

Microbiological Profile of Packaging Leaves of Cassava "Chikwangue" Stored in Warehouses and Sold in Brazzaville (Congo)

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Abstract: In order to contribute to the evaluation of the hygienic quality of the different packaging sheets of chikwangue (cassava leg stick) stored in warehouses and sold in Brazzaville, in view of the food safety of consumers, the different sheets were tested in their microbiological quality and the physico-chemical parameters of the warehouses were determined by classical methods. Five warehouses located in different markets in the districts of Brazzaville were chosen for the study: These were: Château d'eau, Bourreau, Commission, Tsiémé and PK. The results obtained showed that the humidity level varies from one warehouse to another depending on their location. The values of the humidity levels vary from 54.53% (Château d'eau market) to 70.46% (PK market). As for the pH values, they show an acidification of the leaves with a decrease of the pH from 6.6 (PK market) recorded on the first day of storage to 3.8 observed at the Bourreau market on the sixth day. On the basis of cultural, morphological and biochemical characteristics, several bacteria were isolated from the leaves were: Enterobacteriaceae, *Bacillus*, Staphylococci, *Pseudomonas*, Streptococci, *Clostridium*, *Candida*. After identification, Enterobacteriaceae consisted of *Salmonella* spp, *Shigella* spp, *Escherichia coli*. *Bacillus* were *Bacillus cereus* and *Bacillus* spp. Staphylococci were represented by *Staphylococcus aureus* and *Staphylococcus* spp. The following species were also identified: *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Candida albicans* and *Lactobacillus* spp, *Streptococcus* spp. The percentage of mesophilic flora isolated at each market ranged from 11% (Château d'eau) to 25% (PK) These results show that, whatever the storage market and the origin of the product, the packaging sheets used are subject to abiotic factors responsible for the proliferation of germs, some of which are pathogenic and can harm the health of consumers. Hence the need to act on the application and enforcement of hygiene rules to prevent food-borne diseases.

Keywords: Green Leaves, Packaging, Microbial Ecology, Food Safety, Food Contamination

1. Introduction

Packaging is an important and determining step in the preservation and safety of food in the food industry. It guarantees the delivery of the food to the consumer under optimal hygienic conditions. Packaging plays a role in the preservation of flavours, organoleptic quality, prevention and integrity of food. Packaging serves to transport and store the

product but also to provide relevant information about the product (brand name, expiry date, list of ingredients, producer or importer, preparation method, recipes, etc.) [1]. For many products, such as food or perfumes, the packaging aims to attract and entice the buyer as much as the contents. It seeks to give a positive image of the product. Any producer or importer whose products are marketed in packaging is obliged to contribute to or provide for the disposal of all such packaging waste. Several types of packaging are in common use: boxes,

tubes, jars, bags, envelopes and aluminium foil. Packaging materials should not transmit any toxic substances, odours or flavours to the product [2]. In Africa, people have long used the leaves of certain plants to package semi-solid or solid foods for storage for a few hours or even days in all tropical regions. These packaging materials of plant origin contain natural substances that are responsible for their mechanical, physicochemical, organoleptic and microbiological properties [3]. Several green leaves of plants are used for cassava packaging such as: *Tectona grandis*, *Musa paradisiaca*, *Megaphrynium macrostachyum*, *Haumania liebrechtsiana*, *lisiomorpha senegalensis*,... Some leaves have been found to contain active compounds-aromatics, colouring agents, enzymes (e.g. papain), antimicrobial agents (essential oils) that migrate from the plant leaf into the food product [2].

In the Congo, chikwangu, a dense paste (35 to 45g of dry matter per 100g of tubers), with an elastic texture, resulting from the wet processing of cassava tubers, is consumed by more than 84% of the population in both rural and urban areas [4]. Several leaves are used as packaging for cassava (chikwangu), the main ones being Amarantaceae. The

current situation of manufacturing, transport, storage and marketing of the products in the markets is a major concern for the microbiological quality of the stored and sold chikwangu. No microbiological study on the quality of packaging in the protection of cassava has been done to date. It is with this aim that this work aims to contribute to the evaluation of the hygienic quality of the different packaging sheets of chikwangu stored in the warehouses of Brazzaville for a better food safety of consumers.

2. Materials and Methods

2.1. Biological Material

The biological material consisted of leaves used for packaging cassava.

2.2. Collection of Samples

Chikwangu were purchased in the arrival markets of four districts of the city of Brazzaville (Table 1) during the dry season for a period of three months.

Table 1. Sample collection sites.

