Original Research Article

Effect of Wood Ash In The Preparation of "*Soumbala*" From *Parkia biglobosa* Seeds

ABSTRACT

Aims: Evaluation of the biochemical and microbiological characterization of *Parkia biglobosa* seeds added with wood ash during the fermentation for the preparation of "soumbala" (traditional condiment used across West Africa).
Study design: Food safety
Methodology: 32 soumbala samples due to eight samples per commune were collected in the markets of Dimbokro (city in the center of Côte d'Ivoire) and three communes from Abidjan district, namely Adjamé, Abobo and Cocody. Also, 5 kg of *Parkia biglobosa* seeds divided into 14 samples were collected on Adjamé's market. The loads of 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 analyzes showed that the Bacillus loads varied from 5.2 to 9.9 log (CFU/g) for the control and 4.2 to 10.9 log (CFU/g) with the addition of wood ash with a respective optimum of 60 hours, those of lactic acid bacteria varied respectively by 2.3 log (UFC/g) and 3.1 log (UFC/g) in the control and supplemented with wood ash to reach an optimal load similar of 5.5 log (CFU/g). Yeast and mold loads varied from 2 to 5.2 log (CFU/g) for the control and from 3 to 4.5 and 5.6 log (CFU/g) respectively with the addition of wood ash. *Bacillus* was the only microorganism with the highest load both in commercial soumbala [10.2 log (CFU/g)] and in laboratory-produced soumbala [9.7 log (CFU/g)].
Conclusion: Wood ash had a enhancing effect on the fermentation of *Parkia biglobosa* seeds where *Bacillus* are in the majority. "Soumbala" produced in the laboratory were free of lactic acid bacteria and fungi at the end of fermentation.

Keywords: Parkia biglobosa seeds, *Fermentation, Wood ashes, Microflora, "Soumbala"*

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, prepare porridge or couscous with millet flour [3] and 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 use derived from African locust bean seeds is "*soumbala*". It is a food additive or fermented condiment with flavours that is widely known by various names: "*soumbala*" in Côte d'Ivoire, Burkina Faso and Mali [4-5], "*soumbara*" in Guinea Konakry [1], "*nététou*" in Senegal [6]**)**, "*dawadawa*" in Nigeria [7], "*afitin*", "*iru*" and "*sonru*" in Benin [8-9]. "*soumbara*" 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. The fermentation of most local food products is a spontaneous fermentation in acidic or basic medium which is done by the intervention of several microorganisms including lactic acid bacteria, *Bacillus*, yeasts and molds [10]. At the level of “Soumbala”, it is a spontaneous and uncontrolled traditional alkaline fermentation of *Parkia biglobosa* seeds [11]. Its preparation is long (4 to 5 days) and includes two essential steps, namely double cooking of *Parkia biglobosa* seeds and fermentation of the cotyledons from these seeds.

"*soumbala*" 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 clostridum [12]. However, *Bacillus*, lactic acid bacteria, yeasts and moulds are recognised as the main micro-organisms responsible for the spontaneous fermentation of many fermented foods, including *Parkia biglobosa* seeds, guinea sorrel and cassava [10-12]. The fermentation of African locust bean seeds to make "*soumbala*" 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 potash 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, "*soumbala*" is not subject to any sanitary or biochemical quality control before being put on the market. Only the organoleptic quality and mainly the 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 "Soumbala" 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. To our knowledge, no scientific research has so far been undertaken in Côte d'Ivoire concerning the use of wood ash to accelerate the fermentation of *Parkia biglobosa* seeds. It is in this context that this study was initiated to assess the contribution of wood ash in the fermentation process of *Parkia biglobosa* seeds for the preparation of "Soumbala", traditional condiment used across West Africa.

