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ASIO Journal of Microbiology, Food Science & Biotechnological Innovations (ASIO-JMFSBI)

Volume 7, Issue 1, 2024: 01-11

[STUDY OF THE SENSORY, MICROBIOLOGICAL AND BIOCHEMICAL](https://albertscience.com/journals/article_detail/291) [STABILITY OF ORANGE AND PINEAPPLE JUICES PRODUCED BY STREET](https://albertscience.com/journals/article_detail/291) [VENDORS IN CÔTE D'IVOIRE](https://albertscience.com/journals/article_detail/291)

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ARTICLE INFO ABSTRACT

Article's History

Received: 14th May, 2024 **Accepted**: 7th July, 2024

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Untreated fruit juices sold on street corners often exhibits flavor deterioration during storage. In this study, the sensory, microbiological, biochemical changes in orange and pineapple juices stored under two different temperature conditions (4°C and ambient temperature) were investigated. The results showed that appearance, odor, and taste of the orange and pineapple juices were spoiled after 48 h of storage at ambient temperature (28 to 30 $^{\circ}$ C). At 4 $^{\circ}$ C, orange and pineapple juices were not judged as spoiled after the 72 h of storage. The initial microbial counts of the orange juice sample were 4.44 log (CFU/mL), 4.60 log (CFU/mL), 4.98 log (CFU/mL), and 4.15 log (CFU/mL) for AM, LAB, Yeast and AAB, respectively. As for hygienic flora the loads were 3.89 log (CFU/ml) for *Enterobacteria* and 3.86 log (CFU/mL) for *Staphylococcus*. The counts observed in fresh pineapple juice sample were 6.58 log (CFU/mL), 6.13 log (CFU/mL), 5.27 log (CFU/mL), 5.38 log (CFU/mL), 6.02 log (CFU/mL), and 4.76 log (CFU/mL) for AM, LAB, Yeast, AAB, *Enterobacteria*, and *Staphylococcus* respectively. During storage at ambient temperature, the microbial counts of AM, Yeast, and LAB increased after 24 h for pineapple juice and after 24 or 48 h for orange juice. A gradual decrease in TSS value was observed from 10.2 to 9.2 °Brix for the orange juice and from 13.3 to 11.1 ° Brix for pineapple juice. With regard to volatile compounds in orange juice, increases in concentration of acetic acid, 2,3-butanediol, octanol, phenylethyl alcohol, ethyl decanoate, ethyl dodecanoate were observed in deteriorated juices. For pineapple juice acetic Acid and 2,3-Butanediol were observed quantitative modification. These compounds can therefore be considered as indicators of spoilage in orange and pineapple juices.

Keywords: Pineapple juice, orange juice, spoilage, Volatile organic compounds, sensory analysis.

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INTRODUCTION

Fruit juices play an important role in the modern diet of people from different communities and social classes around the world. These juices are fermentable liquids extracted from the edible part of healthy, ripe, fresh fruit [1]. Fresh fruit juices are very popular with the public because of their pleasant aroma and flavour and their many functional properties [2]. According to [3], all natural fruit juices contain more than 5% of the recommended dose of vitamin C, folic acid, magnesium, and potassium. For example, the daily intake of orange juice improved blood biochemical parameters, such as low-density lipoprotein-cholesterol, glucose, and insulin sensitivity and positively modulated the composition and metabolic activity of microbiota, increasing the population of fecal *Bifidobacterium* spp. and *Lactobacillus* spp [4]. In citrus juice, [5] reported the presence of antioxidant, anti-inflammatory, anti-tumour, anti-fungal, and blood coagulation inhibiting activity, as well as numerous bioactive compounds such as ferrulic acid, hydrocinnamic acid, cyanidin glucoside, hisperidin, vitamin C, carotenoids, and naringin. In view of all these properties, fruit juices are good for health and considerably reduce the risk of illness [6].