Arrondissement	Makélékélé		Baongo	Ouenzé	Mfilou
	Markets		Market	Market	Market
	Château d'eau	Bourreau	Commission	Texaco Tsiémé	Marché PK
Type of Chikwangu	Fabriqué	Ngudi yaka	1. Ngudi yaka 2. Ivoumba 3. Mungwélé du Nord 4. Mungwélé de Brazzaville	1. Mungwélé du Nord 2. Ivoumba	Mungwélé de Brazzaville

After purchase, the different cassava were stored in storage facilities located in the same markets. The samples analysed were of two kinds: (1) Three samples were freshly purchased and deposited in the different depots for six days and then analysed; (2) six freshly deparcelled (arrived) samples were purchased and five are deposited in the depot and the sixth one is directly brought back to the laboratory for analysis, then the stored samples were analysed successively from day one to day six.

2.3. Physico-Chemical Analysis

2.3.1. Determination of the Humidity in the Warehouse

Humidity monitoring in the warehouses was carried out using a HYGROCHECK® brand Hygrometer from HANNA instrument® with TFPC (Thin Film Polymer Capacitance) technology. The relative humidity measurements were read automatically and converted to percentage relative humidity.

2.3.2. Measurement of Leaf pH

The analyses were carried out using a pH meter type pHep HI 98107. In a beaker containing 40mL of sterile distilled water, 10g of leaves were introduced. After homogenisation, the pH meter was introduced and the pH value was read.

2.4. Microbiological Analysis

2.4.1. Isolation and Identification

Eight specific culture media were used for isolation and

identification of strains:

- (1) Sabouraud chloramphenicol Agar for isolation and identification of *Candida albicans*;
- (2) TSN Agar (Triton-Sulfate-Neomycin) for the isolation and identification of bacteria of the genus *Clostridium*, mainly *Clostridium perfringens* species;
- (3) Mossel Agar for the isolation and identification of bacteria of the genus *Bacillus* including *Bacillus cereus* species (pink-red colonies) and *Bacillus* spp (yellow colonies);
- (4) *Salmonella - Shigella* Agar (SS) for the isolation and identification of bacteria of the genus *Shigella* and *Salmonella* including *Shigella* spp (red colonies) and *Salmonella* spp (colonies with black centre);
- (5) Eosin Methylene Blue Agar (EMB) for the isolation and identification of bacteria of the genus *Enterobacteriaceae* including *Escherichia coli* species (metallic sheen colony);
- (6) Cetrimide Agar for the isolation and identification of bacteria of the genus *Pseudomonas* and mainly the species *Pseudomonas aeruginosa*;
- (7) Mannitol Salt Agar for the isolation and identification of bacteria of the genus *Staphylococcus* including *Staphylococcus aureus* species (golden yellow) and *Staphylococcus* spp (white and small);
- (8) M17 Agar for the isolation and identification of bacteria of the genus *Streptococcus* and *Lactobacillus*

including *Streptococcus* spp (white colonies) and *Lactobacillus* spp (small yellow colonies).

2.4.2. Enumeration

In a beaker bottle containing 40mL of distilled water covered with aluminium foil, 10g of cassava leaves were introduced after weighing. 1mL of this stock solution was used for decimal dilutions. The inoculations were done on Petri dishes in better specific culture. The plates were incubated at 37°C for 24 hours in the oven under aerobic conditions. After the appearance of the colonies, the count was carried out and the enumeration carried out according to the following formula [5].

$$CFU = N / VD$$

CFU/mL = colony forming units, N = number of colonies, V = volume sown, D = dilution factor.

3. Results

3.1. Study of Physico-Chemical Parameters

3.1.1. Moisture Content

The averages of the moisture contents in the different sites are shown in Figure 1. The results of the statistical analysis reveal that the rates are very high and greater than or equal to 50% with a dominance at the PK market depot where the average is 70.46% followed closely by Bourreau (67.66%) and Commission (65.66%), while the Château d'eau (54.53%) and Tsiémé (55.76%) markets have almost identical average moisture contents.

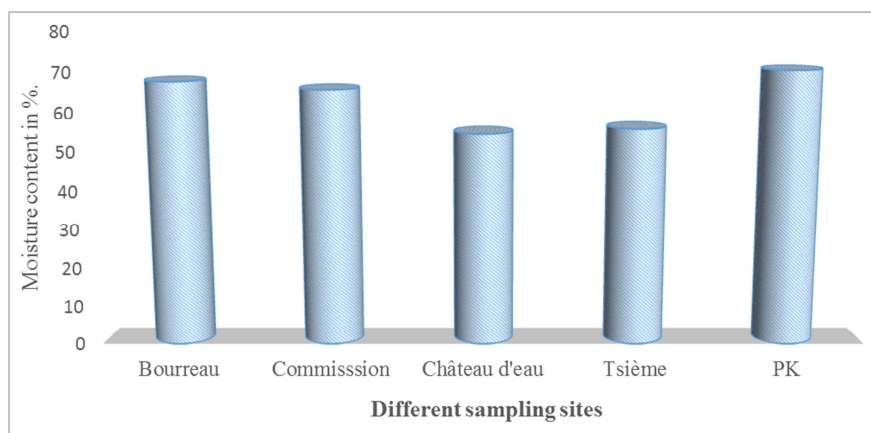


Figure 1. Valeurs moyennes du taux de l'humidité dans les dépôts des différents marchés.