2. MATERIAL AND METHODS

2.1 Plant Material

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

Fig. 1A. *Parkia biglobosa* seeds

Fig. 1B. "*Soumbala*"

Fig. 1. Photograph of Parkia biglobosa seeds (1A) and "Soumbala" (1B)

2.2 Sampling of *Parkia biglobosa* seeds and commercial "*soumbala*"

Parkia biglobosa seeds of about five kilograms were collected in the market of Adjamé, a commune in the district of 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. With regard to commercial "Soumbala", 24 samples were collected in the markets of three communes, namely Adjamé, Abobo and Cocody in the district of Abidjan located in the south of Côte d'Ivoire. Subsequently, 8 samples of commercial "Soumbala were collected in the market of Dimbokro, a city in the center of Côte d'Ivoire. This makes a total of 32 samples of commercial "Soumbala" for all of the two cities (Abidjan and Dimbokro). These commercial "*soumbala*" 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.

2.3 Preparation of "*soumbala*" from *Parkia biglobosa* 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 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. The diagram for preparation of soumbala from *Parkia biglobosa* seeds is shown in figure 2.

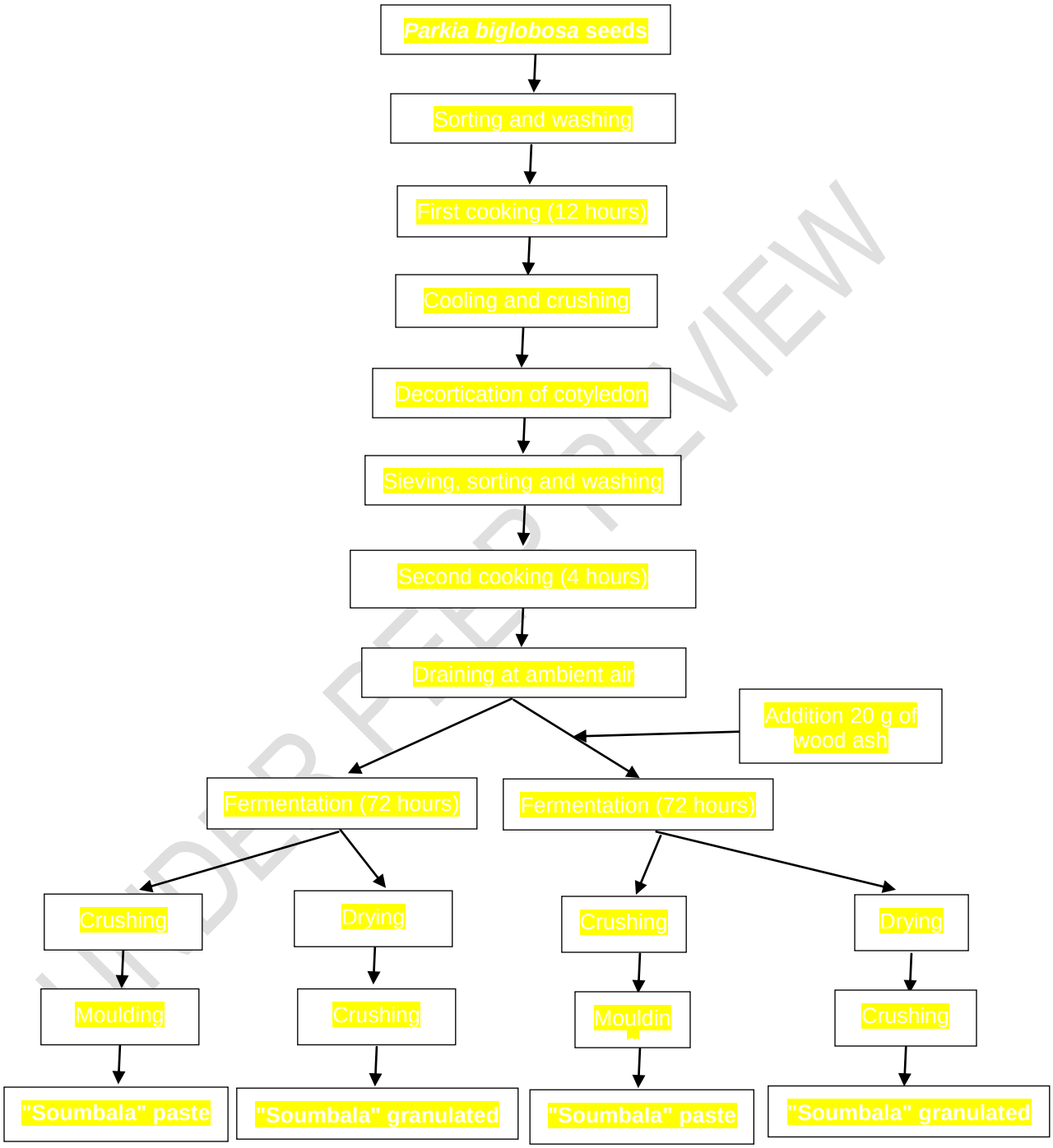

Fig. 2. Diagram of preparation of "Soumbala" from *Parkia biglobosa* seeds

2.4 Physical and biochemical analysis of *Parkia biglobosa* seeds and "*soumbala*

