**Microbiological and physicochemicalevaluation of pineapple fruit and itsderivatives**


## Abstract

Fruit juices are unfermented but probably fermentable fruit extracts, storedfresh and consumedaround the world due to theirgreatnutritional importance. The objective of thisstudy~~is~~ wastodetermine the pesticide residues, the physicochemical and nutritionalparameters of pineapplejuice and pineapplederivatives in Benin. The samplesweretakenfrompineapplejuiceproducers and pineappleproducers. Thesesamplesweresubjected to microbiological and physicochemicalanalyzes in the laboratory.

The results show that the average pH value of wholepineappleis 4.1228±0.0130. While and the average pH value of pineapplejuiceis 4.0762±0.0222. Wholepineapplesampleshad an averagetitratableacidity of 85.78±2.27. The pineapplejuicesampleshad an averagetitratableacidity of 85.73±4.52. Importedwholepineapplesampleswerefound to have the highestaverage concentration compared to pineapplejuice (0.60±0.009 g/L). The averageetephon concentration ishigher in wholepineapplesamples (0.76±0.05) than in pineapplejuicesamples (0.49±0.06). The averagelogarithmic contamination rate on wholepineapplesamplesisaround 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 averagelogarithmic contamination rate on wholepineapplesamplesislessthan 10 log10 CFU/g respectively for Coliforms, FaecalColiforms and *E coli*. The suspicion of salmonella ~~is~~ wasaround 0% for wholepineapplesamples.

While the averagelogarithmic contamination rate on pineapplejuicesamplesisaround 0.71 log10 CFU/ml for FAMT. Nevertheless, the averagelogarithmic contamination rate on pineapplejuicesamplesislessthan 10 log10 CFU/ml respectively for Coliforms, FaecalColiforms, E coli, yeasts and molds and Staphylococcus aureus. The suspicion of salmonella isaround 0% for the pineapplejuicesamples.

This studyrevealsthatpineapplejuice and pineapplederivativescontainedvariousmicroflora, nutritionalelement and pesticide residues. It also notes that the microbiological and nutritionalquality of pineapplejuice and pineapplederivatives varies according to the producers and depends on the hygienic conditions of production.



**Keywords:**P~~p~~ineapplejuice ;~~,~~pineapplederivatives ;~~,~~microbiologyquality;~~,~~ pesticide residues;~~,~~nutrient.


## 1- INTRODUCTION

The fruits and industrial by-products of pineapple (*Ananas comosus*(L.) Merril) have a strongflavor and intense aroma and are used for freshconsumption and the production of variousindustrialderivedproducts, includingjuices( Krause et al., 2013). Pineappleis a good source of fiber, vitamins, minerals and phenolic compounds, with notable antioxidantcapacities and

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potentialhealthbenefits (Couto et al., 2011). In Benin, pineapplejuice has recentlybecome one of the major products of the foodindustry. Severalcompanies have specialized in their production and marketing. The pineapplejuicemarket in Africaisgrowing and the Republic of Benin is no exception to thisdynamic. The pineapplejuicesector in Benin allows the creation of new economicactivitiesthatcreate jobs and therefore the fightagainstunemployment (Noumavo et al., 2022).

Although fruit juices are highlyrecommended due to their effective nutritional values, concerns over theirmicrobiological and physicochemicalquality (Nemer and Benseghir, 2019). In light of the global concern about foodsafety over the pastdecades, researcherswanted to conductstudies on the risksassociatedwith the use of fruit juices and foodscontaminatedwithpathogens, trace metals and othertoxins (Couto et al., 2011). Fruit contamination can occurbeforeharvestfrom a variety of sources, includinghuman handling, fertilizers, pesticides, waste and polluted water (Couto et al., 2011). In addition, juices are contaminatedwithpathogensfrom the rawmaterials and equipmentused for juice extraction. The atmosphere in whichjuiceisprepared and the unhygienic practices of vendors can alsobe the source of contamination (Elez-Martinez et al., 2006). These types of spoiledjuices can cause diet-relatedillnesses in people who consume themfrequently. In developing countries, E. coli (whichisusuallypresent in contaminated fruit juices) isresponsible for 25% of childhooddiarrhea (Couto et al., 2011).