3.1.2. Evolution of pH

These results show that the pH decreases with the number of days; on the first day, the pH is close to neutral with a dominance of the PK market deposit (6.6) followed by the

Tsiémé (6.5) but it is 6 at the Bourreau deposit, which is therefore less acidic. On the sixth day, the pH becomes more acidic, especially in the deposits where the pH is lower than 4 compared to the deposits (Figure 2).

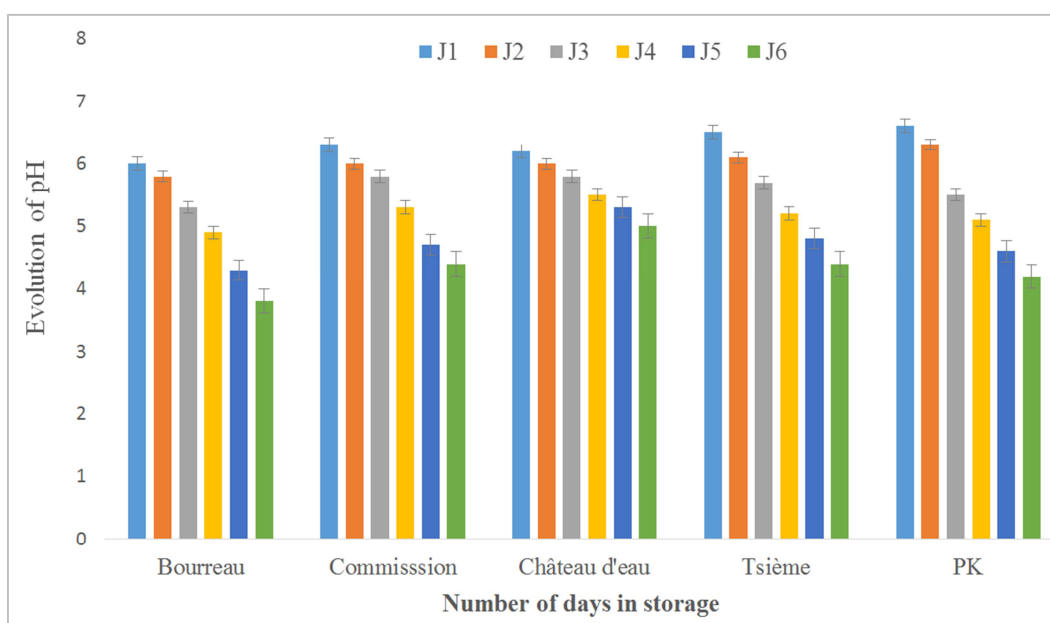


Figure 2. Evolution of pH as a function of the number of days in storage.

3.2. Microbiological Analysis

3.2.1. Isolation and Identification

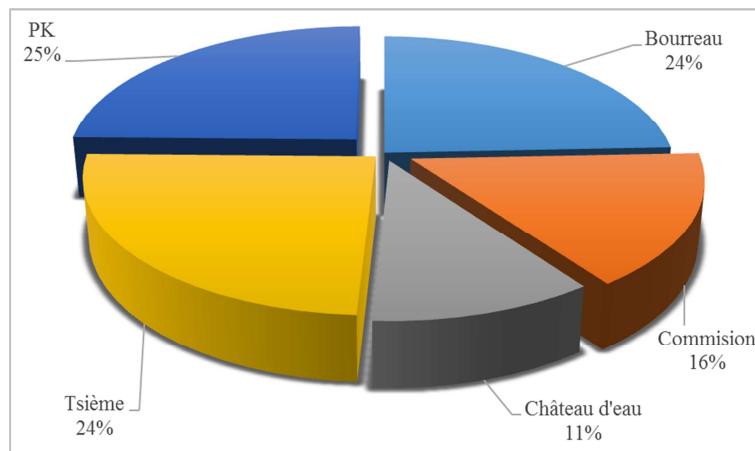


Figure 3. Level of contamination by deposits.

