

Microbiological and physicochemical evaluation of pineapple fruit and its derivatives

Comment [Ma1]: juice

Abstract

Fruit juices are unfermented but probably fermentable fruit extracts, stored fresh and consumed around the world due to their great nutritional importance. The objective of this study is to determine the pesticide residues, the physicochemical and nutritional parameters of pineapple juice and pineapple derivatives in Benin. The samples were taken from pineapple juice producers and pineapple producers. These samples were subjected to microbiological and physicochemical analyzes in the laboratory.

Comment [Ma2]: Need to mention the research question or problem statement (related to the hygiene of each producers)

The results show that the average pH value of whole pineapple is 4.1228 ± 0.0130 . While and the average pH value of pineapple juice is 4.0762 ± 0.0222 . Whole pineapple samples had an average titratable acidity of 85.78 ± 2.27 . The pineapple juice samples had an average titratable acidity of 85.73 ± 4.52 . Imported whole pineapple samples were found to have the highest average concentration compared to pineapple juice (0.60 ± 0.009 g/L). The average etephon concentration is higher in whole pineapple samples (0.76 ± 0.05) than in pineapple juice samples (0.49 ± 0.06). The average logarithmic contamination rate on whole pineapple samples is around $2.329 \log_{10}$ CFU/g for FAMT, $0.18 \log_{10}$ CFU/g for yeasts and molds and $0.014 \log_{10}$ CFU/g for *Staphylococcus aureus*. Nevertheless, the average logarithmic contamination rate on whole pineapple samples is less than $10 \log_{10}$ CFU/g respectively for Coliforms, Faecal Coliforms and *E. coli*. The suspicion of salmonella is around 0% for whole pineapple samples.

Comment [Ma3]: What concentration ? vitamin C ?

While the average logarithmic contamination rate on pineapple juice samples is around $0.71 \log_{10}$ CFU/ml for FAMT. Nevertheless, the average logarithmic contamination rate on pineapple juice samples is less than $10 \log_{10}$ CFU/ml respectively for Coliforms, Faecal Coliforms, *E. coli*, yeasts and molds and *Staphylococcus aureus*. The suspicion of salmonella is around 0% for the pineapple juice samples.

Comment [Ma4]: Should be in the same paragraph

This study reveals that pineapple juice and pineapple derivatives contain various microflora, nutritional element and pesticide residues. It also notes that the microbiological and nutritional quality of pineapple juice and pineapple derivatives varies according to the producers and depends on the hygienic conditions of production.

Comment [Ma5]: It is not mentioned before and suddenly stated here. Better to mention the hygienic condition of each producers

Keywords: pineapple juice, pineapple derivatives, microbiology quality, pesticide residues, nutrient.

1- Introduction

The fruits and industrial by-products of pineapple (*Ananas comosus* (L.) Merrill) have a strong flavor and intense aroma and are used for fresh consumption and the production of various industrial derived products, including juices (Krause et al., 2013). Pineapple is a good source of fiber, vitamins, minerals and phenolic compounds, with notable antioxidant capacities and potential health benefits (Couto et al., 2011). In Benin, pineapple juice has recently become one of the major products of the food industry. Several companies have specialized in their production and marketing. The pineapple juice market in

Africa is growing and the Republic of Benin is no exception to this dynamic. The pineapple juice sector in Benin allows the creation of new economic activities that create jobs and therefore the fight against unemployment (Noumavo et al., 2022).

Although fruit juices are highly recommended due to their effective nutritional values, concerns over their microbiological and physicochemical quality (Nemer and Benseghir, 2019). In light of the global concern about food safety over the past decades, researchers wanted to conduct studies on the risks associated with the use of fruit juices and foods contaminated with pathogens, trace metals and other toxins (Couto et al., 2011). Fruit contamination can occur before harvest from a variety of sources, including human handling, fertilizers, pesticides, waste and polluted water (Couto et al., 2011). In addition, juices are contaminated with pathogens from the raw materials and equipment used for juice extraction. The atmosphere in which juice is prepared and the unhygienic practices of vendors can also be the source of contamination (Elez-Martinez et al., 2006). These types of spoiled juices can cause diet-related illnesses in people who consume them frequently. In developing countries, *E. coli* (which is usually present in contaminated fruit juices) is responsible for 25% of childhood diarrhea (Couto et al., 2011).