Reports have citedthatapproximately 40% of fresh fruit isprocessedintojuice and thatimproper handling and storage conditions lead to contamination of theseproductswithpathogenicbacteria, which poses a potentialrisk to consumers (Simforian et al. al., 2015; de Sousa Guedes et al., 2016). The pathogenicbacteriapresent in fruit juicesoriginatefrommicrofloranormallypresent on the surface of the fruit or which have been contaminatedduringharvesting, post-harvest handling and distribution, whichinvade the pulp of the fruit duringprocessing, or from post-processing contamination (de Sousa Guedes et al., 2016). According to the European Union in 2019, 33.5% of foods of plant origincontained pesticide residues at or belowlegallypermitted maximum residuelimits (MRLs) and 1.6% containedresidue concentrations exceeding the legallyauthorizedMRLs ( (EFSA, 2019). As we have seen, fruit juices are comparativelyveryexposed to contamination by microbes and toxicelements, whichmakes a physico-chemical and biologicalevaluation of the juicesnecessary of fruit consumed by the general public and suggests the adoption of safe and standardizedprocedures.

For example, the United States Food and Drug Administration (FDA) has implemented Hazard Analysis and Critical Control Point (HACCP) regulationsthatinclude a performance criterion for juicesafety, commonlyreferred to as regulations. HACCP on juices (US-FDA, 2001). The regulationsrequirejuice processors to achieve a 5 log reduction of the targetmicroorganism(s) of public health importance whenmakingjuice, but thisregulationdoes not require the use of a specificmethod to achievethislevel of inactivation. However, consumer concern about the potentialrisks of foodscontainingchemicals and pathogenicmicroorganisms (Somolinos et al., 2009) has led to demand for safer, fresher and healthierfoodswithlittle or no chemicalpreservatives, thuscreating a marketdemand for natural, non-thermal products and feasible technologies to ensure the microbialsafety of juices (Kapoor et al., 2009).

In thiscontext, thisworkaims to assess the physicochemical, nutritional and microbiologicalquality of wholepineapple and pineapplejuiceconsumed in Benin. Indeed, the consumption of wholepineapple and pineapplejuice continues to increase in Benin.

## ~~2-~~2. MATERIEL ET METHODES

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### ~~2-1-~~2.1. Study area

The Atlantique departmentis one of the smallest of the twelve (12) departments of Benin and stretchesalmost 100 km from the coasttowards the interior of the country. It has an area of 3,233 km² and includes 500 villages and eightmunicipalitieswhich are Abomey-Calavi, Allada, Kpomassè, Ouidah, So-Ava, Toffo, Torri-Bossito and Zè. The Atlantic Oceanforms the southernlimit of the departmentwhichislimited to the west by the department of Mono. Lake Ahémé, the Couffo River and the Aho River constitute the naturallimits of itsborders. To the north, the department of the Atlantic isbordered by the department of Zou. This border islocated at the geographicallevel of the villages of Sêhouè, Kpomè and Djigbé and passes through the Lama depression. To the east, itisbordered 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.

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### 2.2. Sampling

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A total of 231 wholepineapplesamples and 52 pineapplejuicesamplesweretaken for microbiological, physicochemical and nutritional analyses.

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### ~~2-2-~~2.3. Microbialanalysis

The sampleswereanalyzedaccording to standard methods. Enumeration and research of Total MesophilicAerobic Flora (AFNOR-NF-4833, 2005), *Staphylococcus aureus* (AFNOR NF V 08-057, 1999), Total Coliforms (AFNOR NF 08-050, 2006), ThermotolerantColiforms (AFNOR NF V 08-060, 2009), Escherichia coli (AFNOR NF V 08-017, 1980), Salmonella (AFNOR V 08-052, 2004) werecarried out according to AFNOR recommendations.

