United States)

Comment [MAA11]: It is my opinion that this is not the correct form of including a direct citation of the work of other authors in the text, or hte methods used. It should be written, for example, "...described by Martínez-Herrera et al. [15]..." rather than just "...described by [15]..."

Formatted: English (United States)

Formatted: Font: 10 pt

2.5 Microbiological analysis of African locust bean seeds and "soubala"

Formatted: Font: 10 pt

Loads of lactic acid bacteria, *Bacillus*, yeasts and moulds were determined on culture media Man Rogosa Sharpe (MRS), Plate Count Agar (PCA) containing 1% starch, Sabouraud with chloramphenicol respectively. Isolation of each microbial load was done by introducing 10 g of sample into labelled sterile Stomacher bags containing 90 mL of autoclaved buffered peptone water (121 °C, 15 min). After ~~hemegenisation~~homogenization and complete dissolution of the sample, the mixture obtained corresponds to the stock suspension. From this stock suspension, serial decimal dilutions up to 10^{-9} were performed. The lactic bacteria were inoculated according to the NF ISO 6611 standard. The inoculum of the different decimal dilutions (0.1mL) was spread on Man Rogosa Sharpe agar and then incubated in an anaerobic jar at 30°C for 48 hours. The characteristic colonies appeared circular with a regular outline, milky, shiny, medium in size and their number was expressed in Colony Forming Units per gram (CFU/g). *Bacillus* loads were determined according to NF ISO 7218. A volume (0.1 mL) of the stock suspension and decimal dilutions were inoculated on the Plate Count Agar (PCA) containing 1% starch and incubated at 30°C for 48 to 72 hours. Characteristic *Bacillus* colonies are flat, opaque with serrated edges, transparent, whitish and their number was expressed as Colony Forming Units per gram (CFU/g). The inoculation of yeasts and moulds was carried out according to the NF ISO 21527-2 standard on sterile chloramphenicol Sabouraud agar by spreading 0.1 mL of the stock suspension and successive decimal dilutions. Petri dishes were incubated at 30°C for 48 to 72 hours for yeasts and 48 to 120 hours for moulds.

Formatted: Font: 10 pt

2.6 Statistical analysis

Formatted: Font: 10 pt

One-way analysis of variance (ANOVA) was performed with Statistica 7.1 (StatSoft) software to compare the variables measured on the different samples. Differences between values from physico-chemical analyses are considered significant for values of $P < 0.05$.

Formatted: Font: 10 pt

3. RESULTS AND DISCUSSION

Formatted: Font: 11 pt

3.1 Physical and biochemical characteristics of African locust bean seeds during fermentation

Formatted: Font: 10 pt

The evolution of pH during the fermentation of African locust bean seeds with and without the addition of wood ash is presented in figure 3. During the fermentation of the seeds, the pH increased progressively both in the control from 5.5 to 7.9 and with the addition of wood ash from 8.2 to 8.7. However, the pH of the seeds with the addition of wood ash is higher than that of the control without wood ash. Our results are in line with the observations of [16] who reported that the acidic pH on the first day of fermentation of seeds reaches an alkaline pH at the end of fermentation. The increase in pH observed during the fermentation of seeds could be explained by the activity of *Bacillus* which constitute the majority and predominant flora. These microorganisms degrade the proteins of the seeds and lead to the release of peptides, amino acids and the formation of ammonia. The released ammonium ion is the basis for the increase in pH during the fermentation [17]. The results obtained corroborate those of several authors [18-9] who observed an increase in pH during the fermentation of seeds. The slight decrease in pH observed after 60 hours of fermentation would be due to a decrease in the metabolic activity of *Bacillus* linked to the phase of microbial decline observed in all microorganisms during this alkaline fermentation [19].

Formatted: Font: 10 pt

Comment [MAA12]: This explanation does not seem very reasonable. I have tried to find more information in the article of Pelig-Ba [17] but this author does not mention the release of ammonia. In general fermentations decrease the pH because of the formation of organic acids. It is try that the mineralization of proteins lead to hte formation of ammonium which under high pH will be released as ammonia, but what cause the release of ammonia is an alkaline agent. Could be possible that the alkaline nutrients of the locust seed are the responsible of the increase in the pH? (e.g potassium.)

