



Biopreservation of Home Eggplants, Potatoes and Chikwangue Using Biosurfactants Produced by Bacteria of the Genus *Bacillus*

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Abstract

Thousands of informations on food preservation field have been at the crossroads of scientific orientations in the agri-food industry. This study aims to increase knowledge of proper food preservation practices by studying the role of biosurfactants extracted from *Bacillus* spp isolated from traditional fermented food in Republic of Congo. Our study has shown that biosurfactants are secreted in the extracellular medium with EI24 ranging from 80% to 100%. The antibacterial and antifungal properties of a biosurfactants mixture of *Bacillus subtilis* B17, *B. pumilus* B26, *B. amyloliquefasciens* B18 were effective with diameters ranging from 1.5 ± 0.1 to 3.5 ± 0.2 . Furthermore, the coating of the biosurfactant consortium on the surface of home chikwangue (fermented cassava), potatoes and eggplants allowed an extension of their shelf life while preventing their freshness and microbiological quality for 4 weeks at 25 °C and 37°C. Even when home chikwangue, eggplants and potatoes were likely contaminated with pathogenic microorganisms including *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhirium*, *B. cereus*, *Staphylococcus aureus*, *Shigella flexenri* and *Aspergillus* sp., the biosurfactant mixture showed 100% efficacy. The biosurfactants mixture produced by *Bacillus* could be the subject of industrial application in the Republic of Congo in the preservation and processing of food.

Keywords: Biopreservation; Biosurfactants; *Bacillus* spp; Eggplants; Chikwangue; Potatoes

Introduction

Food is one of the basic necessities of life because food contains nutrients, substances necessary for growth, maintenance of body tissues, and regulation of vital processes [1]. Nutrients provide the energy our body needs to function. However, it is important to note that food is an extremely perishable commodity [2]. In fact, it carries many bacterial species, yeasts, or molds that, by developing during preservation and storage, can alter it [3-5]. The degradation of food produced for human consumption represents a great danger to the human population due to the increase in population [6].

International organisations such as the FAO and WHO [7], which indicate that 30 to 50% of global food production would be lost between the field and the consumer's plate [6]. This is responsible for significant economic losses, as well as famine and malnutrition problems encountered in developing countries. Ensuring food safety and reducing losses related to food production remain a major challenge for the agri-food sector. Therefore, food preservation helps prevent food deterioration after harvest. Today, biological preservatives are preferred over chemical preservatives considered toxic [2,8-13]. Among recently reported biological preservatives, biosurfactants of bacteria of the genus *Bacillus* are

prominent [14,15]. Biosurfactants are low-toxicity antimicrobial, antioxidant, and biodegradable agents that are used in food preservation [16,17]. This project is part of biopreservation and food safety. LABs have been extensively studied and used in a variety of industrial and food fermentations [5,18,19].

Many studies have been carried out using LAB and bacteriocins in the context of food biopreservation [5,19,20]. Pathogenic bacteria and fungal contaminations and related diseases in local food are an urgent problem to solve in developing countries, including the Republic of Congo [21,22]. The fecal indicator bacteria *E. coli*, *enterococci* (ENT), and Bacteroidales have been found on the surfaces of eggplants [18].

Biosurfactants of *B. subtilis* are amphiphilic metabolites with pronounced surface activity with a wide range of chemical structures (such as glycolipids, lipopeptides, polysaccharide-protein complexes, phospholipids, fatty acids, and neutral lipids) with several advantages over chemical surfactants, i.e. low toxicity, biodegradable, and effective at different temperature and pH [23,24].

Many microbial species can cause food spoilage or even food-borne disease. Perishable foods are often attacked by different microorganisms. Among the microorganisms involved in food contamination and spoilage are bacteria; *B. cereus*, *Clostridium botulinum*, *E. coli*, *Pseudomonas* spp., *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp. and *S. aureus* [25-28]. Similarly, molds such as *Aspergillus* spp. are involved in food contamination and spoilage [29,30].

