

Assessment of the Physicochemical and Nutritional Parameters of Pineapple Fruits (*Ananas comosus* L.) and Post-harvest Bioconservation Test

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Abstract Pineapple (*Ananas comosus* (L)) is a monocotyledon, herbaceous, of the Bromeliad family. Côte d'Ivoire is the leading supplier of fresh pineapple to the European market. For reasons of sanitary quality and also because of the deterioration under the action of several factors of the marketable quality of the fruits, pineapple suffers a slump in the European market. To deal with the problem of fruit deterioration under the action of microorganisms, phytosanitary products are used. However, these foods present risks for consumers and may be responsible for public health problems. The objective of the present study is to reduce postharvest losses of pineapple fruit due to fungal contaminants using bacterial biopesticides such as *Bacillus subtilis* GA1, *Pseudomonas fluorescens* F19 and *Pseudomonas fluorescens* CI. Physicochemical analyzes were carried out on 200 samples composed of healthy pineapple fruits in order to determine the nutritional value of these fruits and to carry out conservation tests using the biomass and the supernatant of these 3 biopesticides. The physico-chemical analysis of the fresh pineapple fruits showed high humidity levels (83.23 to 85.5%), an acidic pH (3.79 to 3.88), levels of reducing sugars and total sugars of the order of 20.70 to 26.79 g / 100g and 59.42 to 62.32 g / 100g favourable to fungal growth. The conservation achieved made it possible to extend the shelf life of the fruits over fourteen (14) days for all the biopesticides against seven (7) days for the control. This study contributed to the development of biopesticides for post-harvest conservation of fruits in Côte d'Ivoire.

Keywords: pineapple fruit, physico-chemical, biopesticides, conservation, Côte d'Ivoire

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1. Introduction

Pineapple (*Ananas comosus* (L) M.) is a monocotyledon, herbaceous, of the Bromeliad family. It is the eleventh most cultivated fruit, with a global production of 25.8 million tonnes in 2016 [1]. This world production has grown steadily and increased by more than 8 million tonnes between 2000 and 2013. Pineapple cultivation is highly developed in the south-eastern part of the Côte d'Ivoire. It occupies an area of 16,000 ha and contributes 0.6% to the national GDP [2]. With 33,976 tonnes of fruit exported in 2014, Côte d'Ivoire ranks first among African exporters ahead of Ghana, which produces 33,175 tonnes [3]. Pineapple *comosus*, a species cultivated for its fruit, includes several varieties, eight of which are cultivated in Côte d'Ivoire. The best known are the "Smooth Cayenne", the "Queen" and the "MD2". These species are cultivated

by 2,500 small planters with an average farm area of 5 ha. They carry out 80% of the production and are affiliated for the most part to cooperatives for the grouping, packaging and transport of their production for export. In contrast, there are industrial-type farms owned by large fruit distribution groups around the world. This is the case of BCS (Banana Cultivation Society) belonging to the international group DOLE, practicing intensive production of fruits for export, with farms averaging 500 ha. Côte d'Ivoire, the leading supplier of fresh pineapple to the European Union with a coverage rate of 97% in the years 1986 [4], has experienced a sharp drop in production for several years. Pineapple production has declined steadily since 2001, dropping from 200,000 tonnes in 2001 to around 35,000 tonnes in 2012 [4]. This regression is due to several problems including non-compliance with the maximum residual limits for ethephon (chemical substance used for ripening) set at 2 mg / kg on pineapple [5] and post-harvest losses. Indeed, the quality of tropical fruits

such as pineapple is generally affected by post-harvest diseases such as fruit rot, which are mainly caused by improper handling and storage during transport and marketing [6]. About 20-25% of harvested fruits are spoiled by microorganisms during post-harvest handling, even in developed countries; this leads to a depreciation of the economic value of the fruits. Post-harvest losses represent about 40-50% of world production annually [3]. In order to have on the one hand, pineapple fruits of good sanitary quality and on the other hand, to preserve their taste quality while extending their shelf life, many proposals have been made. These include storage in a modified atmosphere, the use of antioxidants such as ascorbic acid, citric acid and calcium, and the use of firming agents [7]. However, despite these concerns, there is growing demand from consumers and suppliers to demand a reduction in the use of chemical pesticides. Although these chemicals are considered the most effective means of controlling pests unfortunately they have negative consequences [8,9]. On the one hand, at the level of the environment through the accumulation of residues and soil pollution and on the other hand, the appearance and generalization of the mechanisms of resistance in pathogens and ecological imbalance, due to the fact that these synthetic compounds have a wide spectrum of action. These chemicals destroy not only harmful agents, but also other populations in the ecosystem. In view of these harmful consequences, it is important to find alternative solutions which will make it possible to continue to fight against phytopathogens while reducing the use of chemicals. These may involve the rationalization of agricultural practices, the use of resistant plant varieties and / or the development of biopesticides [9]. There are plant, animal and bacterial biopesticides. Among the latter, there are microorganisms used in biological control. Pineapple is a highly perishable fruit because of its water and sugars content, favourable to development and to the spoilage actions of microorganisms such as yeasts and molds. In addition, failure to comply with good handling and transport practices promotes the action of these microorganisms through shocks and injuries. The use of chemical pesticides has drawbacks related to residues in food. These products, which are often misused, pose dangers to humans and the environment. A treatment widely used to slow down the inevitable senescence of fruits is the treatment of fruits in the cold, also called refrigeration [10]. However, this heat treatment, under certain conditions, causes internal browning of the pineapple [11]. In order to provide consumers with products of good microbiological and sanitary quality, biological preservation tests are carried out on the fruits healthy pineapples.