Reports have cited that approximately 40% of fresh fruit is processed into juice and that improper handling and storage conditions lead to contamination of these products with pathogenic bacteria, which poses a potential risk to consumers (Simforian et al. al., 2015; de Sousa Guedes et al., 2016). The pathogenic bacteria present in fruit juices originate from microflora normally present on the surface of the fruit or which have been contaminated during harvesting, post-harvest handling and distribution, which invade the pulp of the fruit during processing, or from post-processing contamination (de Sousa Guedes et al., 2016). According to the European Union in 2019, 33.5% of foods of plant origin contained pesticide residues at or below legally permitted maximum residue limits (MRLs) and 1.6% contained residue concentrations exceeding the legally authorized MRLs (EFSA, 2019). As we have seen, fruit juices are comparatively very exposed to contamination by microbes and toxic elements, which makes a physico-chemical and biological evaluation of the juices necessary of fruit consumed by the general public and suggests the adoption of safe and standardized procedures.

For example, the United States Food and Drug Administration (FDA) has implemented Hazard Analysis and Critical Control Point (HACCP) regulations that include a performance criterion for juice safety, commonly referred to as regulations. HACCP on juices (US-FDA, 2001). The regulations require juice processors to achieve a 5 log reduction of the target microorganism(s) of public health importance when making juice, but this regulation does not require the use of a specific method to achieve this level of inactivation. However, consumer concern about the potential risks of foods containing chemicals and

pathogenic microorganisms (Somolinos et al., 2009) has led to demand for safer, fresher and healthier foods with little or no chemical preservatives, thus creating a market demand for natural, non-thermal products and feasible technologies to ensure the microbial safety of juices (Kapoor et al., 2009).

In this context, this work aims to assess the physicochemical, nutritional and microbiological quality of whole pineapple and pineapple juice consumed in Benin. Indeed, the consumption of whole pineapple and pineapple juice continues to increase in Benin.

2- MATERIEL ET METHODES

Comment [Ma6]: Should be in English

2-1- Study area

The Atlantique department is one of the smallest of the twelve (12) departments of Benin and stretches almost 100 km from the coast towards the interior of the country. It has an area of 3,233 km² and includes 500 villages and eight municipalities which are Abomey-Calavi, Allada, Kpomassè, Ouidah, So-Ava, Toffo, Torri-Bossito and Zè. The Atlantic Ocean forms the southern limit of the department which is limited to the west by the department of Mono. Lake Ahémé, the Couffo River and the Aho River constitute the natural limits of its borders. To the north, the department of the Atlantic is bordered by the department of Zou. This border is located at the geographical level of the villages of Sèhouè, Kpomè and Djigbé and passes through the Lama depression. To the east, it is bordered by the department of Ouémé. The border passes through the middle of the Ouémé Valley and crosses Lake Nokoué to reach the coast at the limit of the Coastal Department.

Sampling

A total of 231 whole pineapple samples and 52 pineapple juice samples were taken for microbiological, physicochemical and nutritional analyses.

Comment [Ma7]: Please mention this in the abstract so the abstract will be more informative

2-2- Microbial analysis

The samples were analyzed according to standard methods. Enumeration and research of Total Mesophilic Aerobic Flora (AFNOR-NF-4833, 2005), Staphylococcus aureus (AFNOR NF V 08-057, 1999), Total Coliforms (AFNOR NF 08-050, 2006), Thermotolerant Coliforms (AFNOR NF V 08-060, 2009), Escherichia coli (AFNOR NF V 08-017, 1980), Salmonella (AFNOR V 08-052, 2004) were carried out according to AFNOR recommendations.