The Republic of Congo is a country with interesting agronomic potential. More than 60% of the agricultural products consumed in Brazzaville households come from agrifood products, in particular vegetables, tubers and fruits. These foods contribute 50% to the food security of the city's population. Among the foods consumed and grown in the Republic of Congo are fermented foods, including cassava (chikwangue), and vegetables such as eggplants, and potatoes. However, these foodstuffs, after harvest or production, are subject to deterioration due to the activity of microorganisms following the lack or insufficiency of appropriate processing and preservation technologies. Food deterioration is therefore a recurring national problem with the consequences of increasing undernourishment, loss of economic opportunities, and the emergence of microbial food poisoning. Several studies have been conducted

in the Republic of the Congo on the preservation of foodstuffs. However, the latter uses traditional techniques such as fermentation, pasteurization, pickling and candied fruit.

The objective of this paper is to study the biotechnological potential of *Bacillus* spp. To contribute to solving this problem, two questions should be asked whether biosurfactants produced from *Bacillus* can prevent home food contamination and extend their shelf life. We propose to evaluate the biopreservative potential of *Bacillus* spp. biosurfactants on three home foods (Chikwangue, potato, and eggplant).

Methods

Biological material

The plant material used in this study consisted of African eggplants (green), potatoes, and chikwangue (cooked cassava) collected locally. The biological material used in this study consisted of three strains of *Bacillus* previously isolated during previous studies in our laboratory *B. subtilis* B17, *B. pumilus* B26, and *B. amyloliquenifasciens* B18 [21]. The reference pathogenic strains had been isolated from the hospital and were used to test antagonistic effects. This includes *S. typhimurium*, *E. coli*, *B. cereus*, *Ps. aeruginosa*, *P. mirabilis*, *S. aureus*, and *Aspergillus* sp.

Biosurfactant production assay and antagonistic effect

To assess the biosurfactant production capacity of *B. subtilis* B17, *B. pumilus* B26, and *B. amyloliquenifasciens* B18, we used the method previously described for enterobacterial strains. The extraction from the *Bacillus* strain was performed following the method described by Kinouani Kinavouidi, *et al.* [31]. In summary, after an overnight culture, the supernatant was centrifuged at 11,000 rpm for 11 minutes and then acidified to pH 2.0 with concentrated HCl. The resulting precipitate was left at 4 ° C overnight and then again centrifuged to obtain pellets. These pellets were dissolved in PBS and tested for their ability to emulsify hydrocarbons. Biosurfactant precipitates from the three *Bacillus* species were mixed together to achieve greater efficiency. The antibacterial activity of the Biosurfactant extract mix was assessed against pathogenic strains (*Ps. aeruginosa*, *S. aureus*, *E. coli*, *S. typhimurium*, *S. flexneri* 5a M90T, *B. cereus*, and *Aspergillus* sp.) according to the method described by Bokamba Moukala, *et al.* [32].

Potato biopreservation assay using biosurfactant extracts

In order to extend the shelf life of foods, we have established a consortium of biosurfactant extracts from (3) strains of *Bacillus*; B.

subtilis (B17), *B. pumilus* (B26), and *B. amyloliquefasciens* (B18). This consortium, whose inhibitory activity has been tested, was tested in this report on 3 foods consumed in the Republic of Congo, including Potato, African Eggplants, and Chikwangu (cooked cassava).

To highlight the ability to increase the shelf life of foods, we first sterilized the jars (containers) in which the food should be placed. The food samples were treated differently and aseptically. Finally, left in ambient conditions 25 ° C and 37 ° C in the incubator.

The potatoes were carefully cleaned with clean water and disinfected with alcohol under the host. The aseptic potatoes were cut in half using a sterile kitchen knife. Before any treatment, the generated halves of the potatoes were subjected to the measurement of their fresh mass. Then each half of potato was placed in sterile jars after undergoing different treatments.