2. Material and Methods

2.1. Material

The study material consists of healthy pineapple fruits of the MD2 variety obtained directly on the markets of Yopougon, Abobo, Adjamé and Plateau and three bacterial biopesticides including *Bacillus subtilis* GA1

obtained from the collection of the Wallon Centre for Industrial Biology (WCIB-Belgium), *Pseudomonas fluorescens* F19 isolated from tomatoes from Algeria and *Pseudomonas fluorescens* CI isolated from tomatoes from Côte d'Ivoire.

2.2. Methods

Two hundred (200) samples of pineapple fruits taken directly from the markets of 4 municipalities in the city of Abidjan (Abobo, Adjamé, Plateau, Yopougon,) at a rate of 50 fruits per site. Pineapple fruits taken directly from the markets were collected during the period from June to December 2016, ie 6 months of sampling.

2.2.1. Physico-chemical Analyzes of Pineapple Fruits

- Humidity

The method used to determine the humidity level is that proposed by [12], the principle of which is based on the loss of mass of the sample in an oven at 105°C until a mass is obtained. constant. Thus, 5 g of healthy pineapple pulp are introduced into a glass capsule of known mass (m_0). The capsule containing the sample of total mass m_1 was placed in an oven (Memmert, Germany) which was set at $105 \pm 2^\circ\text{C}$ for a period of 24 hours. Subsequently, the capsule was removed from the oven and cooled in desiccators. After cooling, the whole (sample + capsule) was weighed and the mass is noted. The operation was then repeated every 2 hours until a constant mass (m_2) was obtained. The water content is determined by the following formulas:

$$\text{Humidity}(\%) = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100$$

For each sample, the test was repeated 3 times and the average of the three tests was retained.

- Ash content

The method used for the determination of ash is that described by [12] which consists of incinerating a sample until white ash is obtained. Five (5) g of pineapple pulp was placed in a clean, dry incineration capsule of known mass (m_0). The capsule containing the sample (total mass m_1) is placed in an automatic regulator muffle furnace (Pyrolabo, France) and then incinerated at $550 \pm 15^\circ\text{C}$ for a period of 12 hours. The capsule is then removed from the muffle furnace and cooled in a desiccator. The whole (sample and dish) was weighed after cooling in the desiccator; which corresponds to the mass m_2 . The ash content is expressed as a percentage by mass as follows:

$$\text{Ash}(\%) = \frac{(m_1 - m_2)}{(m_1 - m_0)} \times 100$$

The tests were carried out in triplicate and the average of the 3 tests was retained for each sample.

- pH and acidity

The pH and acidity were determined according to the [12]. To do this, 100 g of pineapple pulp (m_2) is used for juice extraction. The resulting extract was filtered through filter paper (Whatman) in an Erlenmeyer flask. The pH was read directly in the filtrate collected using a pH meter

(Hanna, Spain) previously calibrated with buffer solutions pH 4.00 and pH 7.00. Ten milliliters (10 mL) of the filtrate (V₀) were taken and introduced into an Erlenmeyer flask. This test sample, previously calibrated with 2 drops of phenolphthalein, is titrated with a NaOH solution (Sigma Aldrich, France) of 0.1 N normality until it turns pink. Let V_{eq} (mL) be the volume of NaOH added in equivalence. The acidity in meq / 100 g of sample is calculated using the following formula:

$$\text{Acidity}(\text{meq}/100\text{g}) = \frac{N \times V_{eq} \times 10^5}{m_e \times V_0}$$

The tests were carried out in triplicate and the average of the 3 tests was retained for each sample.