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Once in the laboratory, 10 g of eachwholepineapplesamplewasadded to 90 ml of sterilebacteriological peptone (Oxoid, Hampshire, England), thenincubated at 37°C for 1-3 h for enrichment (Akoachere et al., 2009). Regarding the pineapplejuicesamples, 10 ml of eachsamplewasadded to 90 ml of sterilebacteriological peptone (Oxoid, Hampshire, England), thenincubated at 37°C for 1 to 3 h for enrichment (Akoachere et al., 2009). In thisstudy, microbiologicalanalyzesfocused on staphylococci, E. coli, and mesophilicaerobic flora (FAM) werecounted. From the incubated suspension, a decimal dilution wascarried 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) witheggyolk (Baird-Parker, 1990;Dennaï 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 wasused to isolate the E. coli. The research of. coliwassupplemented by an indole production test (Riegel et al., 2006). For identification of Salmonella spp., 10 g of eachsamplewascutintosmallpieces in a sterile blender jar containing 90 mL of peptone water as pre-enrichmentbroth and incubated at 37°C for 24 h. After incubation, 0.1 ml of pre-enrichment culture wastransferred to sterile tubes containing 10 ml of RappaportVassiliadisbroth, and incubated at 42°C for 24 h. After incubation, one loopful of each tube wascultured on Salmonella Shigella agar and incubated for 24 h at 37°C. A typical Salmonella colonyappears as transparent colonies with or without a black center (depending on the speciesisolated).

## 2-3-2.4. Physico-chemicalanalysis

### 2-3-1-.4.1.Determination of pH

The pH wasdetermined in tenmilliliters of juicedispensedinto a beakerafter calibration with phosphate buffer of pH 4.0 and 7.0 (Adubofuor et al., 2010).

### 2-3-22.4.2.-Determination of total titratableacidity (TTA)

For the measurement of titratableacidity, the standard method of Talasila et al. (2012) wasused. Five grams of concentrated fruit juicewasdilutedwithdistilled water (20 ml) and filteredusingfilterpaper (Whatman #1). The indicator (two drops of phenolphthalein) wasadded to 20 mL of the filtrate and titratedagainst 0.05 M NaOH. The total titratableaciditywascalculated (Eq. (1)).

TA = (M NaOH x C NaOH x 0.064 × 100)/V (1)

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Where: TA: titratableacidity; M NaOH: Molarity of the NaOHused; V NaOH volume of NaOHused; 0.064: Citricacidequivalentweight V: volume of juice.

### ~~2-3-3-~~2.4.3.Determination of vitamin C in wholepineapple fruit and fruit juicesamples by HPLC~~.~~

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The dosage of vitamin C was made according to the methodused by Alsarahe and Saeed, (2022). To do this, the homogeneoussolidsamplewasmeasuredaround 10 to 30g and mixed with 60 to 80 ml 6% metaphosphoricacid (HPO3) for one minute. As for the juice of the samples, itwas mixed with 30 ml of metaphosphoricacid and mixed welluntilhomogeneous, then the volume was made up to 100 ml. The obtainedextractwasfilteredthroughfilterpaper and washedseveral times using vacuum pump filtration. Then the filtratewasquantitativelytransferred to a 100 ml volumetricflask and 6% metaphosphoricacid (HPO3) wasadded up to the 100 ml volumetric mark. All sample solutions wererefilteredthrough a 0.45 µm syringe filter. Afterthat, the sampleswere run through the HPLC system. The separationswerecarried out on a carbon 18 (C18) column of (25 x 0.46) µm, temperature 30°C and the mobile phase usedwas 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 detectionwavelengthwas 254 nm.

First, the columnwaswashedwith the mobile phase for 45 minutes, then the standard solutions wereanalyzed. Then, the columnwaswashedwith the mobile phase for 45 minutes in order to eliminate all traces of standard solution, then the sampleswereanalyzed and theirretention time wascomparedwiththat of the standard solution.

### ~~2-3-4-~~2.4.4.Determination of ethephon by LC-MS/MS in samples of wholepineapple fruit and fruit juice by HPLC,

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An internal standard isaddedbefore the ethephon extraction. This iscarried out usingacidifiedmethanol (0.1% formicacid). After centrifugation, the extractisfiltered on a micro-disc and injected onto a HILIC type column in UPLC-MS/MS.

• 10g of samplescut and homogenizedaccording 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 Wathmanfilterflask, 5 µl of 25% ammoniaisaddedwith the eVol in the bottom of the flask. 500 µl of the extractisadded and the vials are closed and mixed.