In Côte d'Ivoire, the leading fruit exporter in West Africa, some of the fruit produced, notably oranges and pineapples, was used to produce juice in the traditional way along the streets. In the economic capital (Abidjan), every crossroads and backstreet has its own stand selling natural drinks at 0.76 ϵ . To obtain the juice, the fruit was washed, squeezed, filtered and bottled in a matter of seconds, before being sold to the consumer on the spot. The task was to place the cut piece of fruit inside the homemade juice machine and pull down the other handle to extract the juice.

Fruit juices were regularly consumed at traditional ceremonies (weddings, births, christenings, etc.), religious celebrations, seminars and workshops. Despite their socio-economic importance, these juices are less stable after production and can rapidly lose their organoleptic characteristics due to the proliferation of spoilage micro-organisms [7]. According to [8], spoilage of juices, resulting in changes to organoleptic and physico-chemical parameters, leading to rejection of the product by consumers and financial losses for producers. This was characterized by the production of unpleasant flavors, color change and product deterioration. This results in heavy losses being incurred by the local brewers since the unsold batches have to be discarded. So, the challenge for producers is to find an effective way of extending the shelf life of juices.

Shelf life of any product can be determined by monitoring physical, chemical, microbiological and sensory changes occurring to the food during storage whereby measurable deterioration characteristics maybe chosen [9].

In pineapple and orange juices no investigation has dealt in detail with the indicators that can be used to

characterize the alteration. Therefore, this study attempts to investigate the effects of different storage conditions on sensory, microbiological, biochemical stability in order to screen the deterioration markers of orange and pineapple juices during storage.

MATERIALS AND METHODS:

Samples Collection and Storage Conditions:

Untreated orange and pineapple juices sold at the roadside of Abidjan (Côte d'Ivoire) were purchased from three producers. Immediately after collection, the samples were cooled with ice and transported to the laboratory within 2 h. Each sample was subdivided into volumes of 200 mL in sterile bottles and stored at ambient temperature (28 to 30°C) or 4°C in a refrigerator. The juices were analyzed on 0, 8, 24, 48, and 72 h. All experiments were carried out independently in triplicate.

Enumeration of Microorganisms:

One mL of each sample was subjected to serial dilution and microbiological analysis was carried out immediately on the following media: plate count agar (PCA; Conda, Madrid, Spain) for aerobic mesophilic bacteria (AM), Man Rogosa and Sharpe agar (MRS; Biokar Diagnoses, France) supplemented with cycloheximide for lactic acid bacteria (LAB), Sabouraudchloramphenicol Agar (Biorad, France) for yeast, and GYC agar (glucose 50 g/L; yeast extract 10 g/L; CaCO₃ 3 g/L; agar 15 g/L) supplemented with nystatin and penicillin for the isolation of acetic acid bacteria (AAB). The plates for LAB were incubated anaerobically for 48 h at 30°C; those for AM and yeasts were incubated for 48 to 72 h at 30°C, while the plates for AAB were incubated for five to seven days at 30°C.

Physico-Chemical Analysis:

The pH was determined using a digital pH-meter (P107 Consort). The total titratable acidity (TTA) was determined by titrating 5 mL of the sample against 0.1 M NaOH using phenolphthalein as the indicator. The total titratable acidity was calculated as the percentage of lactic acid. The Total Soluble Solids (TSS) content, expressed as Brix value, was measured by a hand refractometer (Atago, Japan).

Volatile Compounds Analysis:

The silica fiber 65 μm PDMS/DVB purchased from Supelco (Supelco, Sigma-Aldrich, France) were used for extraction of volatile compounds in this study, following the method previously described by [10] with slight modifications. Before extraction, the fiber was preconditioned at 2500C for 30 min in the injection port of the GC as indicated by the manufacturer. Extractions were performed by placing in a 20 mL glass vial 1 g of sample, 1 g of NaCl, and 3 µL of 3-heptanol (concentration of 2.454 g/L) used as an internal standard (IS) for semi-quantification of VOCs. The vial was tightly capped with a PTFE-silicon septum on a

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heating platform. The SPME fiber was exposed to the headspace for 30 min at 60 °C with agitation at 400 rpm. Then the fiber was removed and the components were desorbed in the GC injection port for 5 min.