The different microorganisms isolated and identified on the basis of cultural, morphological and biochemical characteristics on the different chikwangue packaging sheets are as follows *Salmonella* spp, *Shigella* spp, *Escherichia coli*, *Bacillus cereus*, *Bacillus* spp, *Clostridium perfringens*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus* spp, *Streptococcus* spp, *Candida albicans* and *Lactobacillus* spp. Figure 3 shows the percentages of mesophilic flora isolated in each market. It shows a dominance of the PK market (25%), while the Bourreau and Tsiémé markets have an average of 24%. The Commission

market has an average of 16% compared to the Château d'eau market which has a very low average of 11%.

3.2.2. Enumeration

The average microbial load of the cassava wrapping sheets kept for six days in the depot, gave the microbial profile represented by figure 4. This figure shows that *Bacillus* spp. were predominant in the samples purchased at the PK market. *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Shigella* spp, *Lactobacillus* and *candida albicans* were isolated in all samples.

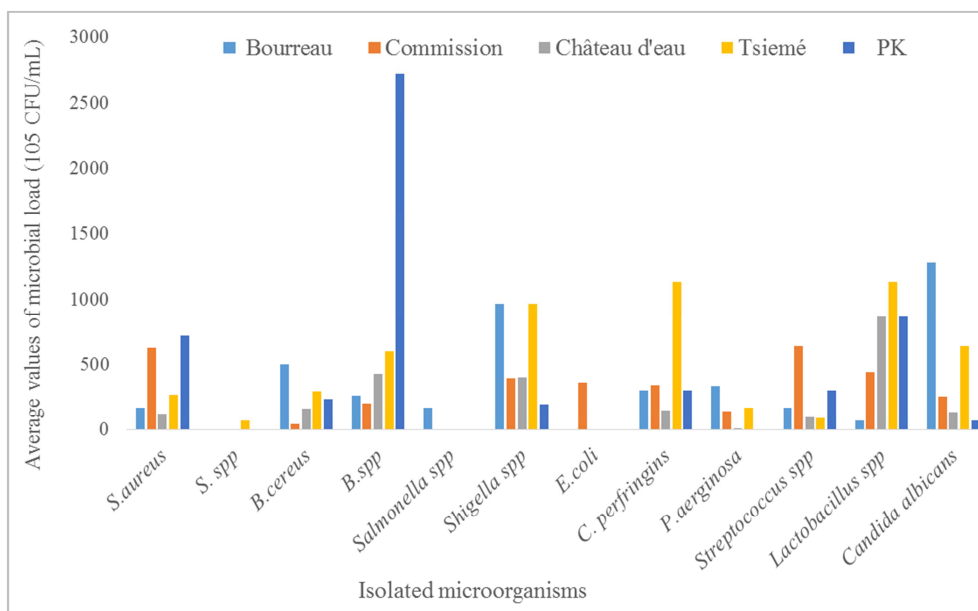


Figure 4. Variation in microbial flora by site.

Evolution of the Microbial Flora over Time by Market

a. The Bourreau Market

Figure 5 shows the evolution of microorganisms as a function of the number of days. *Staphylococcus aureus*,

Bacillus cereus, *Lactobacillus* spp. and *Streptococcus* spp. were present prior to deposition until day six, *Staphylococcus* spp. were mostly isolated on day one prior to deposition and then decreased until it disappeared on day four. *Clostridium*

perfringens microorganisms were absent before plating and appeared only after the first day of cassava storage and evolved until day six. For *Bacillus* spp, the microbial load was highest before plating and on the first day of plating, then decreased on the third day and increased normally from

the fourth day until the sixth day. For *Shigella* spp, they appear from the third day and evolve until the sixth day, whereas *Salmonella* spp do not appear until the sixth day. *E. coli* are completely absent. *Candida albicans* evolve progressively from day two to day six after deposition.