• Injection

### ~~3~~2.5.Statistical analyses

The statisticalprocessing of the data wascarried out with the R software version 4.2.1. A p-value of 0.05 wasused for the Mann-Whitney test. Correlation tests wereperformedwith the Pearson coefficientwith a p-value of 0.05 (quantitative variables)

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## 3.- RESULTS

### ~~4-1-~~3.1. Physicochemicalparameters

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The physico-chemicalparameters of wholepineapple and pineapplejuicesamplesrevealedthat the average pH value of wholepineappleis 4.1228±0.0130. While and the average pH value of pineapplejuiceis 4.0762±0.0222. There ~~is~~was no significantdifferencebetween pH of wholepineapple and pineapplejuice ($p > 0.05$) (Figure 1). Wholepineapplesampleshad an averagetitratableacidity of 85.78±2.27. The pineapplejuicesampleshad an averagetitratableacidity of 85.73±4.52. There is no significantdifferencebetweenaciditybetweenwholepineapple and pineapplejuice ($p > 0.05$) (Figure 2).

The solutions of each of the wholepineapple and pineapplejuicesampleswereprocessed for the determination of ascorbicacid. These surface values wererelated to the corresponding concentrations in the calibration curves (Table 1). HPLC resultsrevealvaryingamounts of vitamin C in wholepineapple and pineapplejuicesamples. Importedwholepineapplesampleswerefound to have the highestaverage concentration compared to pineapplejuice (0.60±0.009 g/L). The average concentration of pineapplejuiceis 0.54±0.020 g/L. The averageetephon concentration ~~is~~ washigher in wholepineapplesamples (0.76±0.05) than in pineapplejuicesamples (0.49±0.06). There ~~is~~ wasa highlysignificantdifference ($p < 0.002$) between the presence of etephonbetweenwholepineapple and pineapplejuice(Figure 3)

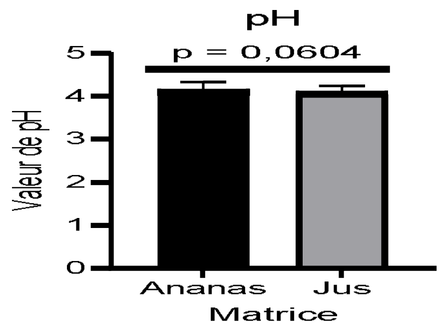


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**Figure 1 :pH of wholepineapple and pineapplejuice.**

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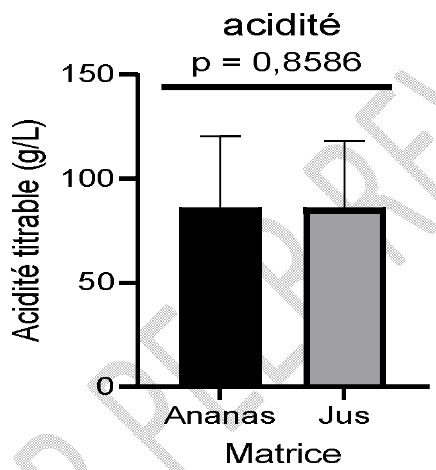


**Figure 2:Acidity of wholepineapple and pineapplejuice**

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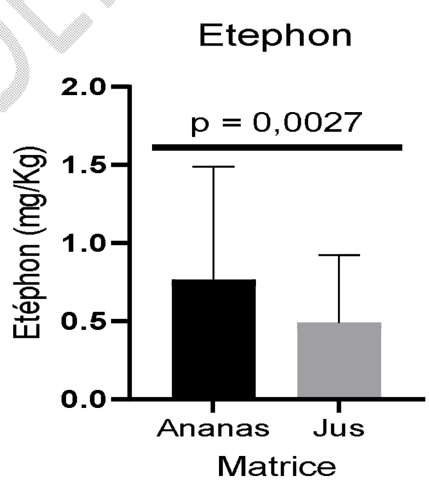

**Figure 3:Etephon in wholepineapple and pineapplejuicesamples**

## ~~4-2-~~3.2.Microbiologicalparameters of wholepineapple and pineapplejuice~~.~~

The averagelogarithmic contamination rate on wholepineapplesamplesisaround 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 averagelogarithmic contamination rate on wholepineapplesamples~~is~~ waslessthan 10 log10 CFU/g respectively for Coliforms, FaecalColiforms and *E coli*. The suspicion of salmonella isaround 0% for wholepineapplesamples.