For separation of volatile compounds, the GC MS system used was a PerkinElmer Clarus SQ 8C GC/MS with an Agilent 5975 mass spectrometer. A DB-5MS capillary column (5% diphenyl cross-linked 95% dimethylpolysiloxane, 30 m \times 0.25 mm \times 0.25 µm) (Agilent Technologies, Santa Clara, CA, USA) was used and the separation conditions were as follows: initial column temperature of 40°C for 2 min, then increased by 4 ◦ C/min to 130°C for 1 min, then increased by 7°C/min to 230°C, where it was maintained for 4 min. High purity Helium (99.999%) was used as the carrier gas at a flow rate of 1.2 mL per minute. The temperature of the transfer line was set at 250°C, and the temperature of the ion source at 230°C. The ions were generated by a 70 eV electron beam. The mass range was scanned from m/z 33 to 500 Da.

The volatile compounds were identified by comparing the mass spectra with commercial database Wiley275 or using the NIST mass spectral search program (NIST/EPA/NIH database version 2.0). Then, the retention indices of the compounds were calculated relative to the n-alkanes (C8–C20) and compared to those of compounds in the NIST online database (https://webbook.nist.gov/chemistry/cas-ser.html,

accessed on 27 August 2021). Peak areas were used for relative quantification using the MSD Chemstation software (version E.02.02.1431, Agilent Technologies).

Sensory Evaluation:

Sensory analysis was performed following the method used by [11]. A panel was composed of 10 semi-trained judges (four females and six males), 20–30 years of age (mean = 24), all belonging to Nangui Abrogoua University. They were familiarized with the scoring scale and the sensory attributes to be evaluated during the preliminary training session. At each testing session, the judges were served a plate containing randomized threedigit coded cups with juice samples. A sample of a fresh Juice was used as the reference. The panelists noted the samples for odor, appearance and taste using a threecategory rating scale corresponding to fresh (samples similar to control juice or without any off-odor), marginal (sample having slight difference or slight offodor but still being acceptable), and deteriorated (sample largely different from control juice or producing strong off-odor). Percentages were obtained by dividing the number of panelists in a category by the total number of panelists. The juice has been classified in a category when at least 50% of the panelists evaluated the samples in this category. The experiment was conducted in triplicate for each sample with three independent tests.

Statistical Analysis:

Analysis of variance (ANOVA) and Tukey HSD tests were performed with XLSTAT software 2014 (Addinsoft Inc., Brooklyn, NY, USA) to compare the variables analyzed on the fresh and deteriorating samples. Statistical differences with $p < 0.05$ were considered significant. Then the parameters with variation after 48 h storage at ambient temperature were submitted to a Principal Component Analysis (PCA).

RESULTS AND DISCUSSION:

Microbial Flora:

The initial microbial counts of the orange juice sample were 4.44 log (CFU/mL), 4.60 log (CFU/mL), 4.98 log (CFU/mL), and 4.15 log (CFU/mL)for AM, LAB, Yeast, and AAB respectively (**Fig.1**).The counts observed in fresh pineapple juice sample were 6.58 log (CFU/mL), 6.13 log (CFU/mL), 5.27 log (CFU/mL), 5.38 log (CFU/mL) , 6.02 log (CFU/mL) , and 4.76 log (CFU/mL) for AM, LAB, Yeast, AAB, *Enterobacteria*, and *Staphylococcus* respectively (**Fig. 2**). Overall, the current study found AM count higher than the maximum permitted level of [12] (> 4 log CFU/mL). As for hygienic flora the loads were 3.89 log (CFU/mL) for *Enterobacteria* and 3.86 log (CFU/mL) for *Staphylococcus* in orange juice (**Fig. 1**) and respectively 6.02 log (CFU/mL) and 4.76 log (CFU/mL) for *Enterobacteria* and *Staphylococcus* in pineapple juice*.* [13] showed that fresh pineapple juice is generally not exempt from high levels of pathogenic as well spoilage microorganisms. The presence of these microorganisms is potentially dangerous to public health. [14] Point out that fruit juices facilitate the survival of foodborne pathogens due to their carbohydrate content. According to [15], street foods, particularly juices, were considered to be potential reservoirs of food-borne diseases as they contain high microbial loads due to poor hygiene practices.