Formatted: English (United States)

Formatted: Font: 10 pt

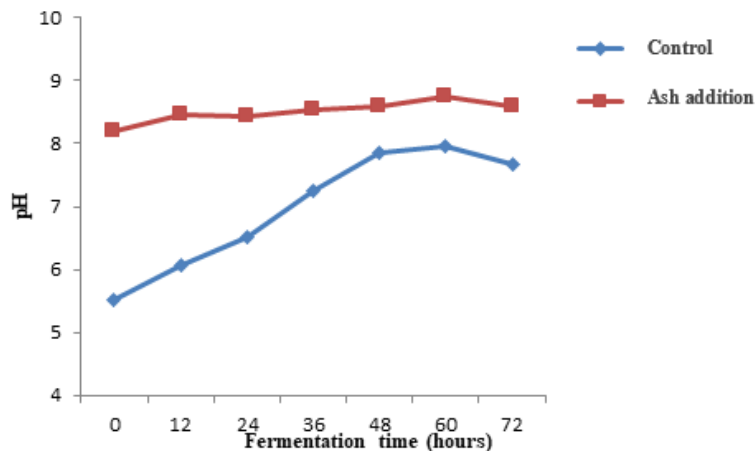


Fig. 3. Evolution of pH during fermentation of African locust bean seeds with and without the addition of wood ash

The evolution of the biochemical characteristics of the African locust bean seeds during fermentation is presented in Table 1. Before fermentation (T_0), the dry matter content was 38.8% in the control seeds and 38.14% in the seeds with wood ash added. During the 72 hours of fermentation, the dry matter content of the seeds started to decrease progressively in both the ash and control samples to reach values of 34.3% and 35.0% respectively. The protein content increased slightly during fermentation for all types of seeds, from 19.3% and 20% to 20.8% and 22.6% for the control and ash addition respectively. At the same time, the initial total carbohydrate content of the control (41%) and the wood ash addition (40.6%) decreased to 38.1% and 32.7% respectively at the end of fermentation. Reducing sugars ranged from 8 to 39.3% for the control seeds and 10.2% to 29.2% for the seeds with wood ash after 72 hours of fermentation. The lipid content also increased from 3.0 to 4.4% in the control and from 2.5 to 4.5% in the wood ash addition. The ash content increased from 0.8 to 1.3% in the control and from 3.6 to 4.5% before returning to its initial value of 3.6% in the wood ash addition at the end of fermentation.

Formatted: Font: 11 pt

Formatted: English (United States)

Formatted: Font: 10 pt

Comment [MAA13]: The addition of wood ash was supposed to increase the dry matter content.

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt

The analysis of the results showed that the dry matter content decreased during the fermentation of seeds. This decrease could be explained by the absorption of water during the cooking stage. As for the protein content of the seeds, it increases progressively during the whole fermentation process. This could be due either to the variety of seed or to the microorganisms that participate in the fermentation. Indeed, according to [4], the high protein percentage during the fermentation of seeds could be related to the varietal characteristics and/or the types of microorganisms that proliferated during the fermentation process of this product. Furthermore, according to [19], the nature of the species and the strain of bacteria that develop have a strong influence on the protein content of the product. Indeed, according to [17] and [20], microbial proteins from *Bacillus* and lactic acid bacteria could significantly increase the contents of these biological compounds. Also, the production of microbial enzymes such as proteases and lipases can be an additional source of protein [21]. The analysis of lipid content shows very low values in both the control and the wood ash addition samples. The low lipid content observed in this study could be explained by the possibility of oxidation reactions occurring [11].

Formatted: Font: 10 pt

Comment [MAA14]: It would be great to offer more details about these oxidation reactions.