While the averagelogarithmic contamination rate on pineapplejuicesamples~~is~~ wasaround 0.71 log10 CFU/ml for FAMT. Nevertheless, the averagelogarithmic contamination rate on pineapplejuicesamplesislessthan 10 log10 CFU/ml respectively for Coliforms, FaecalColiforms, *E coli,*yeasts and molds and Staphylococcus aureus. The suspicion of salmonella isaround 0% for the pineapplejuicesamples. Figure 4 shows the meanlogarithmic contamination rate on wholepineapple and pineapplejuicesamples. This figure shows thatthere~~is a~~washighlysignificantdifference ($p < 0.001$) between the logarithmicmean of the contamination rate on the wholepineapple and pineapplejuicesamples (Figure 4). But there~~is~~ wasno significantdifference ($p > 0.05$) between the meanlogarithmicyeast and mold contamination rate on the wholepineapple and pineapplejuicesamples (Figure 5).

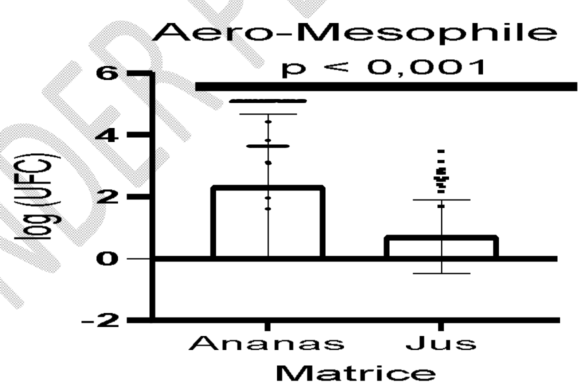


**Figure 4 :Averagelogarithmic FAMT contamination rate on samples of wholepineapple and pineapplejuice.**

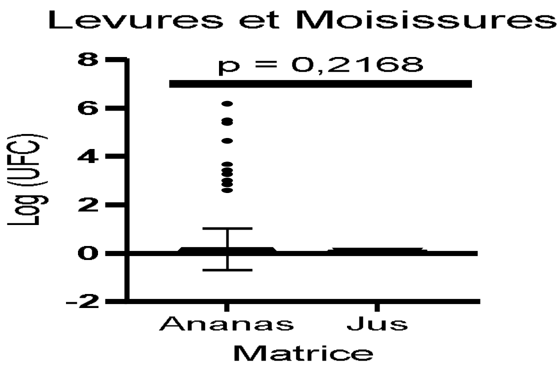


**Figure 5:Meanlogarithmicyeast and mold contamination rate on wholepineapple and pineapplejuicesamples.**





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**Table 1:Physicochemicalcharacteristics of wholepineapple and pineapplejuice**

| **Variable** | **Echantillons** | **Moyenne** | **Ecart type** | **Standard deviation** | **Coefficient ~~de~~ of 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 |
| VitamineC | 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 |

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**Table 2:Microbiologicalcharacteristics of wholepineapple and pineapplejuice**

| **Variable** | **Echantillons** | **Moyenne (log10 UFC)** | **Ecart type** | **Standard deviation** | **Coefficient de of 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 |
| *Faecal coliforms* | Ananas (231) | <10 | 0,000000 | 0,000000 | * | 0,000000 | 0,000000 |
| | Jus (52) | <10 | 0,000000 | 0,000000 | * | 0,000000 | 0,000000 |
| *Ecoli* | Ananas (231) | <10 | 0,000000 | 0,000000 | * | 0,000000 | 0,000000 |
| | Jus (52) | <10 | 0,000000 | 0,000000 | * | 0,000000 | 0,000000 |
| *Staphylococcus aureus* | 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 |



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## 4. DISCUSSION

Althoughpineapplejuiceis the source of manybenefits for the body, and participates in certain functions due to its composition. Knowledge of its composition isnecessary for its valuation.