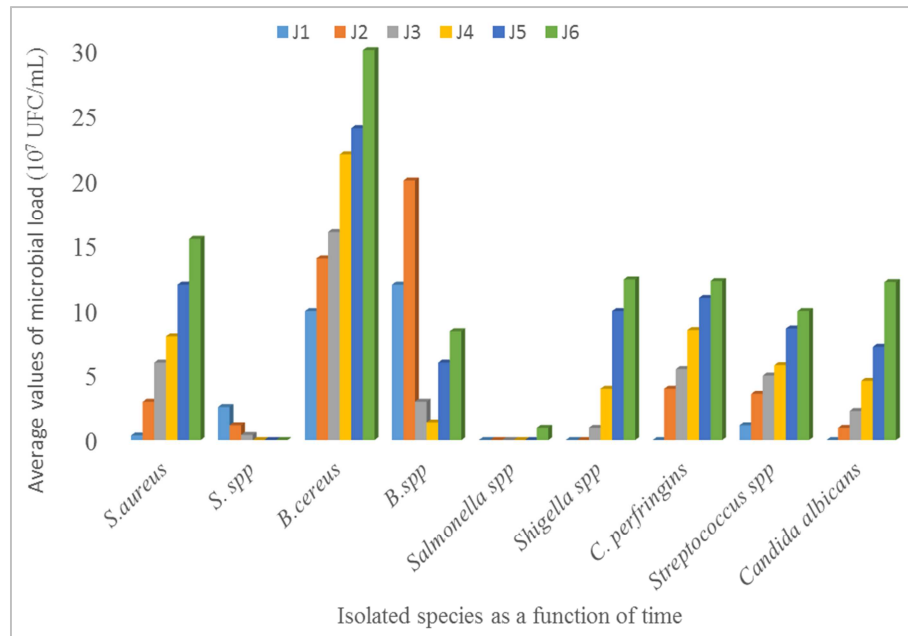


Figure 5. Evolution of the microbial flora as a function of the number of days of storage at the Bourreau market.

b. Market Commission

Figure 6 shows the evolution of microorganisms in relation to the number of days. *Staphylococcus aureus* only appear after the deposition, i.e. from the second day until the sixth day, their loads are very low. *Streptococcus* spp. exist even before deposition. These loads evolve until the sixth day when they become more important, especially for the last two days. *Staphylococcus* spp. are completely absent. *Bacillus cereus*, *Bacillus* spp. and *Lactobacillus* spp. are present from

the first day to the sixth day with very low loads. *Clostridium perfringens* does not appear until day five with a very high value on day six and *Pseudomonas aeruginosa* does not appear until after plating on day six. *Shigella* spp appear from day three to day six and *Escherichia coli* appear from day five but the microbial loads are higher on day six. *Candida albicans* in relation to the number of days with a progressive increase per day from day three to day six where the microbial load is higher.

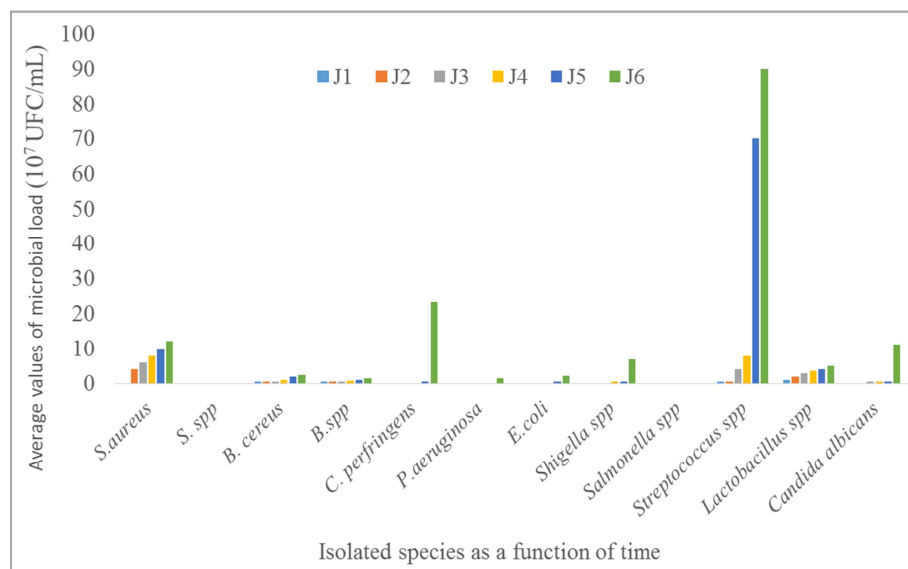


Figure 6. Evolution of the microbial flora as a function of the number of days of storage at the Commission market.

c. Château d'eau Market

The evolution of microorganisms per day isolated at the water tower is shown in Figure 7. *Staphylococcus aureus* and *Streptococcus* spp. are present from before deposition until the sixth day with very high loads on the sixth day. On the other hand, the microbial load of *Staphylococcus* spp. is more important on the first day before plating and then decreases until it disappears on the fourth day. *Bacillus cereus*, *Bacillus* spp. and *Lactobacillus* spp. are present before plating and

evolve until the sixth day. *Clostridium perfringens* only appear after plating from day four but the load value is very high on day six and *Pseudomonas aeruginosa* only appear on day six. *Shigella* spp. appear from day five with a higher microbial load on day six. *Salmonella* spp and *E. coli* are completely absent. The evolution of *Candida albicans* in relation to the number of days shows a progressive increase. It is on day four that they appear with a higher microbial load on day six.