Formatted: English (United States)

The fat contents obtained in this study are significantly lower than those reported (19.39-22.56%) by [3] for raw *Parkia biglobosa* seeds in use in Benin. This difference could be due to the seed preparation processes and heat treatment which have a major influence on fat oxidation. The decrease in total carbohydrate content of the control as well as the addition of wood ash during fermentation could be explained by microbial activity. Indeed, microorganisms use carbohydrates as nutrients for their growth [22]. This more pronounced decrease in seeds with the addition of ash could be due to microbial metabolism during fermentation of seeds. This could be explained by the fact that ash could be considered as a catalyst for microbial activity. Conversely, there is an increase in the reducing sugar content of the seeds due to the increase in fermentable simple sugars. This was observed by [19] in their work on the biochemical composition of African locust bean seeds during fermentation. The increase in the content of reducing sugars is thought to be related to the metabolic activity of the yeasts during their growth. However, given the inhibition of fungal growth by *Bacillus*, the transformation of neoformed reducing sugars would be partial. Regarding the ash content, our results are in agreement with [11] who reported an increase in the ash content of African locust bean (*Parkia biglobosa*) produced in Niger. The high ash content of the fermented seeds in the presence of wood ash compared to the control seeds is due to the addition of wood ash before fermentation.

Formatted: Font: 10 pt

Comment [MAA15]: Please, rewrite this sentence. As it is not grammatically correct.

Formatted: English (United States)

Formatted: Font: 10 pt

Table 1. Evolution of biochemical parameters of seeds nere during fermentation

Formatted: Font: 10 pt

proteins of the fermenters and therefore multiply significantly for the production of enzymes [23].

Formatted: English (United States)

Lactic acid bacteria have a load of 2.3 log (CFU/g) and 3.1 log (CFU/g) respectively in the control and wood ash-added seeds at the beginning of fermentation. This load increased progressively for both the ash addition and the control, with a similar load of 5.5 log (CFU/g) during the first 24 hours of fermentation, before decreasing to reach almost zero values at 48 hours for the ash addition and 60 hours for the control (Figure 4b). The lactic acid bacteria load increases very little during the fermentation of seeds. This lactic flora is less important in the fermented seed in the presence of wood ash compared to the control seeds. After 48 hours of fermentation, the lactic acid bacteria population decreases drastically until it reaches non-detectable values. These results are almost similar to those reported by [20] in African locust bean seed samples. This inhibition of lactic acid bacteria growth could be justified either by the basic pH of the fermentative medium or by the competitive action of *Bacillus*.

Formatted: Font: 10 pt

At the beginning of fermentation, the yeasts load were 2 log (CFU/g) in the two samples before to increase to reach values of 5.2 log (CFU/g) for the control and 4.5 log (CFU/g) for the sample with wood ash added during the first 24 hours of fermentation. After 24 hours of fermentation, the yeast load progressively decreases in the control and wood ash samples to reach non-detectable loads at 48 and 36 hours of fermentation respectively (Figure 4c). Mould loads in the samples, which were 2.6 log (CFU/g) similarly for fermented seeds from the control and wood ash addition at the beginning of fermentation, increase sharply before reaching a growth optimum of 5.2 log (CFU/g) at 12 hours of fermentation in both types of samples. However, after 12 hours of fermentation, these loads enter a steep decline to zero loads at 36 hours of fermentation (Figure 4d). The results of this study also showed the presence of fungi (yeasts and moulds) during the first 24 hours of fermentation followed by a drop. These results could be explained by a relatively high moisture content at the beginning of fermentation just after cooking. This would favour the initial development of moulds during 36 hours. Their low contamination rate in the samples produced in the laboratory confirms the results of the work of [24] and could be explained by a significant reduction of mould spores during the cooking stage [25]. The sharp decrease in fungal load after 24 hours of fermentation could be explained by the antifungal activity of *Bacillus* and lactic acid bacteria. Indeed, several species of *Bacillus* produce antifungal substances, notably mycosubtilin, surfactin and iturin, as a result of their secondary metabolism [26].

Formatted: English (United States)

Formatted: Font: 10 pt

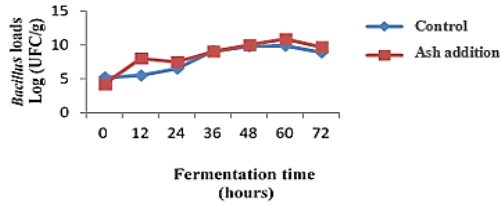


Figure 4a : Evolution of *Bacillus* sp. load during fermentation of *Parkia biglobosa* seeds with the addition of wood ash and the control