Biochemical characteristics of *Parkia biglobosa* seeds and "*soumbala*" 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 (Memmert, 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 ethanosoluble sugars described by Martinez-Herrera *et al.* [15] using 3,5 - dinitrosalycilic acid (DNS). The total carbohydrate content was determined by the following formula [14] : TC = 100 - (% moisture + % protein + % fat + % ash) (1)

2.5 Microbiological analysis of *Parkia biglobosa* seeds and "*soumbala*"

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 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.

2.6 Statistical analysis

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 $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physical and biochemical characteristics of *Parkia biglobosa* seeds during fermentation

The evolution of pH during the fermentation of *Parkia biglobosa* 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 abundant production of ammonia. The increase in pH during *Parkia biglobosa* seeds fermentation can be attributed to the abundant production of ammonia from the desamination of amino acids [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].

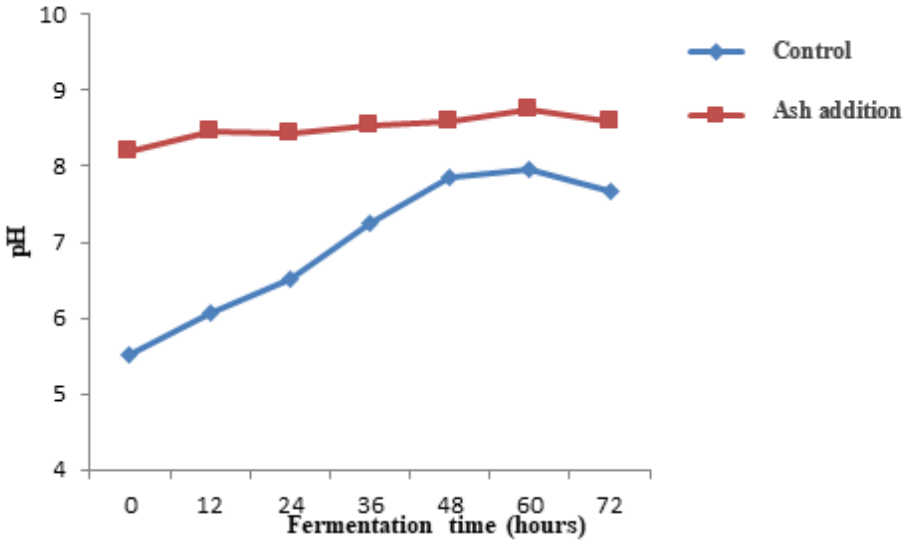

Fig. 3. Evolution of pH during fermentation of *Parkia biglobosa* seeds with and without the addition of wood ash

The evolution of the biochemical characteristics of *Parkia biglobosa* seeds during fermentation is presented in Table 1. Before fermentation (T_0), the dry matter content was

38.1% in the control seeds and 38.8% 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 achieve similar values of 35.0%. 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.

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].

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.