During storage at ambient temperature, AM and LAB counts increased ($p < 0.05$) from 4.44 to 6.27 log (CFU/mL) and from 4.60 to 6.32 log (CFU/mL) after 48 h in the orange juice. An increase ($p < 0.05$) was noted for yeast count after 24 h of storage from 4.98 to 6.09log (CFU/mL). For pineapple juice sample stored at ambient temperature AM, LAB and yeast counts observed significant variation. These loads increased significantly from 6.58 to 8.20 log (CFU/mL) for AM and from 6.13 to 7.99 log (CFU/mL) for LAB after 24 h. For yeast, the counts increased from 5.27 to 6.53 log (CFU/mL) after 48 h. AAB counts slightly increased to reach maximum value of 6.53 log (CFU/mL) and 5.96 log (CFU/mL) respectively after 48 h of storage. These microorganisms, in particular yeast, LAB and AAB, can cause fruit juices to deteriorate. Indeed, microbial growth in fruit juices was often characterized by the production of unpleasant flavors and product deterioration that was commonly caused by yeast [16].

According to [17], yeasts were the main cause of deterioration of freshly prepared citrus juices.[18] reported that the metabolic activity of yeast at a cell

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concentration of up to 4 log (UFC/mL) is insufficient to produce any appreciable difference in food. In orange juice, [19] demonstrated that detectable spoilage required a concentration of yeast that was, approximately 5–6 log (CFU/mL). In addition to deteriorating the product, high counts of yeast can have adverse health effects in consumers [20]. Elsewhere, [21] reported that the metabolic activities of mesophilic lactic acid bacteria are primarily responsible for spoilage. According to these authors, lactic acid bacteria, along with other undesirable bacteria (*Acetobacter*), produce acetic acid, volatile off-flavors, fruity odors, and pellicles, which render the taste, odor, and texture of the beer unacceptable to consumers.

Fig. 1: Evolution of microbial counts (log CFU/mL) in artisanal orange juice during the storage at ambient temperature (A) and 4°C (B)

Fig. 2: Evolution of microbial counts (log CFU/mL) in artisanal pineapple juice during the storage at ambient temperature (A) and 4°C (B).

Changes in pH, Total Acidity, and Soluble Solids:

As shown on **Fig. 3**, the fresh orange juice pH, TTA, and TSS was 3.4, 1.3%, and 10.2 °Brix respectively. These values are in agreement with those of [22] who reported 3.23, 1.06%, and 8.20 °Brix for the pH, titrable acidity, and total soluble solids respectively. The initial values of the pH, TTA, and TSS of the pineapple juice were 4.0, 0.7%, and 13.3 °Brix respectively (**Fig.4**). These values were not different from 3.75 for the pH, 0.47% for the TTA, and 9.5 °Brix for the TSS reported by [23]. During the storage at ambient temperature, a gradual decrease in TSS value was observed from 10.2 to 9.2 °Brix for the orange juice and from 13.3 to 11.1 °Brix for pineapple juice. The change in TSS may be attributed to the presence of the microorganisms that cause the fruit juice to deteriorate as a result of sugar fermentation [24 & 25] The TTA values also remained stable but the pH value slightly increased to 3.6 after 24 h and then reached to 3.7 after 48 h in orange juice. For pineapple juice the pH significantly decreased after 48 h of storage to reach 3.6 and TTA observed significant increase passing from 0.7 to 1.4% after 48 h of storage, and then 2% after 72 h. These variations could have an impact on the organoleptic characteristics of orange and pineapple juices, because according to [26] the acidity in fruit juice reflects fruit taste, smell and aroma.

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From production to the 72 h of storage at 4°C no significant variation ($p \ge 0.05$) was observed in the pH, TTA and TSS of the juices.