- Total sugars and reducing sugars

The determination of the total and reducing sugars was carried out after the extraction of the ethanolsoluble sugars. The ethanolsoluble sugars were extracted according to the method described by [13] as follows: 0.5 g of sample was dissolved and homogenized in 10 mL of ethanol (Fluka, France) (80%, v / v). To this mixture was added 2 mL of lead acetate (10%, w / v) and the whole was centrifuged at 3000 rpm for 10 min using a centrifuge (Hettich, Germany). Then the supernatant was taken up with 2 mL of oxalic acid (Sigma Bioblock Scientific, France) (10%, w / v) and centrifuged at 3000 rpm for 10 min. The combined supernatants were transferred to a 50 mL volumetric flask and the excess ethanol was evaporated in a sand bath for 10 min using a water bath (Heidolph, Germany). The resulting solution was then made up to 50 mL with distilled water and the whole corresponds to the extract of ethanolsoluble sugars.

• Determination of total sugars

The total sugars content was determined according to the phenol-sulfuric method as described by [14]. A volume of 100 µL of ethanol-soluble extract was taken and placed in a test tube. To this solution were successively added 0.9 mL of distilled water, 1 mL of phenol (Sigma Aldrich, France) (5%, w / v) and 5 mL of concentrated sulfuric acid (Sigma Aldrich, France). The tube was left to stand for 10 min. The optical density (OD) reading was taken at 490 nm against a blank using a spectrophotometer (Spectrometer T80, Germany). A calibration range from a 0.1 mg/mL glucose standard solution was carried out for the determination of the total sugars content. The tests were carried out in triplicate and the average of the three tests was retained.

• Determination of reducing sugars

The quantification of reducing sugars was carried out according to the method of [15]. To 1 mL of ethanol-soluble extract introduced into a test tube, were successively added 0.5 mL of distilled water and 0.5 mL of dinitrosalicylic acid (DNS) (Merck, France). The mixture was heated in a boiling water bath for 5 min and after cooling, 5 mL of distilled water was added. The optical density reading at 540 nm against white was thus carried out. A calibration range from a 0.1 mg / mL glucose standard solution allowed the quantification of reducing sugars. The tests were carried out in triplicate and the average of the three tests was retained.

- Total lipid

The lipid content was determined as described by [16], using the Soxhlet as an extractor. A mass of 10 g of pineapple pulp (**m_e**) was introduced into a previously tared cellulose extraction cartridge. The cartridge containing the sample was plugged with cotton and the whole was placed in the Soxhlet type extractor. Then, the total lipids were extracted with 300 mL of hexane at reflux for 7 hours, at the boil. The hexane was then evaporated using a rotary evaporator (Heidolph, Germany). The previously tared extraction flask (**m₀**) was dried in an oven (Memmert, Germany) at 100°C for 20 min. Subsequently, the whole (extraction flask + lipids) of mass **m₁** was weighed and the total lipid content was determined by the following formula:

$$\text{Lipid}(\%) = \frac{(m_1 - m_0) \times 100}{m_e}$$

The tests were carried out in triplicate and the average of the three tests was retained for each sample.

- Total protein

Total protein was determined from the determination of total nitrogen according to the Kjeldhal method [12]. It includes a mineralization phase, followed by a distillation phase and a sulfuric acid titration phase. A mass of 1 g of sample (**m_e**) is mineralized in a Kjeldahl flask (Bloc Digest 6, JP Selecta, Spain) at 400°C for 2 h with 20 mL of concentrated sulfuric acid in the presence of a pinch of mineralization catalyst (selenite + potassium sulphate). After cooling the tube to room temperature, the mineralate was transferred to a 100 mL flask and made up with distilled water. Subsequently, 10 mL of the collected mixture was added to 10 mL of 40% (w / v) NaOH solution and the whole is placed in the tank of the still. The extension of the still condenser was then immersed in a beaker containing 20 mL of boric acid supplemented with a mixed indicator (methyl red + bromocresol green). The distillation was carried out for 10 min. The resulting distillate was titrated with sulfuric acid solution (0.1N) until it turned from green to pink (**V₁**). A blank test (**V₀**) was carried out and the total protein content was determined by the following formula:

$$\text{Total Protein}(\%) = \frac{(V_1 - V_0) \times 14 \times 6,25 \times N}{m_e}$$

V₀: volume (mL) of sulfuric acid solution (0.1 N) poured in for the blank test.

V₁: volume (mL) of sulfuric acid solution (0.1 N) poured for the test (sample).

N: normality of the sulfuric acid solution (0.01 N).

m_e: mass (g) of the sample.

6.25: conversion factor of nitrogen to protein (FAO, 1978).

The tests were carried out in triplicate and the average of the three tests was retained for each sample.