Once in the laboratory, 10 g of each whole pineapple sample was added to 90 ml of sterile bacteriological peptone (Oxoid, Hampshire, England), then incubated at 37°C for 1-3 h for enrichment (Akoachere et al., 2009). Regarding the pineapple juice samples, 10 ml of each sample was added to 90 ml of sterile bacteriological peptone (Oxoid, Hampshire, England), then incubated at 37°C for 1 to 3 h for enrichment (Akoachere et al., 2009). In this study, microbiological analyzes focused on staphylococci, E. coli, and mesophilic aerobic flora (FAM) were counted. From the incubated suspension, a decimal dilution was carried out with peptone water (BioRad, Paris, France) and used for the identification and counting of the bacteria. Each of the dilutions (0.1 ml) was spread on Baird-Parker agar (Biokar Diagnostics, France) with egg yolk (Baird-Parker, 1990; Dennai et al., 2001) before incubation at 37° C for 48 h for Gram-positive cocci. TBx agar (Biokar Diagnostics, France) incubated at 37° C. for 24 h was used to isolate the E. coli. The research of E. coli was supplemented by an indole production test (Riegel et al., 2006). For identification of Salmonella spp., 10 g of each sample was cut into small pieces in a sterile blender jar containing 90 mL of peptone water as pre-enrichment broth and incubated at 37°C for 24 h. After incubation, 0.1 ml of pre-enrichment culture was transferred to sterile tubes containing 10 ml of Rappaport Vassiliadis broth, and incubated at 42°C for 24 h. After incubation, one loopful of each tube was cultured on Salmonella Shigella agar and incubated for 24 h at 37°C. A typical Salmonella colony appears as transparent colonies with or without a black center (depending on the species isolated).

2-3- Physico-chemical analysis

2-3-1- Determination of pH

The pH was determined in ten milliliters of juice dispensed into a beaker after calibration with phosphate buffer of pH 4.0 and 7.0 (Adubofuor et al., 2010).

Comment [Ma8]: Did you use pH meter ? please provide the information of pH meter device (type, country of producer)

2-3-2- Determination of total titratable acidity (TTA)

For the measurement of titratable acidity, the standard method of Talasila et al. (2012) was used. Five grams of concentrated fruit juice was diluted with distilled water (20 ml) and filtered using filter paper (Whatman #1). The indicator (two drops of phenolphthalein) was added to 20 mL of the filtrate and titrated against 0.05 M NaOH. The total titratable acidity was calculated (Eq. (1)).

$$TA = (M \text{ NaOH} \times C \text{ NaOH} \times 0.064 \times 100) / V \quad (1)$$

Comment [Ma9]: Better to use Equation type

Where: TA: titratable acidity; M NaOH: Molarity of the NaOH used; V NaOH volume of NaOH used; 0.064: Citric acid equivalent weight V: volume of juice.

2-3-3- Determination of vitamin C in whole pineapple fruit and fruit juice samples by HPLC.

The dosage of vitamin C was made according to the method used by Alsarahe and Saeed, (2022). To do this, the homogeneous solid sample was measured around 10 to 30g and mixed with 60 to 80 ml 6% metaphosphoric acid (HPO₃) for one minute. As for the juice of the samples, it was mixed with 30 ml of metaphosphoric acid and mixed well until homogeneous, then the volume was made up to 100 ml. The obtained extract was filtered through filter paper and washed several times using vacuum pump filtration. Then the filtrate was quantitatively transferred to a 100 ml volumetric flask and 6% metaphosphoric acid (HPO₃) was added up to the 100 ml volumetric mark. All sample solutions were refiltered through a 0.45 µm syringe filter. After that, the samples were run through the HPLC system. The separations were carried out on a carbon 18 (C18) column of (25 x 0.46) µm, temperature 30°C and the mobile phase used was a mixture of methanol - water (50.950, v/v) with a flow rate of 1ml/min. The injection volume was 20 µl and the UV detection wavelength was 254 nm.