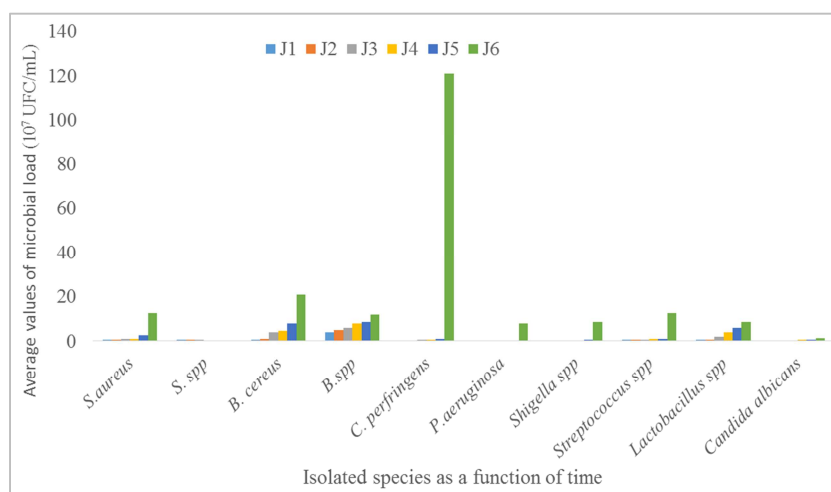


Figure 7. Evolution of the microbial flora as a function of the number of days of storage at the Château d'eau market.

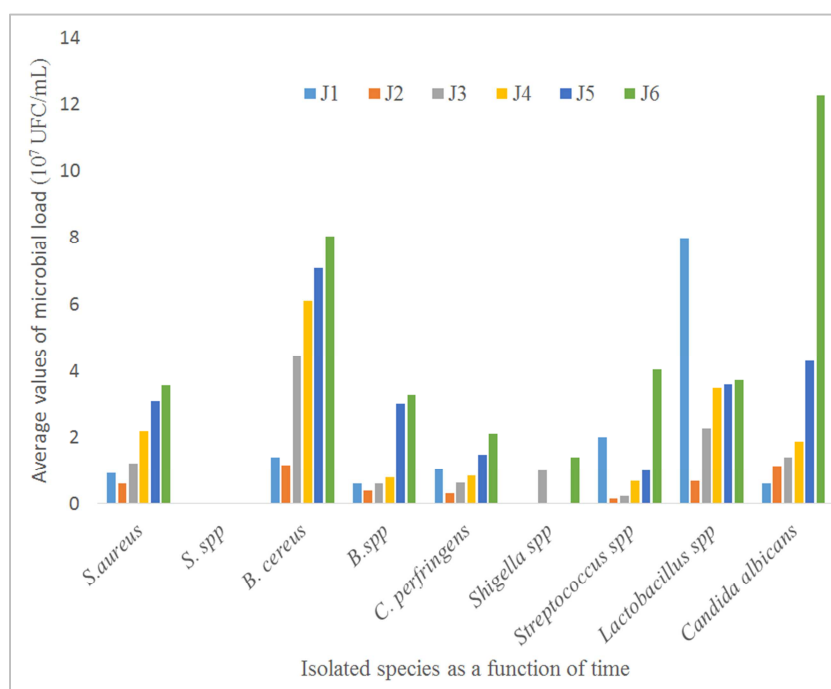


Figure 8. Evolution of the microbial flora as a function of the number of days of storage at the Tsiémé market.

d. Tsiémé Market

Figure 8 shows the evolution of the microorganisms isolated in relation to the number of days. *Staphylococcus aureus* and *Streptococcus* spp. are present before deposition, i.e. on the first day, the loads are high compared to the

second day when they decrease, and increase until the sixth day. As for *Streptococcus* spp, their load is higher on the sixth day, and *Staphylococcus* spp. are completely absent. *Bacillus cereus*, *Bacillus* spp, *Clostridium perfringens* and *Lactobacillus* spp are present before the deposit until the

sixth day, but we note a decrease of the loads on the second and from the third day onwards, the evolution is progressive. The load is very high, especially for *Lactobacillus* spp on the first day. *Pseudomonas aeruginosa* was completely absent until day six. *Shigella* spp. only appear on the third and sixth day with a very remarkable absence between the fourth and fifth day. *Salmonella* spp and *E. coli* are completely absent. *Candida albicans* in relation to the number of days shows a progressive increase per day. This occurs before deposition until the sixth day after deposition.