Table 1. Evolution of biochemical parameters of *Parkia biglobosa* seeds during fermentation

Samples	Times of fermentation (hours)	Physico-chemical charactteristics					
		Dry matter (%)	Protein (%)	Carbohydrates (%)	Reducing sugars (%)	Lipids (%)	Ash (%)
Seeds nere without wood ash (control)	0	38,1±1,3[a]	19,3±0,6[a]	41,0±0,2[a]	8,1±0,1[a]	3±0,1[a]	0,8±0,4[a]
	48	35,4±0,3[b]	18,9±0,6[b]	38,2± 0,2[b]	30,4±1,1[b]	3,9±0,2[b]	0,8±0,1[a]
	72	35,0±0,1[b]	20,8±1,2[c]	38,1± 0,4[b]	39,3±0,1[c]	4 ,4±0,1[c]	1,3±0,1[b]
Seeds nere with added wood ash (control)	0	38,8±0,6[a]	20±1,1[a]	40,6±0,3[a]	10,2± 0,2[a]	2,5±0,4[a]	3,6±0,3[a]
	48	36,3±0,3[b]	20,6±1,8[a]	33.0±0,2[b]	26,8±0,1[b]	4,1±0,2[b]	4,5±0,2[b]
	72	35,0±0,1[b]	22,6±1,2[b]	32,7±0,1[b]	29,2±0,7[c]	4,5±0,4[c]	3,6±0,1[a]

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

3.2 Microbiological characteristics during fermentation of *Parkia biglobosa* seeds

The microbial load curves during the fermentation of seeds are shown in Figure 4. At the beginning of fermentation, *Bacillus* sp. load was estimated at 5 log (CFU/g) in the control seeds and 4 log (CFU/g) in the wood ash addition. From 12 hours of fermentation, the increase in this microbial population enters its exponential growth phase to reach their maximum value (10.9 log CFU/g) for the ash addition and 9.9 log (CFU/g) for the control at 60 hours of fermentation. Thereafter, the *Bacillus* sp. load began a phase of decline with values falling by 9.7 log (CFU/g) for the addition of ash and 8.9 log (CFU/g) for the control (Figure 4a). Microbiological analyses provided significant *Bacillus* loads during seed fermentation. Our results confirm those of [6] who indicated that the microflora associated with the fermentation of *Parkia biglobosa* seeds were mainly spore-forming germs of the genus *Bacillus*. The high level of this flora may be due to the fact that it is described as an initiator of fermentation of seeds during the production of "*soumbala*" [19]. The slight decrease in *Bacillus* load after the 60 hours of fermentation could be due either to the depletion of available substrates [9] or to physico-chemical changes such as alkalinisation of the fermentation medium. *Bacillus*, thanks to their high proteolytic activity, degrade the proteins of the fermenters and therefore multiply significantly for the production of enzymes [23].

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*.

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].

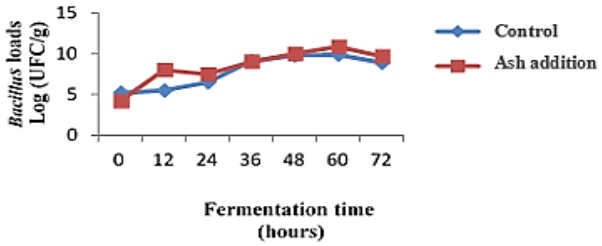

Figure 4a : Evolution of *Bacillus sp.* load during fermentation of *Parkia biglobosa* seeds with the addition of wood ash and the control

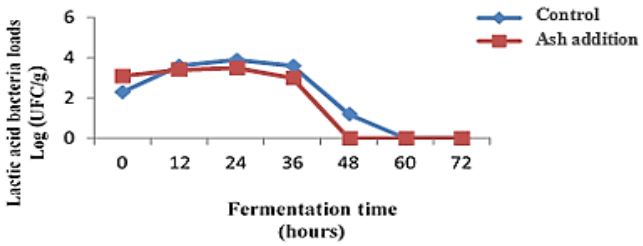

Figure 4b : Evolution of lactic acid bacteria load during fermentation of *Parkia biglobosa* seeds with wood ash addition and the control