Comment [Ma10]: please provide the information of device (type, country of producer)

First, the column was washed with the mobile phase for 45 minutes, then the standard solutions were analyzed. Then, the column was washed with the mobile phase for 45 minutes in order to eliminate all traces of standard solution, then the samples were analyzed and their retention time was compared with that of the standard solution.

Comment [Ma11]: What standart solution ? please mention it and it's information (producer name, country of producer)

2-3-4- Determination of ethephon by LC-MS/MS in samples of whole pineapple fruit and fruit juice by HPLC.

An internal standard is added before the ethephon extraction. This is carried out using acidified methanol (0.1% formic acid). After centrifugation, the extract is filtered on a micro-disc and injected onto a HILIC type column in UPLC-MS/MS.

Comment [Ma12]: please provide the information of device (type number, country of producer)

• 10g of samples cut and homogenized according to SOP 24/1203/F are weighed to the nearest 0.1g in a 50ml Falcon

• Addition of 200 µl of the intermediate solution of the internal standard at 10 µg/ml • Addition of 10 ml of the extraction solution

• Shake for 2 min using the Multi Reax (Heidolph or equivalent) or the ultra-turrax (IKA or equivalent)

- Centrifuge (Centrifuge 5810 R, Eppendorf or equivalent) at 5°C and 4000 rpm for 5 min.

- In a Wathman filter flask, 5 µl of 25% ammonia is added with the eVol in the bottom of the flask. 500 µl of the extract is added and the vials are closed and mixed.

- Injection

Comment [Ma13]: Please write in a paragraph form

3- Statistical analyses

The statistical processing of the data was carried out with the R software version 4.2.1. A p-value of 0.05 was used for the Mann-Whitney test. Correlation tests were performed with the Pearson coefficient with a p-value of 0.05 (quantitative variables)

3- Results

4-1- Physicochemical parameters

The physico-chemical parameters of whole pineapple and pineapple juice samples revealed that the average pH value of whole pineapple is 4.1228 ± 0.0130 . While and the average pH value of pineapple juice is 4.0762 ± 0.0222 . There is no significant difference between pH of whole pineapple and pineapple juice ($p > 0.05$) (Figure 1). Whole pineapple samples had an average titratable acidity of 85.78 ± 2.27 . The pineapple juice samples had an average titratable acidity of 85.73 ± 4.52 . There is no significant difference between acidity between whole pineapple and pineapple juice ($p > 0.05$) (Figure 2).

The solutions of each of the whole pineapple and pineapple juice samples were processed for the determination of ascorbic acid. These surface values were related to the corresponding concentrations in the calibration curves (Table 1). HPLC results reveal varying amounts of vitamin C in whole pineapple and pineapple juice samples. Imported whole pineapple samples were found to have the highest average concentration compared to pineapple juice (0.60 ± 0.009 g/L). The average concentration of pineapple juice is 0.54 ± 0.020 g/L. The average etephon concentration is higher in whole pineapple samples (0.76 ± 0.05) than in pineapple juice samples (0.49 ± 0.06). There is a highly significant difference ($p < 0.002$) between the presence of etephon between whole pineapple and pineapple juice (Figure 3)

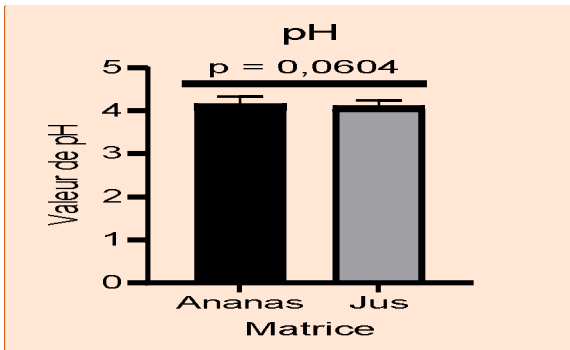


Figure 1 :pH of whole pineapple and pineapple juice.