e. PK Market

Figure 9 shows the evolution of microorganisms as a function of the number of days. *Staphylococcus aureus* and *Streptococcus* spp. are present before deposition until day six, but their microbial load is very high. This increase continues until day 6, especially for *Streptococcus* spp. *Bacillus cereus*, *Bacillus* spp, *Clostridium perfringens* and *Lactobacillus* spp. are present before plating until day 6, but the microbial load of *Clostridium perfringens* and *Bacillus* spp. is very high on day 6. *Pseudomonas aeruginosa* is completely absent. *Shigella* spp. appear from day four to day six, but their microbial load is higher on day six. *Salmonella* spp and *E. coli* were completely absent. *Candida albicans* in relation to the number of days with a progressive increase per day. This evolution takes place from the fifth day after deposition, but the microbial load on the sixth day is very high.

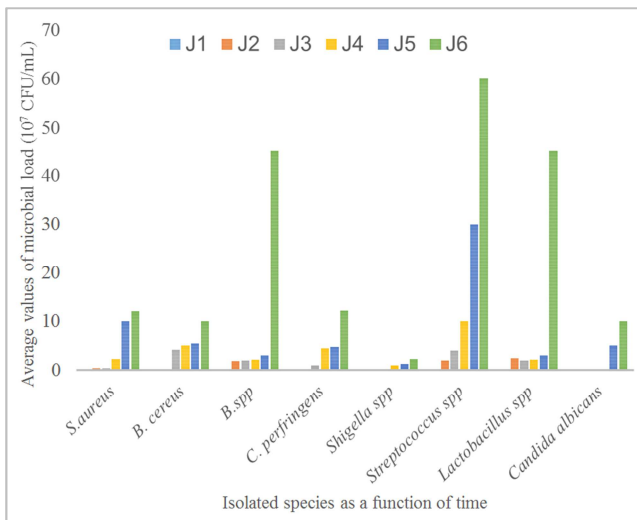


Figure 9. Evolution of the microbial flora as a function of the number of days of storage at the PK market.

4. Discussion

The physicochemical study showed that in all deposits the physicochemical parameters varied in the same order of magnitude. We note a high moisture content and the pH values are acidic but close to neutral and decreasing. These humidity levels favour the growth of psychrophilic microorganisms. The results on moisture content are comparable to those obtained by Clotaire et al. [6] and Bowen et al. [7] or on cassava by Mukandila et al. [8].

The pH values are close to neutral and then decrease,

favouring the growth of microorganisms that tolerate these pH values (Figure 2). These results confirmed those of the acidic pH bread of cassava or chikwangu according to the work of Mukandila et al. [8]; as well as those of Jeantel et al. [9] who prove the presence of lactic and acetic acid produced by lactic bacteria report that the pH range between 0 and 5.5 is favourable for the development of acidophilic microorganisms. Therefore, the pH level observed on the chikwangu favours contamination by this group of microorganisms. Similarly, lactic acid bacteria normally develop on a product with a pH of 4 [10].

Regarding the microbiological study, the results showed that in all the deposits, there is a proliferation of microorganisms in the packaging sheets. Species disappear and others appear as the duration of storage increases. Some bacterial strains are present on the chikwangs before the samples are deposited, but in very low proportions. This is the case for the majority of the microorganisms investigated. On the other hand, others occur after having been kept for a few days in the deposits, such as *enterobacteria*, *Pseudomonas aeruginosa* and *Clostridium perfringens*. The use of soiled equipment, unhygienic working conditions during handling (dirty hands, unhygienic premises, etc.), and poorly adapted storage conditions (damp premises, excessive clutter, poor stock rotation, etc.) may explain the large number of samples heavily contaminated with spoilage germs, hygiene indicator germs and pathogens.

The microbiological loads are very high in all the markets studied. This indicates a strongly engaged microbial spoilage process, especially from the third day onwards, as shown by the work of Bourgeois and Leveau in [11]. This is justified, not only because fermented products contain numerous microorganisms responsible for fermentation such as *Lactobacillus* spp. and *Streptococcus* spp [12]. *Lactobacillus* owe their presence to lactose, which is the basic substance for nutrition and growth. Since cassava is lactose-free, after the hydrolysis of the starch by *Bacillus*, colonisation by these microorganisms is inevitable. Moreover, the environment is always favourable to their presence due to the pH which becomes acidic thanks to their action. Indeed, *Lactobacillus* tolerate very well a very acid pH with a mesophilic temperature of the germs, thus making it easy to understand their preponderance in the cassava ferment [13]; but also because the storage process is conducive to the maintenance of such a microbial load (room packaging bag, transport conditions, storage environment in the warehouses, number of days and temperature conditions), even the high humidity rate (Figure 1).