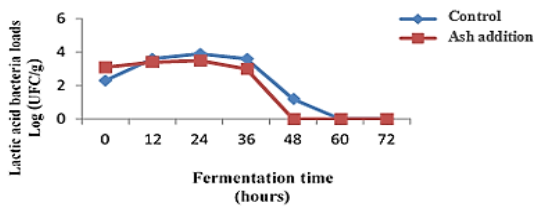


Figure 4b : Evolution of lactic acid bacteria load during fermentation of *Parkia biglobosa* seeds with wood ash addition and the control

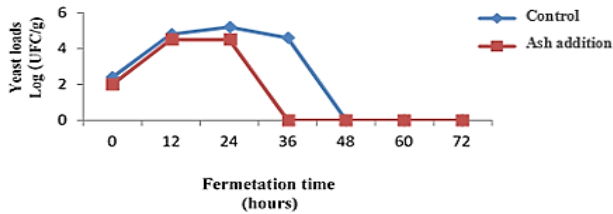


Figure 4c : Evolution of yeast load during fermentation of *Parkia biglobosa* seeds with wood ash addition and the control

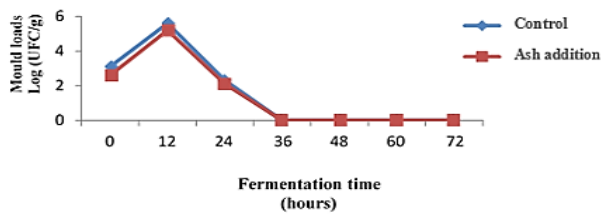


Figure 4d : Changes in mould load during fermentation of *Parkia biglobosa* seeds with added wood ash and the control

Comment [MAA16]: It would be necessary to have 3 – 4 replicates for each condition in order to apply the 2-way ANOVA to determine whether the trends of the control incubation and the ash incubation differ significantly.

Formatted: English (United States)

Fig. 4. Evolution of the microbial load during fermentation of African locust bean seeds

3.3 Comparison of pH and microflora of "sombala" produced in the laboratory in the presence of wood ash and commercial "sombala"

The pH of the commercial samples ranged from 6.4 to 7.1 while that of the sample produced in the presence of wood ash was 8.6. However, a significant difference ($P>0.05$) was observed between the pH values of the commercial samples and that of the "Sombala" sample produced with wood ash after 72 hours of fermentation (Table 2).

The different commercial "sombala" samples have acidic pH values except for those from Abobo which have a pH of 7.1. Our results corroborate those reported by [5] on the samples of "sombala" of African locust bean seeds collected in nine communes of Abidjan. The different acidic pH of the commercial "sombala" samples could be related to the non respect of the fermentation time of the seeds during its production. Indeed, during the production of "sombala", the fermentation process varies the pH of the seeds. These results suggest that most of the commercial "sombala" analysed were certainly produced under conditions other than in the presence of wood ash. In fact, to accelerate the fermentation process of seeds in some localities, producers have resorted to other processes such as corn or millet flour, ash and/or mango leaves [27]. In contrast to commercial "sombala" pH, the pH of "sombala" produced in the laboratory in the presence of wood ash has a pH (8.6). This result is similar to those obtained by [28] who obtained an alkaline pH in samples of "afitin" in Benin, a condiment similar to "sombala".

Table 2. pH of the "sombala" sample produced in the presence of wood ash and commercial "sombala"

Samples of "sombala"	Origin	pH
"Sombala" produced in laboratory in the presence of wood ash		$8,6 \pm 0,1^c$
Commercial "sombala"	Abobo	$7,1 \pm 0,1^b$
	Adjamé	$6,7 \pm 0,1^a$

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Line spacing: 1.5 lines

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Line spacing: 1.5 lines

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Cocody 6,5±0,1^a

Dimbokro 6,4±0,1^a

Values with the same letter in the same pH column do not differ significantly (P<5%) according to the Duncan's test