- Crude fiber

The fiber content was determined according to the method of [17]. A quantity of 2 g of pineapple pulp (**m_e**) was introduced into a flask and homogenized with 50 mL of 0.25 N sulfuric acid. The whole was brought to the boil using a heating cap (JP Selecta, Spain) for 30 min under reflux condenser. Then, 50 mL of 0.31 N sodium hydroxide was added to the contents and the whole was

brought to the boil for 30 min under reflux condenser. The resulting extract was filtered through Whatman No. 00 filter paper and the residue was washed several times with hot water until the alkali was completely removed. After washing, the residue was dried in an oven (Mettler, Germany) at 105°C for 8 h and cooled in a desiccator and then weighed (m_1). The dry residue obtained was incinerated in a muffle furnace (Pyrolabo, France) at 550°C for 3 h and cooled in a desiccator and then weighed again (m_2). The calculation of crude fibers as a percentage of mass was expressed according to the following formula:

$$\text{Crude Fiber (\%)} = \frac{(m_1 - m_2) \times 100}{m_e}$$

All the tests were repeated 3 times and the average of the three tests was taken for each sample.

- Total carbohydrates and energy value

The total carbohydrates and the energy value were determined according to the calculation methods recommended by the [18]. These methods take into account, on the one hand, the moisture, fat, protein and ash contents and, on the other hand, the energy coefficients of [19].

Total Carbohydrates (%)

$$= 100 - [P(\%) + E(\%) + L(\%) + C(\%)]$$

Energy Value (kcal / 100g)

$$= [(4 \times P(\%)) + (9 \times L(\%)) + (4 \times G(\%))] + C(\%)$$

P: protein content (%).

E: water content (%).

L: lipid content (%).

G: carbohydrate content (%).

C: ash content (%).

2.2.2. Biomass and Supernatant Production of Bacterial Biopesticides

The production of the biomass and the supernatant of the bacterial biopesticides was used for carrying out the various fruit protection tests.

• Conduct of the pre-culture

250 ml Erlenmeyer flasks containing 65 ml of Yeast Peptone Glucose (YPG) medium were each inoculated with a 24 hour colony of the biopesticides. The pre-cultures were incubated at 30°C. with shaking (shaking water bath) at 105 rpm for 8 hours. These pre-cultures were used to inoculate 2L Erlenmeyer flasks containing 500 ml of YPG medium.

• Production and harvest

Two Erlenmeyer flasks each containing 500 ml of sterile YPG medium were each inoculated with 65 ml of the preculture. The Erlenmeyer flasks were incubated at 30°C with shaking at 105 rpm for 48 hours. The biomass was harvested after centrifugation of the medium at 3500 rpm for 10 minutes. The pellet, which represents the biomass, was separated from the supernatant and washed three times with physiological water (9 ‰ NaCl). The biomass and the supernatant were stored at 4°C for storage tests.

2.2.3. Conservation of Fruit

- Immersion technique

The fruit protection test was carried out by immersion as described by [20]. It consisted of immersing the fruits in a container containing the biopesticide supernatant. Make sure that the entire surface of the fruit is submerged. Thus, the ripe and ripe pineapple fruits were carefully washed in tap water and rinsed three times with sterile distilled water and disinfected by using household paper soaked in 70% ethanol then immersed for 5 minutes. in the biopesticide supernatant and air dried. The controls having undergone the same steps were not treated with the supernatant. The fruits were stored at room temperature (25°C) after immersion.

-Coating technique

The embedding method described by [20] was used. It consisted in applying the biomass of biopesticides to pineapple fruits. Thus, the healthy pineapple fruits were washed thoroughly with tap water and rinsed three times with sterile distilled water. The fruits were disinfected using household paper soaked in 70% ethanol. The fruits were then coated with the biomass of the biopesticides and dried in the open air. The controls having undergone the same steps were not treated with the biomass of the biopesticides. Pineapple fruits after processing were stored at room temperature (25°C).

2.3. Statistical Analyzes

Statistical analyzes were performed with STATISTICA version 7.1 software (StatSoft). An ANOVA study (analyses of variance) associated with the Newman-Keuls test made it possible to analyze the differences observed during the studies of phyco-chemical and nutritional analyzes. Probability values $p < 0.05$ were considered statistically different.

3. Results and Discussion

3.1. Results

3.1.1. Values of Humidity, pH and Acidity of Pineapple Fruits

The humidity, pH and acidity values of the pineapple samples are shown in Table 1. The average moisture content is 85%. However, the differences are not significant between the collection locations. The pH of the pineapple samples ranged from 3.79 to 3.88. The highest value (3.88) was observed in the samples from Yopougon, followed by those from Abobo, Adjame, and Plateau where the values were respectively 3.86, 3.80 and 3.79. The pH does not differ significantly depending on the place of collection. The titratable acidity values vary from 8.97 to 12.23 meq / 100 g of fruit. The highest value (12.23 meq / 100 g) was found in the samples taken at Yopougon, followed by those from Abobo and Adjame where the acidity was respectively 9.55 meq / 100 g and 9.42 meq / 100 g of pineapple fruit. A significant difference ($p < 0.05$) was noted between the acidity samples from the municipality of Yopougon and that of samples from other municipalities.