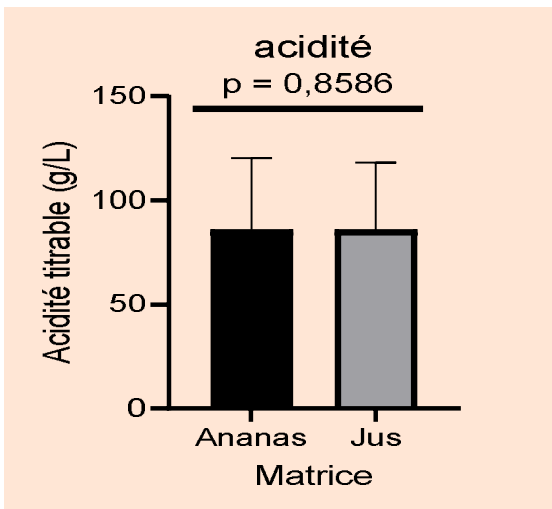


Figure 2: Acidity of whole pineapple and pineapple juice

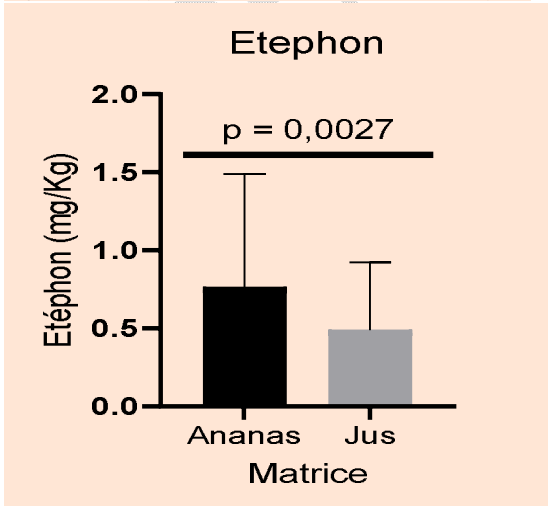


Figure 3: Etephon in whole pineapple and pineapple juice samples

Comment [Ma14]: Please combine into one figure (A, B, and C)
Please change into English (for example Valeur de pH into pH value)

4-2- Microbiological parameters of whole pineapple and pineapple juice.

The average logarithmic contamination rate on whole pineapple samples is around 2.329 log₁₀ CFU/g for FAMT, 0.18 log₁₀ CFU/g for yeasts and molds and 0.014 log₁₀ CFU/g for Staphylococcus aureus . Nevertheless, the average logarithmic contamination rate on whole pineapple samples is less than 10 log₁₀ CFU/g respectively for Coliforms, Faecal Coliforms and E coli. The suspicion of salmonella is around 0% for whole pineapple samples.

While the average logarithmic contamination rate on pineapple juice samples is around 0.71 log₁₀ CFU/ml for FAMT. Nevertheless, the average logarithmic contamination rate on pineapple juice samples is less than 10 log₁₀ CFU/ml respectively for Coliforms, Faecal Coliforms, E coli, yeasts and molds and Staphylococcus aureus. The suspicion of salmonella is around 0% for the pineapple juice samples. Figure 4 shows the mean logarithmic contamination rate on whole pineapple and pineapple juice samples. This figure shows that there is a highly significant difference ($p < 0.001$) between the logarithmic mean of the contamination rate on the whole pineapple and pineapple juice samples (Figure 4). But there is no significant difference ($p > 0.05$) between the mean logarithmic yeast and mold contamination rate on the whole pineapple and pineapple juice samples (Figure 5).

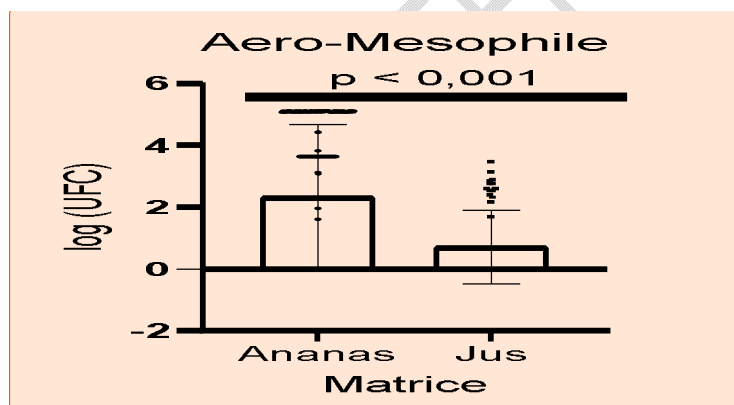


Figure 4 :Average logarithmic FAMT contamination rate on samples of whole pineapple and pineapple juice.