3.4 Microflora of laboratory produced and commercial "sombala"

The figure 5 shows the microbial loads of the laboratory-produced and commercial "sombala" samples. *Bacillus* loads were observed in all samples regardless of the type of "sombala". The highest load was obtained with the commercial "sombala" from Adjamé [10.2 log (CFU/g)], followed by the laboratory-produced "sombala" with added ash [9.7 log (CFU/g)] and the commercial "sombala" from Abobo [9 log (CFU/g)]. The lowest *Bacillus* load was obtained with the commercial "sombala" from Cocody [8.4 log (CFU/g)]. However, no significant difference (P>0.05) was observed between the microbial loads of the "sombala" samples obtained in the laboratory and those collected in the markets. The microbiological analyses showed the presence of the different microbial loads involved in the fermentation of seeds in the "sombala". In terms of *Bacillus*, the different loads were observed in all samples regardless of the type of "sombala". This reveals that *Bacillus* are the most involved microorganisms in the fermentation of African locust bean and the most dominant in its by-products. *Bacillus* are the microorganisms most involved in the production of 'Sombala' from fermented African locust bean seeds [4]. The *Bacillus* load is higher in the "sombala" samples produced in the laboratory than in the commercial samples except those from Adjamé. This suggests that these commercial samples were either produced without the wood ash, or their storage conditions by the sellers are unfavourable to *Bacillus* proliferation.

As for lactic acid bacteria, loads were only observed in commercial "sombala" samples with the highest load in Adjamé [9.9 log (CFU/g)], followed by "sombala" in Abobo [9.2 log (CFU/g)] and "sombala" in Cocody [8.5 log (CFU/g)]. The lowest load was obtained with the "sombala" samples collected in Dimbokro [7 log (CFU/g)]. No significant difference (P>0.05) was observed between the lactic acid bacteria loads of the commercial samples. Loads of lactic acid bacteria, yeasts and moulds were not observed in any of the samples of "sombala" produced in the laboratory. Their loads were only observed in commercial "sombala" samples. These results confirm those of several authors [4-11] who found the presence of lactic acid bacteria, yeasts and moulds in "sombala" samples from African

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: English (United States)

Formatted: Line spacing: 1.5 lines

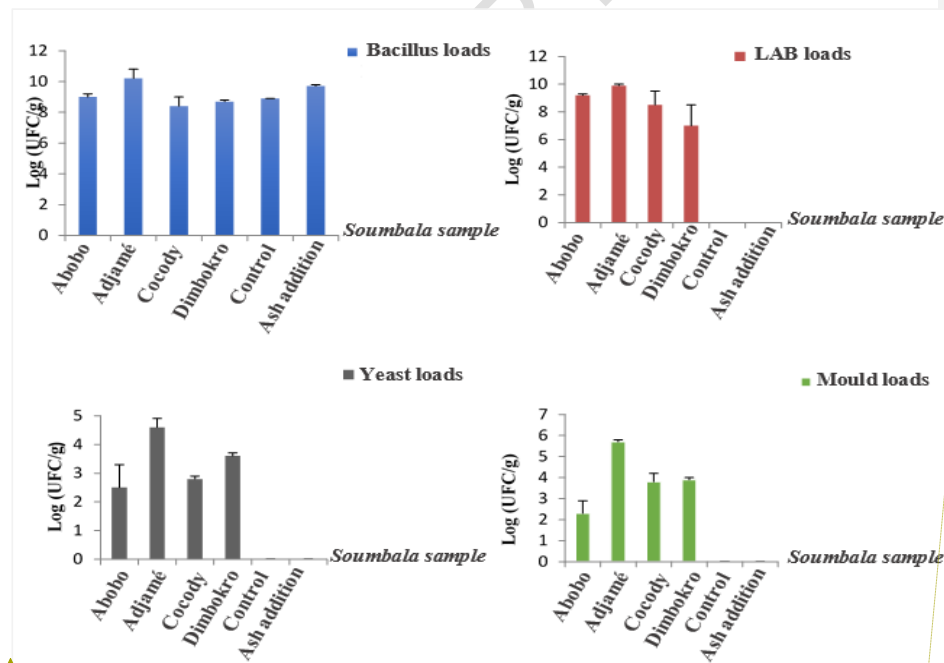
Formatted: Font: 10 pt

Formatted: Font: 10 pt

locust bean seeds collected in markets. The high lactic acid bacteria loads in the commercial samples, while non-existent in those produced in the laboratory, could be explained either by lactic acid contamination during storage or marketing, or by the use of a different technology using cereal flours.