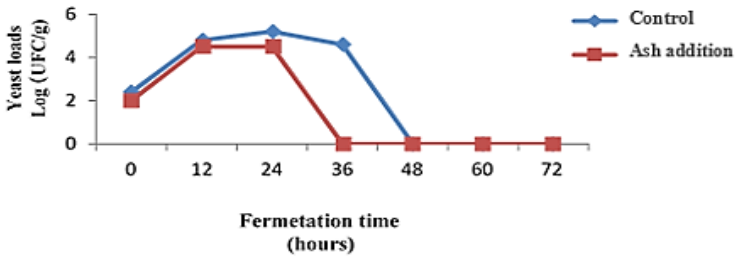

Figure 4c : Evolution of yeast load during fermentation of *Parkia biglobosa* seeds with wood ash addition and the control

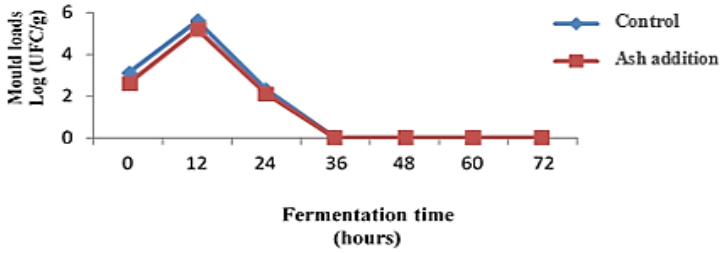

Figure 4d : Changes in mould load during fermentation of *Parkia biglobosa* seeds with added wood ash and the control

Fig. 4. Evolution of the microbial load during fermentation of *Parkia biglobosa* seeds

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

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 "Soumbala" sample produced with wood ash after 72 hours of fermentation (Table 2).

The different commercial "*soumbala*" 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 "*soumbala*" of African locust bean seeds collected in nine communes of Abidjan. The different acidic pH of the commercial "*soumbala*" samples could be related to the non respect of the fermentation time of the seeds during its production. Indeed, during the production of "*soumbala*", the fermentation process varies the pH of the seeds. These results suggest that most of the commercial "*soumbala*" 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 "*soumbala*" pH, the pH of "*soumbala*" 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 "*soumbala*".

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

Samples of "soumbala"	Origin	pH
"Soumbala" produced in laboratory in the presence of wood ash		8,6±0,1[c]
Commercial "soumbala"	Abobo	7,1±0,1[b]
	Adjamé	6,7±0,1[a]
	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 "*soumbala*

The figure 5 shows the microbial loads of the laboratory-produced and commercial "*soumbala*" samples. *Bacillus* loads were observed in all samples regardless of the type of "*soumbala*". The highest load was obtained with the commercial "*soumbala*" from Adjamé [10.2 log (CFU/g)], followed by the laboratory-produced "*soumbala*" with added ash [9.7 log (CFU/g)] and the commercial "*soumbala*" from Abobo [9 log (CFU/g)]. The lowest *Bacillus* load was obtained with the commercial "*soumbala*" from Cocody [8.4 log (CFU/g)]. However, no significant difference (P>0.05) was observed between the microbial loads of the

"*soumbala*" 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 "*soumbala*". *Bacillus* were the only fermentative microorganisms observed in the samples regardless of the type of "*Soumbala*" but with high loads with the addition of wood ash. This reveals that *Bacillus* are the microorganisms most involved in the fermentation of *Parkia biglobosa* seeds and the most dominant in its by products. The high loads in the samples with added wood ash could be explained by the richness of these ashes in minerals. Indeed, according to [29], wood ash is both a basic residue against acidity and a source of essential minerals. Its use allows a good release of nitrogen and could explain the rapid decomposition of organic matter by microorganisms [30]. The predominance of *Bacillus* during the fermentation of *Parkia biglobosa* seeds and the final product, in particular "Soumbala", could be beneficial for research. Indeed, work carried out by [31] have already reported the use of some *Bacillus* strains as potential starters in the production of "*Soumbala*" in Burkina Faso. The *Bacillus* load is higher in the "*soumbala*" 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 "*soumbala*" samples with the highest load in Adjamé [9.9 log (CFU/g)], followed by "*soumbala*" in Abobo [9.2 log (CFU/g)] and "*soumbala*" in Cocody [8.5 log (CFU/g)]. The lowest load was obtained with the "*soumbala*" 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 "*soumbala*" produced in the laboratory. Their loads were only observed in commercial "*soumbala*" samples. These results confirm those of several authors [4-11] who found the presence of lactic acid bacteria, yeasts and moulds in "*soumbala*" samples from African 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 "*soumbala*" samples ranging from 2.5 to 4.6 log (CFU/g) with the highest load obtained in the Adjamé samples and the lowest in those from Cocody. Mould loads were only observed in the commercial "*soumbala*" samples. The highest load was recorded in the Adjamé 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 "*soumbala*" [32]. Several studies [4-5] have also mentioned the presence of fungi including yeasts and moulds in "*soumbala*" samples in similar studies.