Table 1: Physicochemical characteristics of whole pineapple and pineapple juice

Variable	Echantillons	Moyenne	Ecart type	Standard deviation	Coefficient de variation	Minimum	Maximum
Acidité	Ananas (231)	85,78	2,27	34,50	40,22	42,20	127,40
	Jus (52)	85,73	4,52	32,59	38,01	42,20	127,40
Vitamine C	Ananas (231)	0,60818	0,00970	0,14746	24,25	0,42000	0,77000
	Jus (52)	0,5387	0,0202	0,1459	27,09	0,3800	0,7700
Etephon	Ananas (231)	0,7596	0,0478	0,7259	95,56	0,0000	4,8000
	Jus (52)	0,4877	0,0602	0,4338	88,95	0,3000	2,5700
pH	Ananas (231)	4,1228	0,0130	0,1981	4,80	3,8200	4,3900
	Jus (52)	4,0762	0,0222	0,1600	3,92	3,8000	4,4000

Comment [Ma16]: ?

Comment [Ma17]: ? is it in French ?

Comment [Ma18]: ?

Table 2: Microbiological characteristics of whole pineapple and pineapple juice

Variable	Echantillons	Moyenne (log ₁₀ UFC)	Ecart type	Standard deviation	Coefficient de variation	Minimum	Maximum
Total Aerobic Mesophilic Flora (FAMT)	Ananas (231)	2,329	0,154	2,334	100,21	0,000	5,114
	Jus (52)	0,708	0,166	1,197	169,14	0,000	3,477
Yeast and mold	Ananas (231)	0,1756	0,0569	0,8647	492,45	0,0000	6,1761
	Jus (52)	<10	0,000000	0,000000	*	0,000000	0,000000
Coliformes	Ananas (231)	<10	0,000000	0,000000	*	0,000000	0,000000
	Jus (52)	<10	0,000000	0,000000	*	0,000000	0,000000
<i>Faecal coliforms</i>	Ananas (231)	<10	0,000000	0,000000	*	0,000000	0,000000
	Jus (52)	<10	0,000000	0,000000	*	0,000000	0,000000
<i>Ecoli</i>	Ananas (231)	<10	0,000000	0,000000	*	0,000000	0,000000
	Jus (52)	<10	0,000000	0,000000	*	0,000000	0,000000
<i>Staphylococcus aureus</i>	Ananas (231)	0,0136	0,0136	0,2070	1519,87	0,0000	3,1461
	Jus (52)	<10	0,000000	0,000000	*	0,000000	0,000000

Comment [Ma19]: Data are the average of 231 samples ? please give information below the table

Discussion

Although pineapple juice is the source of many benefits for the body, and participates in certain functions due to its composition. Knowledge of its composition is necessary for its valuation.

Comment [Ma20]: comma

The physico-chemical characteristics of the samples of whole pineapple and pineapple juice revealed that the average pH value of whole pineapple is 4.1228 ± 0.0130 . While and the average pH value of pineapple juice is 4.0762 ± 0.0222 . These results are contrary to those obtained by Amin et al. (2022). These authors showed that fruit juices have a low pH (3.55 to 3.8) because they are relatively rich in organic acids. The difference could be explained by the difference in the raw material. Because these actors worked on mango juice and we on pineapple juice. In addition, the average titratable acidity of the whole pineapple samples is 85.78 ± 2.27 and that of the pineapple juice samples is 85.73 ± 4.52 . The total acidity observed in our study, could be due to the presence of a mixture of organic acids, whose composition varies according to the maturity of the pulp used. Organic acids have taken the lead in importance for the characteristics and nutritional value of fruit juices and deliberated individual originality among beverages (Amin et al., 2022).