Yeast loads were only observed in commercial "soubala" samples ranging from 2.5 to 4.6 log (CFU/g) with the highest load obtained in the Adjámé samples and the lowest in those from Cocody. Mould loads were only observed in the commercial "soubala" samples. The highest load was recorded in the Adjámé samples [5.7 log (CFU/g)] followed by those from Dimbokro [3.9 log (CFU/g)] and Cocody [3.8 log (CFU/g)]. The lowest load was obtained in the Abobo samples [2.3 log (CFU/g)]. The yeast and mould loads in the commercial samples could be due to exogenous contamination as these microorganisms do not seem to play a major role in the fermentation of "soubala" [29]. Several studies [4-5] have also mentioned the presence of fungi including yeasts and moulds in "soubala" samples in similar studies.

Formatted: Font: 10 pt



Formatted: Font: 10 pt

Fig. 5. Microbial load of laboratory produced and commercial "soubala".

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

4. CONCLUSION

Formatted: Font: 11 pt

At the end of this study, it was found that the pH of the fermented African locust bean seeds in the presence of wood ash is alkaline from the beginning to the end of the fermentation with values above 8 and remains higher than that of the control without wood ash. Protein, reducing sugars and fat contents increase while dry matter and total carbohydrate contents of African locust bean seeds decrease during fermentation. However, the protein, lipid, fat and ash contents of seeds fermented in the presence of wood ash were higher than those of the control except for carbohydrates. These results indicate that wood ash had a catalytic effect on the fermentation of seeds. This work also indicates that several microbial groups including lactic acid bacteria, *Bacillus*, yeasts and moulds are involved in the fermentation of African locust bean seeds. *Bacillus*, which are mainly responsible for alkaline fermentation, presented the most isolated loads, followed by moulds, yeasts and finally lactic acid bacteria. The microbiological characteristics of the commercial "sombala" samples show exogenous cross and secondary microbial contamination with a high load of lactic acid bacteria and fungal flora compared to the "sombala" sample produced in the laboratory in the presence of wood ash which is free of these microorganisms.

Formatted: Font: 10 pt

REFERENCES

1. Hilly M, Adams ML, Nelson SC. A study of digit fusion in the mouse embryo. M. Touré M, Le néré, un arbre du patrimoine de la Haute Guinée. *Belgeo-Revue belge de géographie*. 2018; ISSN: 2294-9135.
2. Razzavi SMA, Amini MA, Rafe A, Emadzazadeh H. The physical properties of pistachio nut and its kernel as a function of moisture content and variety. *Journal of Food Engineering*. 2007;(81):226-235.
3. Koura K, Ouidoh PIG, Azokpota P, Ganglo JC, Hounhouigan DJ. Caractérisation physique et composition des graines de *Parkia biglobosa* (Jacq.) R. Br. en usage au Nord-Bénin. *Journal of Applied Biosciences*. 2014;(75):6239-6249.

Comment [MAA17]: Please, include the DOI of each reference (if it is available).