Table 1. Values of humidity, pH and acidity of pineapple fruit samples according to the municipalities

| Municipalities | Parameters | | |
|----------------|---------------------------|--------------------------|---------------------------|
| | Humidity (%) | pH | Acidity (meq/100g) |
| Abobo | 85,24 ± 0,82 ^a | 3,86 ± 0,00 ^a | 9,55 ± 0,45 ^a |
| Adjamé | 85,5 ± 0,37 ^a | 3,8 ± 0,01 ^a | 9,42 ± 2,00 ^a |
| Plateau | 84,57 ± 0,09 ^a | 3,79 ± 0,00 ^a | 8,97 ± 1,00 ^a |
| Yopougon | 83,23 ± 0,30 ^a | 3,88 ± 0,22 ^a | 12,23 ± 0,80 ^b |

Values bearing the same letter in the same column do not show a significant difference at the 5% level according to the Newman-Keuls test.

3.1.2. Ash, Fiber, Reducing Sugars and Total Sugars Content of Pineapple Fruit

The ash, crude fiber, total sugars and reducing sugars contents of the pineapple samples collected according to the municipalities were determined (Table 2). The ash contents have evolved from 0.20 to 0.28% in all the communes. No significant difference ($p > 0.05$) exists between the ash contents of the samples taken. The rates obtained for crude fibers vary from 3.24 to 3.49%. The most fiber-rich samples are those collected in Abobo (3.49%), followed by those from Yopougon (3.44%) and Plateau (3.31%). The rates do not show any statistically significant difference.

The total sugar content varied from 59.42 to 62.32 g / 100g of pineapple fruit regardless of the samples analyzed. The highest rate (62.32 g / 100g) was observed in the samples from Yopougon, followed by those from Plateau and Abobo where the total sugar level was 60.19 g / 100g and 60.04 respectively. g / 100g of pineapple fruit. Samples collected at Adjamé showed the lowest rates, an average of 59.42 g / 100g of pineapple fruit. However, the differences are not significant according to the municipalities.

The values obtained for the reducing sugars varied from 20.70 to 26.79 g / 100g of pineapple fruit. The highest level of reducing sugars (26.79 g / 100g) was obtained in the samples taken at Yopougon, followed by those from Plateau (25.58 g / 100g). Contrary to In these 2 towns, the samples obtained in Abobo were less rich in reducing sugars (22.64 g / 100g) as were those from Adjamé (20.70 g / 100g). The differences are not statistically significant.

Table 2. Ash, crude fiber, total sugars and reducing sugars contents of pineapple fruit samples according to the municipalities

| Municipalities | Parameters | | | |
|----------------|--------------------------|--------------------------|---------------------------|---------------------------|
| | Ash (%) | Fiber Crude(%) | Total sugars (g/100g) | Reducing Sugars (g/100g) |
| Abobo | 0,28 ± 0,01 ^a | 3,49 ± 0,17 ^a | 60,04 ± 0,16 ^a | 22,64 ± 0,30 ^a |
| Adjamé | 0,20 ± 0,07 ^a | 3,24 ± 0,28 ^a | 59,42 ± 0,28 ^a | 20,70 ± 0,35 ^a |
| Plateau | 0,22 ± 0,60 ^a | 3,31 ± 0,34 ^a | 60,19 ± 0,32 ^a | 25,58 ± 0,40 ^a |
| Yopougon | 0,26 ± 0,26 ^a | 3,44 ± 0,42 ^a | 62,32 ± 0,41 ^a | 26,79 ± 0,35 ^a |

Values bearing the same letter in the same column do not show a significant difference at the 5% level according to the Newman-Keuls test.

Table 3. Levels of lipids, total proteins, total carbohydrates and energy values of samples of pineapple fruit according to the municipalities

| Municipalities | Parameters | | | |
|----------------|------------|--------------------------|--------------------------|----------------------------|
| | Lipids (%) | Total Proteins (%) | Total carbohydrates (%) | Energy Values (kca/100g) |
| Abobo | 0,1 ± 0,0 | 6,52 ± 0,41 ^a | 7,86 ± 0,57 ^a | 58,42 ± 0,43 ^a |
| Adjamé | 0,1 ± 0,0 | 6,56 ± 0,54 ^a | 7,64 ± 0,41 ^a | 57,70 ± 0,58 ^a |
| Plateau | 0,1 ± 0,0 | 6,65 ± 0,40 ^a | 8,46 ± 0,34 ^a | 61,34 ± 0,35 ^b |
| Yopougon | 0,1 ± 0,0 | 6,69 ± 0,37 ^a | 9,72 ± 0,64 ^b | 66,54 ± 0,45 ^{bc} |

Values bearing the same letter in the same column do not show a significant difference at the 5% level according to the Newman-Keuls test.