Comment [Ma21]: author ?

Comment [Ma22]: then, please compare to pineapple juice pH.

Comment [Ma23]: It is fine

According to the results obtained in the present study, samples of imported whole pineapple had the highest average concentration compared to pineapple juice (0.60 ± 0.009 g/L). The average concentration of pineapple juice is 0.54 ± 0.020 g/L. This difference could be explained by the fact that during processing the juice loses certain nutritious compounds. One of the studies reported that the ascorbic acid concentration of freshly prepared orange juice by the titration method was 58.30 mg/100 ml (Fatin and Azrina, 2017). While the vitamin C concentration of orange juice 43.61 mg/100 ml used the HPLC technique to analyze the vitamin C content of orange juice (Fatin and Azrina, 2017). Vitamin C also acts in favor of the prevention of hypertension. People who suffer from hypertension can thus consume pineapple juice more often, and benefit from its benefits. In short, drinking pineapple juice helps fight against cardiovascular diseases and their development.

Regarding Ethephon in our study, the average concentration of ethephon is higher in whole pineapple samples (0.76 ± 0.05 mg/kg) than in pineapple juice samples (0.49 ± 0.06 mg/kg). Ethephon can inhibit blood cholinesterases and accumulate in the body so that it is harmful to human health, as shown in tests with dogs and rats (Arzam et al. al., 2021). One effort that can be made is to reduce the concentration used. World Food Safety (Codex) has set an ethephon residue threshold at 0.5 ppm.

Regarding the microbiological quality of whole pineapple in our study, reveals that whole pineapple harbor many spoilage microorganisms including yeasts and molds ($0.18 \log_{10}$ UFC/g), FMT ($2.329 \log_{10}$ UFC /g) and Staphylococcus aureus ($0.014 \log_{10}$ CFU/g). But it does not contain Coliforms, Faecal Coliforms, E coli and Salmonella. Unlike whole pineapple, the average logarithmic contamination rate is less than $10 \log_{10}$ CFU/ml respectively for Coliforms, Faecal Coliforms, E coli, yeasts and molds and Staphylococcus aureus. This difference could be explained by the fact that the pasteurization of pineapple juice has an effect on the absence of spoilage microorganisms. In pineapple juice, the presence of these germs could be the result of several factors such as non-compliance with good hygiene practices.

Comment [Ma24]: Please cite the references

Conclusion

Pineapple juice and pineapple fruit are food products rich in vitamins, energy, trace elements and minerals such as potassium necessary for consumer health. Its preparation by simple traditional methods is part of street meals in public markets. This work was carried out to verify the microbiological

Comment [Ma25]: There is no data about this in the manuscript so I think it is not enough to make this words in the conclusion.

and physico-chemical and nutritional quality of pineapple juice and pineapple fruit produced in southern Benin.

The microbiological analyzes carried out show the presence of yeast and mold strains, *Staphylococcus aureus* and *FAMT* on the whole pineapple samples and on the pineapple juice samples. Nevertheless, the presence of strains of total coliforms, faecal coliforms and *E. coli* on the whole pineapple samples and on the pineapple juice samples are less than 10 log₁₀ CFU/g or /ml.

Physicochemical analyzes show that the average pH value of whole pineapple and pineapple juice is 4. The average concentration of ethephon is higher in whole pineapple samples than in pineapple juice samples. .

This study reveals that pineapple juice and pineapple derivatives contain various microflora, nutritional element and pesticide residues. It also notes that the microbiological and nutritional quality of pineapple juice and pineapple derivatives varies according to the producers and depends on the hygienic conditions of production.

Comment [Ma26]: All the data are only compared based on the state (whole and juice). There is no data for each different producers and hygienic condition.

If you want to state that the microbiological and nutritional values varies according to the producers and the hygienic condition please provide or show the data (pH, acidity, vitamin C, ethephon) based on the different producer and hygiene ; not only based on the products (fruit and juice)

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