The biosurfactant consortium was placed and applied strategically on the excised surface

of the potato. In fact, a volume of 400 µL of the consortium was placed and applied specifically to the surface of the excised part of all the package. apples contained in the labelled and encoded containers. LOT1: Potato treated with the biosurfactant consortium, LOT2: Potato treated with the BS consortium in the presence of Molds (100 µL). LOT3: Potato that had not undergone any treatment, LOT4: Potato in the presence of molds (100µL) alone. The different batches obtained were incubated for 3 weeks at 25 ° C in the ambient environment and at 37 ° C in the incubator.

Eggplants biopreservation assay using biosurfactant extracts

In the case of eggplants, they were cleaned with clean water, pasteurised for 40 min at 65 ° C and placed in sterile jars. The samples obtained were subjected to mass measurement before conservation. The biosurfactant consortium was strategically placed and applied on the excised surface of the potato. Different volumes of the consortium were added to the pasteurised eggplants contained in the sterile jars. The following different treatments were carried out; LOT1: Eggplants added to physiological water (10 mL), LOT2: Eggplants added to the biosurfactant control (500 µL), LOT3: Eggplants added to salt (10 g), LOT4: Eggplants added to sugar (10 g), LOT5: Eggplants added to acetic acid (10 mL); LOT6: Eggplants added to the biosurfactant consortium (250 µL), LOT7: Eggplants added to the biosurfactant consortium (500µL), LOT8: Eggplants

added to the biosurfactant consortium (750µL), LOT9: Eggplants added to the biosurfactant consortium (1mL), LOT10: Eggplants added to the biosurfactant consortium (2 mL). The different batches obtained were incubated for 3 weeks at 25 ° C in an ambient environment and 37 ° C at room temperature. the incubator.

Chikwangu biopreservation assay using biosurfactant extracts

After removing these leaves, the cassava was cut into 40 cm³ pieces, weighed, and then placed in sterile jars under the host. The biosurfactant consortium was applied strategically on the excised surface of Chikwangu. In fact, a volume of 400 µL of the consortium was placed and applied specifically to the surface of the excised part of Chikwangu contained in the labelled containers. LOT1: Chikwangu treated with the BS consortium. LOT2: Chikwangu treated with the BS consortium in the presence of Mold (100 µL). LOT3: Chikwangu that had not undergone any treatment. LOT4: Chikwangu in the presence of mold (100 µL). The batches were incubated for 3 weeks at 25 ° C in an ambient environment and 37 ° C in the incubator.

Result

Production and extraction of biosurfactants

The strains of *B. subtilis* B17, *B. pumilus* B26, and *B. amyloliquefiedifasciens* B18 were tested for their ability to produce biosurfactants, by evaluating their ability to emulsify a type of hydrocarbon chosen in the context of this study, namely gasoline (Figure 1). Therefore, by this experimental procedure, we determined the emulsification index after 24 h (E24) using the total culture. Consequently, the three selected strains emulsified at values greater than 83.33% (Figure 1A, B). The three strains with an emulsification index <50 % were subjected to the biosurfactant extraction test by precipitation of hydrochloric acid (HCl). All isolates showed a precipitate at the bottom of the tube, as shown (Figure 1B).

The mixture of three strains tested, from which we extracted the biosurfactants, all showed inhibitory power against the different pathogenic bacteria and on the mold, isolates used in this study. In fact, all the mixture of biosurfactants inhibited the growth of the pathogens tested, including *S.aureus*, *Klebsiella* sp., *E. coli*, *Salmonella* sp. and *Shigella* sp, *B. cereus* and *Aspergillus* sp.. The presence of clear zones or halos around the site of deposition of the biosurfactant extract indicates positive inhibitory activity (+) after 24 h of incubation at 37 ° C (Figure 2A, B). *Staphylococcus aureus* showed