Formatted: Font: 11 pt

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

4. [Camara F, Soro F, Traore S, Brou K, Dje KM. Caractéristiques biochimiques et microbiologiques de moutardes africaines produites à base de graines fermentées de *Parkia biglobosa* et de *Glycine max*, vendues en Côte d'Ivoire. *International Journal of Biological and Chemical Sciences*. 2016; 10\(2\):506-518.](#)
5. [Kambiré O, Boli ZBIA, Yao KM, Ahipo DMP, Koffi-Nevry R. Microbiological quality of "Soubala", an african locust bean \(*Parkia biglobosa*\) condiment sold in the markets of Abidjan, Côte d'Ivoire. *Journal of Advances in Microbiology*. 2020; 20\(10\):67-74.](#)
6. [N'dir B, Lognay G, Wathelet B, Cornelius C, Marlier M, Thonart P. Composition chimique du *nététu*, condiment alimentaire produit par fermentation des graines du caroubier africain *Parkia biglobosa* \(Jacq.\) Benth. *Biotechnologie Agronomie Société et Environnement*. 2000;4\(2\):101-105.](#)
7. [Yisa MG, Fadeyibi A, Hafeez SA. Design, Fabrication and Testing of a Manually Operated Locust Bean Cubing Machine. *Asian Journal Applied of Science*, 2018;11\(2\):56-63.](#)
8. [Azokpota P, Hounhouigan DJ, Nago MC. Microbiological and chemical changes during the fermentation of African locust bean \(*Parkia biglobosa*\) to produce afitin, iru and sonru, three traditional condiments produced in Benin. *International journal of food microbiology*. 2006;107\(3\):304-309.](#)
9. [Azokpota P, Hounbo HY, Akissoe NH. Aptitude stabilisatrice des conservateurs traditionnels de l'afitin, condiment africain à base de graines de néré \(*Parkia biglobosa* Jack. P. Br\). *Cahier Agriculture*. 2011;20\(6\):494-499.](#)
10. [Somda MK, Savadogo A, Tapsoba F, Ouédraogo N, Zongo C, Traoré AS. Impact du processus traditionnel sur la qualité hygiénique du soubala: un condiment fermenté cuit au Burkina Faso. *Journal of Food Security*. 2014;\(2\):59-64.](#)
11. [Roukaya AS, Halima OD, Alio SA, Bakasso Y, Abdourahamane B. Caractérisation Biochimique et Microbiologique de *Soubala* de néré \(*Parkia biglobosa*\) et d'oseille de Guinée \(*Hibiscus sabdariffa*\) Produits au Niger. *European Scientific Journal*. 2020;16\(3\):224-243.](#)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted ...

Formatted: Font: 10 pt

Formatted: English (United States)

Formatted ...

Formatted ...

Formatted ...

Formatted: English (United States)

Formatted ...

Formatted ...

Formatted ...

Formatted: English (United States)

Formatted ...

Formatted: English (United States)

Formatted ...

Formatted ...

Formatted ...

Formatted: English (United States)

vulgaris) seeds during fermentation to condiments. *Journal of Nutrition*. 2004;3(3):140-145.

22. Ameer JB, Venudevan B, Jayanthi B. Storage Fungi in groundnut and the associate seed quality deterioration. *Plant Pathology Journal*. 2013;12(3):127-134.

23. Mo AY, Kwon B, Kamala-Kannan S, Lee KJ, Oh BT, Kim DH, Yang MS, Kim JH, Park SM. Isolation and characterization of *Bacillus polyfermenticus* isolated from Meju, Korean soybean fermentation starter. *World Journal of Microbiology and Biotechnology*. 2010;(26):1099-1105.

24. Unaboi-gbenni PO, Okolie PN, Sobande AO, Alao O, Teniola O, Bessong, PO. Identification of subdominant lactic acid bacteria in dawadawa (a soup condiment) and their evolution during laboratory-scale fermentation of *Parkia biglobosa* (African locust beans). *African Journal of Biotechnology*. 2009;8(25):7241-7248.

25. Gernah DI, Atolagbe MO, Echegwo CC. Nutritional Composition of the African Locust Bean (*Parkia biglobosa*) Fruit Pulp. *Nigerian Food Journal*. 2007;(25):190-196.

26. N'dir B, Gningue RD, Keita NDG, Souane M, Laurent I, Cornelius C, Thonard P. Caracteristiques microbiologiques et organoleptiques du *netetu* du commerce. *Cahiers Agricultures*. 1997;(6):299-304.

27. Millogo F. Analyse socio - économique de la production du *Soumbala* dans la région des Hauts-Bassins avec comparaison des types de production traditionnelle et semi moderne. Diplôme d'Ingénieur du Développement Rural. p. 63, 2008.

28. Hongbété F, Kindossi J, Akissoé N, Hounhouigan DJ. Performance of a wooden box for production of *Afitin*, fermented food condiment from Benin. *International Journal of Agronomy and Agricultural Research*. 2017;11(5):7-15.

29. Ikenebomah MJ, Ingram JM. Processing and fermentation of the African locust bean (*Parkia filicoidea* Welw) to produce dawadawa. *Journal of Sciences Food and Agricultral*. 1986;(37):273-282.

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: English (United States)

Formatted: Font: 10 pt, English (United States)

Formatted: Font: 10 pt

Formatted

Formatted: English (United States)

Formatted: Font: 10 pt

Formatted: Line spacing: 1.5 lines