The presence of these germs indicates contamination of products by *enterobacteria* from the third day onwards, depending on the market, thus confirming the results of Regez and Schmidt in [23]; indeed, their presence is synonymous with poor hygienic quality of the chikwangu during storage, and personal hygiene could be the cause.

As far as the presence of aerobic mesophilic flora is concerned, the result obtained in the present work confirms that obtained by Djoulde et al. [14] in Cameroon. Djoulde et

al [14] report that different post processing operations such as packaging would explain the bacterial contamination. The leaves of *Megaphrynium macrostachyum*, *Haumania liebrechtsiana* and *Lisimorpha senegalensis* used for packaging chikwangué could be associated with contamination by mesophilic aerobic bacteria. According to Oyewe et al [15], the microbial flora on cassava products consists mainly of lactic acid bacteria and has been identified during the fermentation of cassava for the production of gari [16]. Thus, the aerobic mesophilic flora found on cassava products in this work would mainly consist of lactic acid bacteria.

Some of these bacteria are the cause of infectious diseases and collective food poisoning (CFP). Currently, food-borne diseases are a public health problem and represent an important cause of mortality in developing countries [17]. Several germs are responsible, such as those of the genus *Staphylococcus* and specifically *Staphylococcus aureus* and even *Bacillus cereus*, which are among the three bacteria most often implicated in 90% of cases of collective food poisoning [17]. The pathogenicity of germs in the case of CFTIs is manifested either by a high load or by the release of toxins. For example, *C. perfringens* causes many severe diseases in animals, and was identified as the agent responsible for 2.9% of foodborne illnesses in 2001 in France [18]; *Staphylococcus aureus* is responsible for intoxication by ingestion of an enterotoxin [21]. This toxin is destroyed by heat (above 60°C) or cold (below 7°C), its infectious dose is very low, of the order of 1 ng [22]. In 2001, *Staphylococcus aureus* was the cause of 15.8% of foodborne illnesses in France [18]; *B. cereus* is one of the four most important causes of common food poisoning in France [19]. The Ministry of Agriculture, Food and Forestry gives the following definition of CFTI: it is a collective food poisoning defined by the occurrence of at least two similar clustered cases of a symptomatology, usually gastrointestinal. *Bacillus cereus* can cause either vomiting or diarrhoeal symptoms through two types of toxins: cereulide and enteric toxins respectively [20]. The results show that in all markets, the microbiological profile of the packaging exists prior to storage. They show that the level of microorganisms is very low and that there is an absence of other desired microorganisms such as *enterobacteria* and *Pseudomonas*.

Staphylococcus aureus is present in all markets prior to deposition and has a very low load in the PK market compared to the other markets; the load in the PK market is 12.10^7 CFU/80mL (Figure 9), these loads evolve to reach very high values with the Bourreau market dominating. This market has a load of $15.48.10^7$ CFU/80mL (Figure 5) compared to the other markets. The Château d'eau depot has the lowest loads (Figure 7).

This study allowed us to observe that the quality, quantity and even the ease with which cassava packaging is removed greatly influence the contamination and microbiological proliferation of chikwangué. The cassava that is best preserved in the depots is the Fabriques of the Château d'eau depot (11%), the Commission mix (16%), as well as the

Ngudi yaka of Bourreau and the Mungwélé of the north of the Tsiémé. These cassava have proportions of 14%. The large KP manufacturers show proportions of 25% (Figure 3). In general, all the microorganisms that make up our analysis are present in the cassava except for a few that are not present at the cut-off date, i.e., on the seventh day when the chikwangué can still be consumed because the texture and taste are acceptable, especially if the various packaging materials have not yet been removed; this is the case for the packaging materials of the Fabriqués du Château d'eau, which are still in perfect condition until the seventh day.

5. Conclusion

The public has the right to expect that the food they consume is safe and fit for consumption. The objective of this work was to assess the level of contamination of chikwangué by microbiological analysis before and during storage. These microbiological analyses showed that the chikwangué packaging sheets are highly contaminated with a variety of pathogenic microorganisms such as: *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*; *Salmonella*, *Shigella*, *E. coli* and *Candida albicans* with the food-to-packaging exchange ratio, which also leads to the contamination of the chikwangué. The current situation in the manufacture, transport and marketing of products in the markets allows us to highlight the importance of studying the bacteriological quality of chikwangué. This is why the hygienic quality of chikwangué should be monitored and improved in order to protect consumers against food poisoning. Standards are necessary even if they are far from being applied in Congo.

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