The physico-chemicalcharacteristics of the samples of wholepineapple and pineapplejuicerevealedthat the average pH value of wholepineappleis 4.1228±0.0130. While and the average pH value of pineapplejuiceis 4.0762±0.0222. Theseresults are contrary to thoseobtained by Amin et al. (2022). Theseauthorsshowedthat fruit juices have a low pH (3.55 to 3.8) becausethey are relativelyrich in organicacids. The differencecouldbeexplained by the difference in the rawmaterial. Becausetheseactorsworked on mangojuice and we on pineapplejuice. In addition, the averagetitratableacidity of the wholepineapplesamplesis 85.78±2.27 and that of the pineapplejuicesamplesis 85.73±4.52. The total acidityobserved in ourstudy, couldbe due to the presence of a mixture of organicacids, whose composition varies according to the maturity of the pulpused. Organicacids have taken the lead in importance for the characteristics and nutritional value of fruit juices and deliberatedindividualoriginalityamongbeverages (Amin et al., 2022).

According to the resultsobtained in the presentstudy, samples of importedwholepineapplehad the highestaverage concentration compared to pineapplejuice (0.60±0.009 g/L). The average concentration of pineapplejuice is 0.54±0.020 g/L. This differencecouldbeexplained by the factthatduringprocessing the juice loses certain nutritious compounds. One of the studiesreportedthat the ascorbicacid concentration of freshlyprepared orange juice by the titration methodwas 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 alsoacts in favor of the prevention of hypertension. People whosufferfrom hypertension can thus consume pineapplejuice more often, and benefitfromitsbenefits. In short, drinkingpineapplejuicehelpsfightagainstcardiovasculardiseases and theirdevelopment.

RegardingEthephon in ourstudy, the average concentration of ethephonishigher in wholepineapplesamples (0.76±0.05 mg/kg) than in pineapplejuicesamples (0. 49±0.06mg/kg). Ethephon can inhibitbloodcholinesterases and accumulate in the body sothatitisharmful to humanhealth, as shown in tests withdogs 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 ethephonresiduethreshold at 0.5 ppm.

Regarding the microbiologicalquality of wholepineapple in ourstudy, revealsthatwholepineappleharbormanyspoilagemicroorganismsincludingyeasts and molds (0.18 log10 UFC/g), FAMT (2.329 log10 UFC /g) and Staphylococcus aureus (0.014 log10 CFU/g). But itdoes not containColiforms, FaecalColiforms, E coli and Salmonella. Unlikewholepineapple, the averagelogarithmic contamination rate islessthan 10 log10 CFU/ml respectively for Coliforms, FaecalColiforms, E coli, yeasts and molds and Staphylococcus aureus. This differencecouldbeexplained by the factthat the pasteurization of pineapplejuice has an effect on the absence of spoilagemicroorganisms. In pineapplejuice, the presence of thesegermscouldbe the result of severalfactorssuch as non-compliance with good hygiene practices.

## 5. CONCLUSION

Pineapplejuice and pineapple fruit are foodproductsrich in vitamins, energy, trace elements and mineralssuch as potassium necessary for consumer health. Itspreparation by simple traditionalmethodsis part of streetmeals in public markets. This workwascarried out to verify the

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microbiological and physico-chemical and nutritionalquality of pineapplejuice and pineapple fruit produced in southern Benin.

The microbiologicalanalyzescarried out show the presence of yeast and moldstrains, Staphylococcus aureus and FAMT on the wholepineapplesamples and on the pineapplejuicesamples. Nevertheless, the presence of strains of total coliforms, faecalcoliforms and E coli on the wholepineapplesamples and on the pineapplejuicesamples are lessthan 10 log10 CFU/g or /ml.

Physicochemicalanalyzes show that the average pH value of wholepineapple and pineapplejuiceis 4. The average concentration of etephonishigher in wholepineapplesamplesthan in pineapplejuicesamples.

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This studyrevealsthatpineapplejuice and pineapplederivativescontainvariousmicroflora, nutritionalelement and pesticide residues. It also notes that the microbiological and nutritionalquality of pineapplejuice and pineapplederivatives varies according to the producers and depends on the hygienic conditions of production.

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