3.1.3. Lipids, Proteins, Carbohydrates and energy Value of Pineapple Fruit

Table 3 shows the different contents of lipids, total proteins, total carbohydrates and energy values of the samples of the pineapple fruits studied. The lipid content of 0.1% was obtained in all the samples collected from the 4 municipalities investigated.

The values obtained for the proteins vary from 6.52% to 6.69%. The samples taken at Yopougon presented a rate of 6.69%, followed by samples collected at Plateau (6.65%) and Adjamé (6.56%). Abobo's samples showed rates of 6.52%. The different rates do not show any significant difference.

The highest carbohydrate content (9.72%) was obtained in samples taken at Yopougon, followed by those from Plateau (8.46%) and Abobo (7.86%). The samples collected in Adjamé contained less carbohydrate (7.64%). A significant difference ($P < 0.05$) was observed between the carbohydrate content of the samples taken in Yopougon and the other municipalities.

The different samples studied were characterized by energy values ranging from 57.70 to 66.54 kca / 100g of pineapple fruit. The highest energy value (66.54 kca / 100g) was observed in samples from Yopougon, followed by those from Plateau (61.34 kca / 100g). The least energetic samples (57.70 kca / 100g) were obtained in Adjamé. However, a significant difference ($p < 0.05$) exists between the energy values obtained in the samples from the municipalities investigated with the exception of Abobo and Adjamé.

3.1.4. Conservation of Pineapple Fruits Using Bacterial Biopesticides

The various conservation tests were carried out with the biomass and the supernatant of the biopesticides. After 14 days of treatment, the fruits showed spots on the outside without affecting the quality of the pulp while the untreated fruits from 7 days showed signs of deterioration which resulted in a loss of the pulp coloring of the pulp. The microbiological quality of the preserved fruits was evaluated by culturing the pulp on PDA medium. This test noted the absence of fungal strains (Figure 1, Figure 2, Figure 3 and Figure 4).



Figure 1. Untreated pineapple fruit (Control)

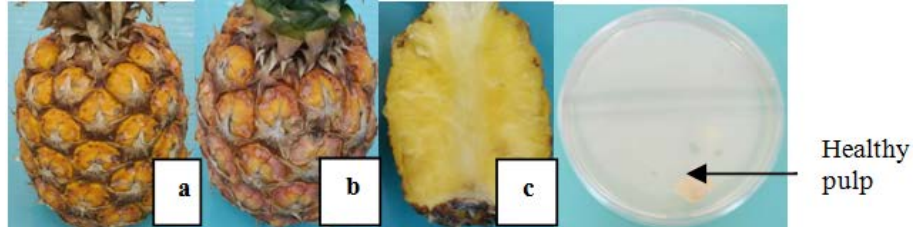


Figure 2. Pineapple fruits preserved with the supernatant (a) and biomass (b) of *Pseudomonas fluorescens* CI (internal view (c))

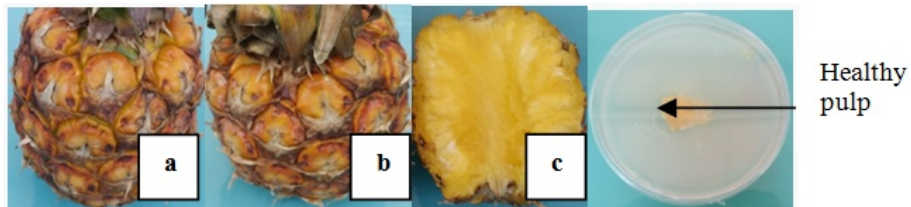


Figure 3. Pineapple fruits preserved with the supernatant (a) and biomass (b) of *Pseudomonas fluorescens* F19 (internal view (c))

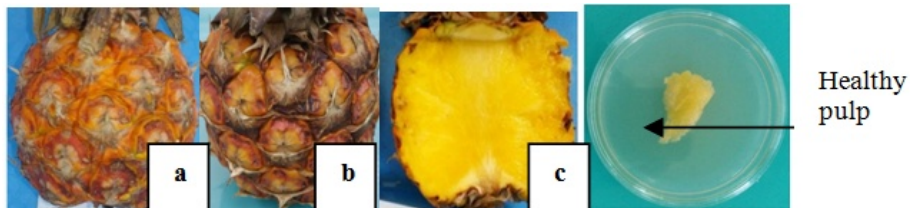


Figure 4. Pineapple fruits preserved with the supernatant (a) and biomass (b) of *Bacillus subtilis* GA1 (internal view (c))

3.2. Discussion

The physicochemical and nutritional characteristics of agricultural products are essential in preservation methods. The pineapple fruit samples studied showed relatively high humidity levels (83.23 to 85.24%). These results could be explained by the fact that most of the fruits are characterized by a much high water content. These moisture levels could also be explained by the intrinsic quality of the fruit. Indeed, according to [21], pineapple fruits being living organs have enough water. Also, it has been reported that all living organisms are mostly water. The values recorded in this study are generally in the same order as those obtained [21]. Water content is an important parameter in the preservation of foodstuffs [22] and, can have an effect on the shelf life of fruits by limiting the proliferation of microorganisms. According to [23], the high moisture content contributes to the survival and growth of moulds.