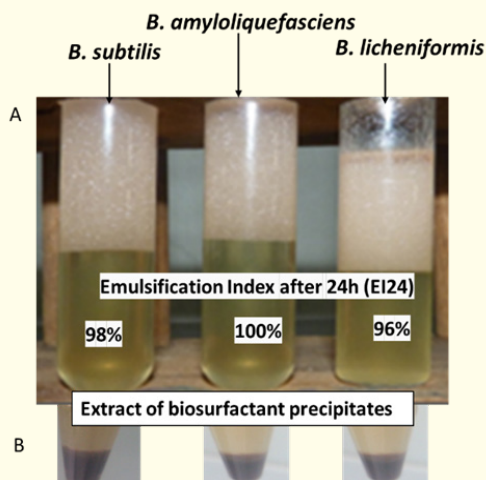


Figure 1: Biosurfactant production (A), Extraction of biosurfactants by hydrochloric acid precipitation (B).

better sensitivity to the biosurfactant mixture (from *B. subtilis* B17, *B. pumilus* B26, *B. amyloliquefasciens* B18) compared to the other strains tested (Figure 2). The diameters of the activities ranged

from 1 to 3.3 cm. Similarly, the biosurfactants tested showed interesting antifungal activity against the mold strains investigated (Figure 2).

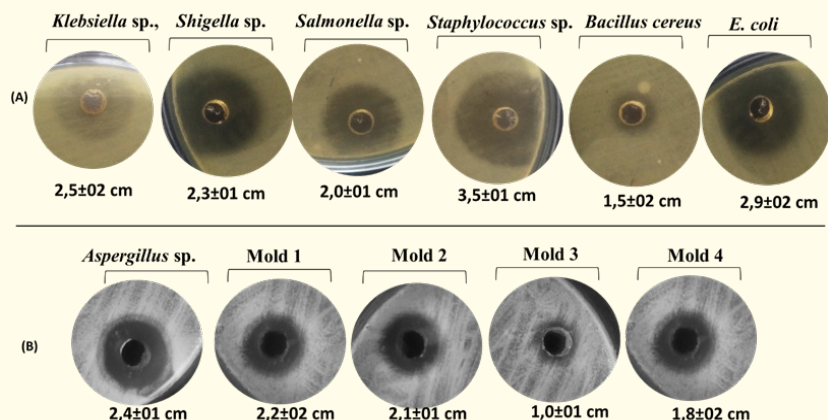


Figure 2: Antagonistic activities of biosurfactants extracted from *Bacillus* strains on pathogenic bacteria (A) and molds (B).

Biopreservation of food potatoes using the consortium

The results obtained during this study show that the presence of biosurfactants during potato storage slows the perishing and degradation of potatoes at temperatures of 25 °C and 37 °C. Similarly, biosurfactants were able to prevent potato contamination when the latter impregnated with biosurfactants is in contact with molds (*Aspergillus* sp.). In addition, the presence of biosurfactants slows down weight loss after storage compared to when the latter are not treated with biosurfactants. It is also interesting

to note that the presence of biosurfactant facilitates the germination of potatoes (Table 1 and Figure 3). At the end of the activities, a loss of water and a loss of mass ranging from 0.5 g to 20.5 g were observed. Indeed, some jars, notably “Potato + Mold” and “Simple Potato” at 37°C and 25°C showed a loss of mass greater than 10g. The table below illustrates the percentage of mass loss of the observed batches (Table 1). This difference is significant (P < 0.005) when potatoes are treated with the biosurfactant.

Macroscopic observations were made at the end of the activities to assess the visual, olfactory, and tactile quality of the products and a culture was also carried out in a box using the SS and PCA mediums at the end of the activity to better assess the mac-

rosopic quality of each jar (Table 1 and Figure 3). At the end of the biosurfactant treatment, the pH of each batch of potatoes was revealed and these results show that all batches of potatoes treated were neutral with values between 7.05 ± 0.10 and 7.84 ± 0.55 .

Table 1: Weight at the start and end of storage at 25 C and 37 C.