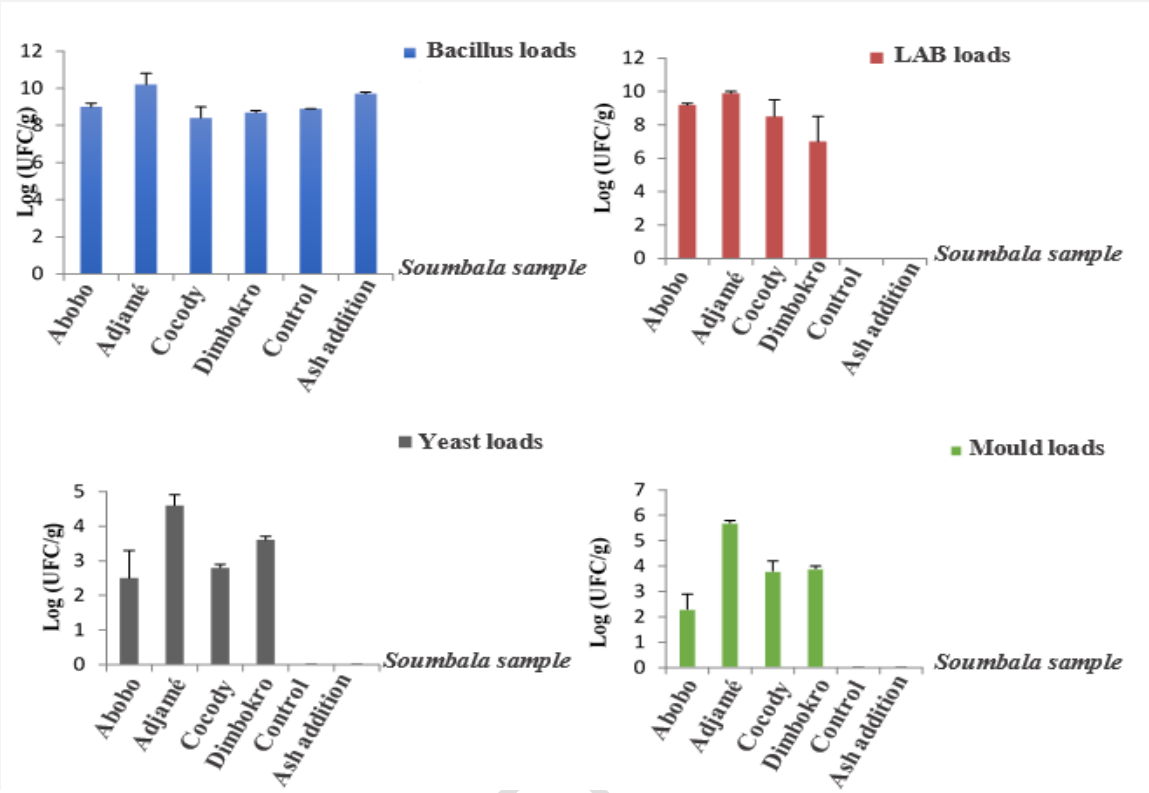

Fig. 5. Microbial load of laboratory produced and commercial "*soumbala*"

4. CONCLUSION

At the end of this study, it was found that the pH of the fermented *Parkia biglobosa* 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 *Parkia biglobosa* 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 "*soumbala*" samples show exogenous cross and secondary microbial contamination with a high load of lactic acid bacteria and fungal flora compared to the "*soumbala*" sample produced in the laboratory in the presence of wood ash which is free of these microorganisms.

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