The pH values obtained (3.79 to 3.88) in the pineapple fruits studied are similar to those obtained by [24] (3.49 to 3.54) and by [25] (3.53 to 3.92) in pineapple fruit in Spain and Ivory Coast respectively. The results of this study fall within the pH range (3.2 to 4.0) of pineapple fruits and

most tropical fruits. According to the [26], the pH of pineapple varies between 3.20 and 4.00. The pH levels depend on the product, the variety and the growing conditions such as the nature and the pH of the soil. The pH values observed in this study were in the range (3.20 - 4.00). These values are positively correlated with acidity. The high acidity values of the samples collected and analyzed could be explained by the presence of several organic acids present in the fruits. Fruit acidity increases with maturity [27]. However, ripening is known to reduce acid levels in fruits [28]. During ripening, acids are involved in protein synthesis resulting in a decrease in acidity. The higher acidity can mean a longer shelf life and higher astringency [29].

The ash contents were lower in all the samples analyzed. However, the presence of ash could be explained by the richness of the samples of pineapple fruit studied in minerals, especially micronutrients, as indicated by [20] and [25]. Indeed, these authors reported that the ash content of pineapple fruits would be an indicator of their richness in mineral elements. The ash values obtained (0.20 to 0.28%) in this work are in the same range as those obtained in pineapple fruits (0.21 to 0.25%) by [25] in Côte d'Ivoire. Minerals are made up in part of calcium,

followed by potassium, magnesium, iron, sodium and zinc. The variation in mineral content observed in pineapple might depend on the type of soil where the plants were grown, the water used for irrigation and the fertilizer applied [30]. For example, molybdenum deficiency can cause high levels of nitrate in fruits, which leads to demineralization of pineapple [31,32].

The crude fiber contents obtained in this study could be due to the fact that pineapple fruits naturally have low amounts of crude fiber. Indeed, according to [33] and [21], fresh pineapple fruits have a fiber content of 1.4%. However, the results obtained in this study are higher. This could be due to either the genetic makeup, the climate and the variety of pineapple. The different values obtained in fiber would be an advantage for normal intestinal digestion, as the lack of fiber in the body leads to cases of constipation and intestinal diseases [34]. Fiber is important for the body. Indeed, large amounts of crude fiber are beneficial for their role in regulating intestinal transit, reducing cholesterol levels, constipation, diabetes, colon and breast cancer [35]. They also prevent the absorption of excess cholesterol [36].

The lipid contents of pineapple fruits studied are lower than those obtained in pineapple fruits (0.2%) by [33] and [21]. The values obtained in this work could be explained by the drying of the pulp of the pineapple, since the extraction of lipids by the Soxhlet requires prior drying. Indeed, [37] reported that the fat content is higher in fresh foods and appears to be lower in dried and roasted foods depending on the intensity of the heat. However, the presence in the samples of pineapple fruits studied in lipids could constitute an alternative for consumers insofar as lipids play an important nutritional role in the human diet in terms of fatty acid intake [38]. Also, [34] reported that the lipids in certain foods are important for the human diet because they facilitate the absorption of fat soluble vitamins.

The pineapple fruit samples studied showed protein contents higher than most of the data found in the literature (0.4 to 5%) [21,25,33]. These results could be due to the nature of the fruits (maturity, variety, etc.). Indeed, according to [39], fruits are very low in protein and have a low quantity. Pineapple, like many other fruits, is very low in protein but contains bromelain, a glycoprotein with protease activity commonly used in the food industry. The amount of bromelain is about half the protein found in pineapple. This protein is unevenly distributed [40], and it is higher at the top of the fruit and lower in the middle and at the base [41]. Also, [42] and [43] reported that the richness of foods in protein could come from varietal differences. The use of pineapple fruit in human food is beneficial. According [44,45], pineapple fruits are considered a good source of minerals and enzymes for improving the nutritional status of humans. The role played by proteins (Bromelain) from pineapple fruits, although in small quantities in the human diet has also been demonstrated by [46]. This author reported that the bromelain in pineapple enters the digestion process as a facilitator. Clinical tests have confirmed that bromelain effectively reduces inflammation in certain conditions such as acute sinus disease, arthritis, gout and accelerates recovery from injuries and surgeries. Moreover, According to [47], the available evidence indicates that

bromelain is well absorbed by the oral route, with an increase in its therapeutic effects depending on the dose.