Mixtures	T(°C)	Weight before incubation (g)	Weight before incubation (g)	Observations and organoleptic characteristics
Potatoes	25	24.0 ± 0.5	16.0 ± 0.8	Significant loss of water, smell of urine, rot, presence of a white layer on the surface (white mold) and black (black mold) and presence of white dots around the potato, softening of the potato
Potatoes Mold	25	24.0 ± 0.5	15.0 ± 0.2	Smell of rot, Presence of mold on the part that was cut and on both sides of the potato, loss of water, softening
Potatoes Mold	37	39.0 ± 0.5	18.5 ± 0.5	Significant loss of water, presence of a black layer on the surface of the excised part, softening, smell of rot
Potatoes Biosurfactants	25	31.0 ± 0.5	27.5 ± 0.4	No softening, no water loss, no mold visible to the naked eye. No rotten smell, presence of powder on its surface
Potatoes Biosurfactants	37	25.5 ± 0.5	23.5 ± 0.7	No particular odor, no mold or other layer or film on its surface, no noticeable water loss
Potatoes Biosurfactants Mold	25	24.0 ± 0.5	23.5 ± 0.4	No water loss, no characteristic rotting odor, no presence of mold around the non-excised part, change of color in the excised part
Potatoes Biosurfactants Mold	37	35.5 ± 0.1	20.5 ± 0.3	No water loss, no characteristic rotting odor, no presence of mold around the non-excised part, change of color in the excised part

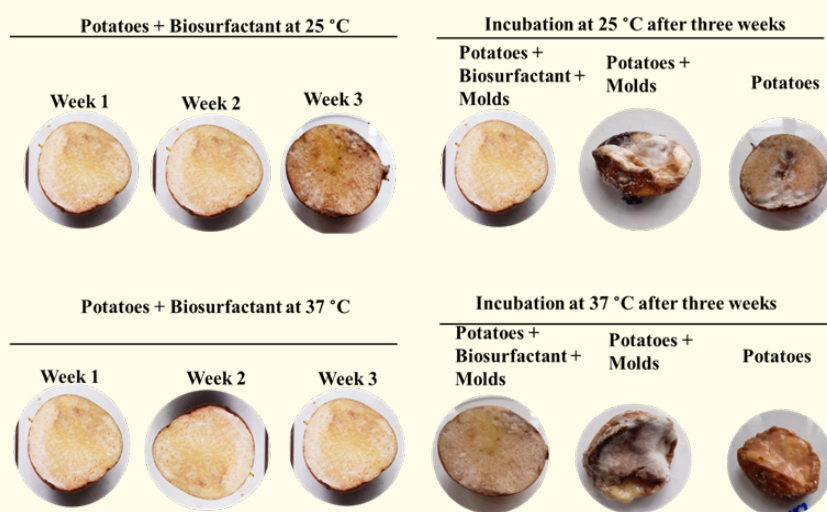


Figure 3: Macroscopic Observations of potato batches during storage.

Biopreservation of eggplants using the consortium

Treatment of eggplants with biosurfactants was done without the use of microbial suspensions. The weight of each portion of eggplants was taken at the beginning and end of the experiment. Table xxx below perfectly illustrates the weight of each reaction mixture in the jars of these latter from the beginning and end of the activities (Table 2).

The results obtained showed that the presence of biosurfactants during the conservation of eggplants slows the process of perishing, degradation, and browning of eggplants. Furthermore,

the presence of the biosurfactant inhibits the growth of epiphytic bacteria, thus preventing the colonization of the surface of eggplants by microorganism mold, normal appearance, no hardening (Table 2 and Figure 4). Additionally, the pH value of each batch was revealed to better judge the impact of the biosurfactant treatment on the food. The pH of the eggplants at the end of the treatment was between 3.84 and 6.99. In fact, the pH of all batches containing the biosurfactant was slightly acidic and approached pH = 7, neutral, and some of the controls had a pH of 3.84 ± 0.08 and 4.58 ± 0.50 very acidic. To illustrate the different pH values observed.

Table 2: Characterization of eggplants in the presence of biosurfactants.

Mixtures	T(°C)	Weight before incubation (g)	Weight before incubation (g)	Observations and organoleptic characteristics
Eggplants	25	53.5 ± 0.5	46.5 ± 0.5	Presence of mold + bacterial film, significant water loss, musty smell, softening
Eggplants + saline liquid, (10 mL)	25	45.0 ± 0.5	48.5 ± 0.7	Odour not characteristic of eggplants, presence of a large amount of bacterial film and mold; with softening
Eggplants + NaCl (10 g)	25	59.5 ± 0.5	53.5 ± 0.1	No softening, no bacterial film or mold, water loss, characteristic eggplant odor
Eggplants + Sucrose (10 g)	25	62.0 ± 0.2	44.5 ± 0.5	The presence of a small amount of bacterial film, large loss of water, softening
Eggplants + Acetic acid (10 ml)	25	74.0 ± 0.5	67.0 ± 0.5	Odeur acidulée, pas de ramollissement, pas de film bactérien ni moisissure
Eggplants + BS (250 µL)	25	62.0 ± 0.5	59.5 ± 0.6	Smell of rot, Presence of mold and bacteria accompanied by a slight loss of water
Eggplants Biosurfactants (500 µL)	25	59.5 ± 0.4	58.5 ± 0.5	Characteristic odor, slight softening, small loss of water, presence of bacterial film or mold.
Eggplants Biosurfactants (750 µL)	25	62.0 ± 0.5	58.0 ± 0.5	Water loss, presence of bacterial film, no softening, characteristic eggplant odor
Eggplants + Biosurfactants (1mL)	25	66.5 ± 0.2	64.5 ± 0.2	The presence of a bacterial film in smaller quantities to the naked eye, no softening, characteristic eggplant odor, loss of water
Eggplantss + Biosurfactants 2mL	25	56.5 ± 0.3	55.5 ± 0.2	Minimal water loss, characteristic eggplant odor, no growth of microorganisms

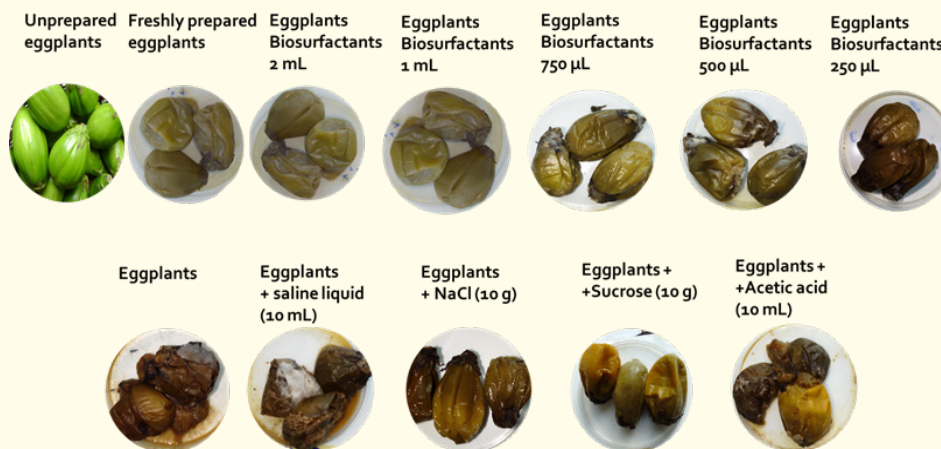


Figure 4: Macroscopic observations after 25 days of batches of eggplants tested in the presence of biosurfactants.

Chikwangue Biopreservation using the consortium

Chikwangue was treated using the biosurfactant consortium and a mold isolate including *Aspergillus* spp. Table 2 illustrates the variability of the weights of the different treatments carried out during storage (Table 3). The weight of each Chikwangue batch was between 18.5g and 31g (Table 3), the packaging temperatures were between 25 ° C and 37°C and unlike eggplants and potatoes, the mass losses were not significant. In fact, only the batches la-

belled “Simple Chikwangue 25 ° C» and “Simple Chikwangue 37 ° C» saw their mass increase by 1g and 0.5g, respectively. To better appreciate the quality of the effects of time on chikwangue and highlight the activity of the biosurfactant consortium, the visual, olfactory, microscopic quality and the appearance or texture of the sample were observed at the end of the activities (Figure 5). Hence, the importance of the table below. We also appreciate these macroscopic observations better. All the variations of the batches according to time, reagents used and the temperature (Figure 5).