The low carbohydrate content of the pineapple fruit samples in this study could be related to varietal selection and stage of maturity. Indeed, in their work on the Kythera apple, [48] showed that the starch content decreased during fruit development, while the sugar content increased considerably. However, after harvest, there was a slight drop in the sugar content. Also, the high temperature of storage and handling increases the decrease in sugar content [49]. The results obtained in this study are for the most part lower than those obtained (11 to 11.6%) in pineapple fruits by [21] and by [33]. Carbohydrates are important in the human diet. According to [43], carbohydrates are the primary source of energy for all humans. The carbohydrate values obtained suggest that the pineapple fruit samples studied cannot be used to address protein-energy malnutrition problems, as they are very low in protein and fat. The energy values obtained from the analyzed pineapple fruit samples (57.70 to 66.54 kcal / 100g) could be explained by their low fat and protein content as indicated by [18]. Water and total sugars have the highest contents among all the components of the fruit pulp. These results are in agreement with the results published by several authors such as [50] on plantain, [51] on mango and [52] on pineapple. These showed that the fruits were in general poor in lipids and proteins and very rich in water and sugars. According to [53], the composition of pineapple fruits would give this product a nutritional value equivalent to that of more well-known tropical fruits such as bananas and mango. Pineapple fruit could be a dietary supplement, just as a dessert for most low-income populations and a source of micronutrients. Storage tests on fresh pineapple fruit have shown that the biopesticides tested exert an inhibitory action on pathogens. Indeed, the colonization of pineapple fruit has prevented the proliferation of pathogenic fungi inside the fruit. This made it possible to preserve the firmness of the fruit as well as its physicochemical, nutritional and organoleptic characteristics. These results are in accordance with those of the work of [54] on the conservation of mangoes with *Bacillus subtilis* GA1. However, the signs of deterioration observed on the fruits after fourteen days could be linked to pathogenic fungi, which, familiar with the environment of the substrate, would again constitute the natural flora of pineapples. These adapt more easily to the product unlike biopesticides which need an adaptation time. The inhibition of the spoilage flora of pineapple by *B. subtilis* is thought to be due to its ability to produce lipopeptides with antibacterial and antifungal properties by bursting the cell wall of fungi [55]. One of the main characteristics of lipopeptides from *B. subtilis* resides in their surfactant properties which can be explained by the decrease in surface tension, modification and disruption of lipid bilayers [56,57]. The lack of significant difference in the treatments carried out with the biomass and its supernatant shows the possibility of biological control with one or the other. Treatment of the fruits with the culture supernatant also provided protection similar to that seen with living cells. This gives a first indication of the role of these lipopeptides in the activity of biocontrol of strains. During the biocontrol, *B. subtilis* GA1 must have produced

lipopeptides. These lipopeptides produced by *B. subtilis* GA1 were demonstrated during a biocontrol study of gray rot caused by *Botrytis cinerea* on apples [54]. The results presented in this work describe the ability of the bacterial biopesticides tested to protect pineapple fruits against diseases caused by fungal strains. Treatment with supernatant or vegetative cells of the biopesticides provided very effective disease control during the first 14 days while defying pathogens. These results confirm the potential of *B. subtilis* species for the control of postharvest diseases already reported in other fruits [59,60]. From a technological point of view, the efficiency of living cells is of little interest because it is more unstable than vegetative cells and therefore the difficulty of maintaining viability for years under appropriate storage conditions of the product. Endospores are also much more resistant to drying processes for powder formulation and are relatively easy to produce with industrial fermentation technology [61]. However, further experiments are needed to assess whether the loss of efficacy observed after 14 days is associated with a decrease in the population of beneficial strains and / or with the spontaneous degradation or active bacterial fungal metabolites involved in inhibition of the pathogen.

4. Conclusion

This study revealed through the various parameters studied (ash, proteins, lipids, carbohydrates, fibers and energy values) that the pineapple fruits sold in the various markets of Abidjan are a food of nutritional quality which could help to solve the problems. Micronutrient malnutrition problems. However, its low protein and lipid content gives it a low calorific value. However, the relatively high humidity of the samples analyzed remains a favourable factor for fungal growth. The various microbial biopesticides isolated and acquired have shown their ability to inhibit the main spoilage germs of pineapple fruits. Their impact on storage made it possible to extend the shelf life of the fruits by 14 days without altering the pulp. These various biocontrol agents can act as biopesticides in the fruit industry, especially in Côte d'Ivoire where there are virtually no biological fungicides.

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