Table 3: Characterisation of eggplants in the presence of biosurfactants.

Mixtures	T°C	Weight before incubation (g)	Weight before incubation (g)	Observations and organoleptic characteristics
Chikwangue	25	26.5 ± 0.1	25.5 ± 0.1	Normal colour and characteristic odor of cassava
Chikwangue	37	19.5 ± 0.8	20.5 ± 0.7	No mold, no bacterial contamination, normal texture
Chikwangue Molds	25	18.5 ± 0.4	18.5 ± 0.5	Presence of mold all over the surface, yellow, black and white, very strong odor
Chikwangue Molds	37	28.0 ± 0.5	28.0 ± 0.6	Presence of white and black mold on the entire surface of the cassava, odor not hig
Chikwangue Biosurfactants	25	21.0 ± 0.5	21.5 ± 0.5	No mold normal color
Chikwangue Biosurfactants	37	31.0 ± 0.4	31.0 ± 0.4	No mold, Maintains normal appearance, characteristic odor, no hardening
Chikwangue Biosurfactants Molds	25	21.0 ± 0.7	21.0 ± 0.6	No mold, normal appearance, no hardening
Chikwangue Biosurfactants Molds	37	27.0 ± 0.6	27 ± 0.5	No mold, normal appearance, no hardening

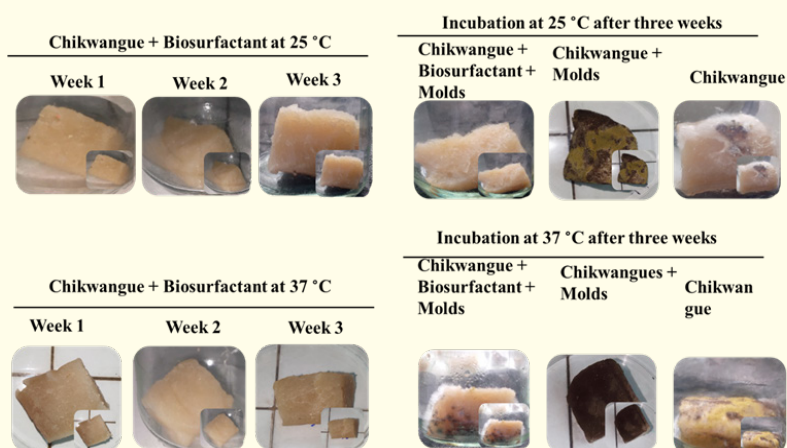


Figure 5: Macroscopic observations at the end of cassava treatment in the presence of surfactants.

In this same perspective, the pH of the batches was taken to better appreciate the action of the biosurfactant and the food. In fact, at the end of the treatment the pH of all batches was acidic, with pH values between 4.29 ± 0.19 and 5.93 ± 0.60 .

Discussion

In recent years, the use of biosurfactants has become fashionable [16]. It is quite obvious that chemical preservations will gradually disappear. Populations are becoming more and more demanding in the composition of their nutritional value. In addition to this, it is necessary to note the understanding of the link between the emergence of metabolic diseases, the emergence of cancer cases [33] and the nutritional quality of foods using preservatives. To reverse this trend, human health should remain at the heart of the attention of all scientists. Fermented foods are widely consumed in the Republic of the Congo [21]. Chikwangue is the most cited example of fermented food [34]. Eggplants are widely consumed [35]. Potatoes are especially grown in the northern part of the country. We are seeing more and more imported products such as potatoes arriving in the Republic of Congo. In state markets, there are many losses related to either the ripening of the eggplants or their rotting. This work is proving to be an added value at home.

This work has shown that *Bacillus*-isolated biosurfactants can extend the shelf life up to a month including protection against pathogenic microorganisms such as *Escherichia coli*, *Ps. aeruginosa*, *P. mirabilis*, *S. typhirium*, *B. cereus*, *S. aureus*, *S. flexenri*, and

Aspergillus sp. Many studies are interested in the biological benefits of biosurfactants [14,17]. The antibacterial properties of biosurfactants against selected Gramme-positive and -negative bacteria have been demonstrated [36].

With the effects of climate change. Preservation of food can help reduce food loss and waste while increasing long-term food security [37,38]. Climate change can negatively affect food storage, especially tuber sprouting and disease in storage chambers [39]. The valorization and protection of agro-resources has become practically a new world order, especially for developing countries. It is no longer necessary to destroy food [40,41]. In this work, we have shown that conservation can be easily tested at 25 °C and 37 °C. The fact that biopreservation is done at room temperature makes it easier to package food in supermarkets.

It is in this context that this work is particularly interesting because it offers easy indigenous solutions using indigenous bacterial species including *B. subtilis*, *B. licheniformis*, *B. amylolichefasciens* and home agrosources. Biosurfactants secreted by *B. subtilis*, *B. licheniformis*, and *B. amylolichefasciens* have been previously characterised. Some biosurfactants have been reported to have antimicrobial properties. In general, antagonistic *B. subtilis* showed significant resource value and offered a promising alternative in the development of food biopreservation [15,42,43]. The ability to prevent adhesion and disrupt biofilm formation preventing food spoilage. *B. subtilis* is widely not underappreciated for its inherent biosynthetic potential. The bioactive secondary metabolites of

B. subtilis highlight potential applications as control agents [44], drugs, and biosurfactants. *Bacillus* spp. are well documented for the production of cyclic lipopeptides that show strong biosurfactant and antimicrobial effects, such as surfactins, iturins, and fengycins [42,43,45-50]. Our indigenous *Bacillus* showed a significant emulsification power of up to 100%. The antagonistic capacity was very significant with diameters up to 3.4 cm. We have no claim to say that the problem of food preservation is entirely solved but this work gives new hope for knowledge on the protection of agro-resources.

Agriculture is an important issue for the Republic of Congo, employing 40% of the country's workforce and contributing 18.3% of non-oil GDP. However, it is characterised by low productivity and insufficient income for producers, and food insecurity affects 14.2% of households. The Republic of Congo remains dependent on food imports. This research on biopreservatives opens the doors and windows for intelligent industrialization because food will have a significant impact on the health of populations. Studies found that most food additives increase cancer risk [51-53]. Some of them may be associated with an increased risk of several major chronic diseases [54]. Therefore, some African countries have not yet integrated dietary guidelines. Research on indigenous curators will facilitate the development of these regulatory frameworks.

Conclusion

This study provides evidence that home food preservation may be beneficial in promoting vegetable intake and food security among the population. Natural resource management is crucial for the future of developing nations, including the Republic of the Congo. This inevitably involves the development of simple, biological, and inexpensive conservation methods using bacteria of the genus *Bacillus*. It is urgent to take advantage of the benefits offered by the microbial ecosystems encountered. This will necessarily involve the financing of multidisciplinary projects with the establishment of monitoring and evaluation mechanisms.

Authors Contributions

Conceptualization, D.N.N.M. and F.Y.O.; methodology D.N.N.M., F.Y.O. and S.N.M.; software F.Y.O.; validation, C.A.K.; formal analysis, S.N.M.; investigation, D.N.N.M. resources, G.A.D.; data curation, C.A.K.; writing—original draft preparation, D.N.N.M.; writing—review and editing, C.A.K.; visualization, C.A.K.; supervision, C.A.K.; project administration, C.A.K.; funding acquisition, C.A.K. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data supporting the findings of this study are available on request from the corresponding author, C.A.K.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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