Fig.3: Changes in pH, Total Acidity (%), and Soluble Solids (° Brix) of artisanal orange juice during the storage at ambient temperature and 4°C

Fig.4: Changes in pH, Total Acidity (%), and Soluble Solids (°Brix) of artisanal pineapple juice during the storage at ambient temperature and 4°C

Evolution of volatile compounds:

In fresh orange juice, a total 24 volatile compounds were identified (**Fig.5 & 6**). Compounds were classified to different chemical classes (organic acids, alcohols, Esters, ketones, aldehydes, and terpenes). Of the 24 compounds, 2 organic acids, 5 alcohols, 7 esters, 4 ketones, 2 aldehydes, and 4 terpenes were identified. During storage at ambient temperature (**Fig. 5**), five compounds notably 2,3-butanediol, octanol, phenylethyl alcohol, ethyl decanoate and ethyl dodecanoate appeared after 24 h. For compounds initially present in fresh juice, the concentration of acetic acid varied significantly (p <0.05). The value increased from 5.4 to 108.5 µg/L after 48 h of storage. The concentration of isobutyl alcohol was also increased from 0.4 to 1.2 µg/L after 24 h of storage. Among esters family, the concentration of ethyl ethanoate, ethyl butanoate, ethyl octanoate were increased after 24 or 48 h of storage. Furthermore, the concentration of methyl vinyl ketone increased regularly from 1.6 to 6.1 µg/L after 24 h, and then reached 35.5 µg/L after 48 h. For terpene compounds, the concentration of D-Limonene was decreased from 733.8 to 228.0 µg/L during the storage. At 4°C (**Fig. 6**), only the concentration of isobutyl alcohol increased after 24 h. On contrary, the concentrations of ethyl hexanoate and methyl butanoate decreased after 24 h. However, composition of volatile compounds was modified by the production of octanol and ethyl decanoate.

A total of 24 volatile compounds were identified in fresh pineapple (**Fig. 7 & 8**). The compounds included 2 organic acids, 5 alcohols, 7 esters, 5 ketones, 2 aldehydes and 2 terpenes. Among the organic acids, the concentrations of acetic acid and hydroxy benzoic acid showed significant variations during the storage at ambient temperature. Indeed, acetic acid concentration passing from 292.24 to 411.14 µg/L after 48 h and hydroxy benzoic acid concentration decreased from 4.89 to 2.39 after 24 h. For alcohol compounds, the concentration of 2,3-butanediol was increased from 11.85 to reach 31.55 µg/L after 48 h. On contrary, the concentration of some ester compounds such as ethyl ethanoate, ethyl butanoate, ethyl octanoate and methyl butanoate observed gradual decrease during the storage. For pineapple juice stored at 4°C (**Fig. 8**), concentrations of 2,3-butanediol, ethyl hexanoate, ethyl octanoate, 2 pentanone and benzaldehyde showed a significant increase ($p \ge 0.05$).

With regard to volatile compounds in orange juice, increases in concentration of acetic acid, 2,3-butanediol, octanol, phenylethyl alcohol, ethyl decanoate, ethyl dodecanoate were observed. For pineapple juice acetic Acid and 2,3-Butanediol were observed quantitative modification. The modification of these volatile compounds content was strictly linked to microbial metabolism [27 & 28]. Any, or a combination, of these modifications could be responsible for the spoilage. Among the compounds modified during storage of pineapple and orange juices, acetic acid was considered to be an indicator of food spoilage. Thus, according to [29], acetic acid is the main volatile acid in fermented beverages, and it is recognized as one of the by-products that have the most negative effects.

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Fig.5: Concentrations (µg/L) of major (A) and minor (B) volatile compounds identified in orange juice during storage at ambient temperature

Fig. 6: Concentrations (µg/L) of major (A) and minor (B) volatile compounds identified in orange juice during storage at 4°C

Fig.7: Concentrations (µg/L) of major (A) and minor (B) volatile compounds identified in pineapple juice during storage at ambient temperature

Fig. 8: Concentrations (µg/L) of major (A) and minor (B) volatile compounds identified in pineapple juice during storage at 4°C

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Principal components analysis:

For orange juice, principal component analysis (PCA) explained 100% of the total variance on the two axes Dim 1 and Dim 2 (**Fig.9A**). Dim 1 opposed the fresh juice (0 h) locating on the left part with deteriorated juice (48 h) locating on the right part. All the variables were positively correlated to this axis accepted ESR and Dlimonene which were negatively correlated (**Fig.9A**). Thus, deterioration of orange juice was characterized by a decrease in ESR and D-limonene content, and on contrary, an increase in concentration of esters (ethyl octanoate, ethyl butanoate, ethyl decanoate, ethyl ethanoate), alcohols (isoamyl alcohol, isobutyl alcohol, octanol, 2-3 butanediol, phenethyl alcohol), methyl vinyl ketone, acetic acid, pH and microbial counts (GAM, Yeast, LAB).

For pineapple juice, Dim 1 opposed the fresh juice (0 h) locating on the right part with deteriorated juice (48 h) locating on the left part (**Fig. 9B**). The variables that contributed negatively to this axis were GAM, LAB, Yeast, acetic acid, 2-3 butanediol. These variables characterized the deteriorated juice and their content increases during storage. On contrary the variables that characterized the fresh juice were ethyl octanoate, ethyl butanoate, ethyl ethanoate, Methyl vinyl ketone, methyl butanoate 3 hydroxybutanone and hydroxybenzoic acid. Their content decreased during the storage.

These results confirm that qualitative and quantitative changes in volatile compounds were linked to microbial growth. To this end, [30] reported that microbial community plays a vital role in forming volatile compounds during storage.

Fig.9: Plot from principal component analysis showing the distribution of parameters with variation after 48 h storage at ambient temperature of orange juice (A) and pineapple juice (B)

Changes in the sensory quality of juices:

The results of the sensory evaluation of orange and pineapple juices during the storage at ambient temperature and at 4°C are presented in **Table 1 and 2.** For orange juice stored at ambient temperature, under 50% (mean percentage of nine independent trials, each assessed by 10 panelists) of the panelists noticed that the appearance was spoiled after the 72 h of storage, while over 50% judged appearance as marginal after 48 h of storage. After 72 h, 70% of panelists reported that the odor was spoiled. More than half of the panelists (52.4%) judged the taste as spoiled after 48 h of storage. For pineapple juice over 50% of the panelists recorded appearance, odor and taste as spoiled after 48 h of storage at ambient temperature and 13.6% judged it as fresh. In any case, the number of panelists who judged attributes as fresh decreased continuously with time. The sensory rejection time, defined as the time when at least 50% of the panelists evaluated the attributes of juice has spoiled. Thus, orange and pineapple juices were spoiled after 48 h of storage at ambient temperature (28 to 30°C).

The results of present investigation are in line with the findings of [31] for the Ivorian sorghum beer tchapalo. Evaluating the appearance, smell and taste of the beer, a panel considered the beers to be spoiled after two days when stored at 28 to 30°C. The change in sensory quality during storage of orange and pineapple juices could be attributed to the reduction in TSS and the change in acidity and volatile compound content [32].

At 4°C less than 20% of the panelists were judged orange juice attributes as spoiled during the 72 h of storage. Taste and odor were judged as marginal by over 50% after 48 h of storage. At 4°C, orange and pineapple juices were not judged as spoiled after the 72 h of storage, these results also show that refrigeration extends the shelf life of fruit juices beyond 72 hours.

Table 1: Notes (in % of panelists) of sensory analysis of artisanal orange juice during the storage at ambient temperature and 4°C

Table 2: Notes (in % of panelists) of artisanal pineapple juice during the storage at ambient temperature and 4°C

CONCLUSION

The deterioration of orange and pineapple juices during the storage at ambient temperature is due to the presence of aerobic mesophiles, yeasts, LAB and AAB that produce volatile compounds such as acetic acid, 2,3- Butanediol, octanol, phenylethyl alcohol, ethyl decanoate, ethyl dodecanoate. These compounds can be considered as markers of spoiled orange and pineapple juices. In addition, changes in most of the biochemical parameters with storage time were relatively slow at the lower temperature. Whereas the refrigeration conditions are difficult to create in the conditions in which fruit juices were produced on the streets, the use of bacteria as bio-preservatives could be explored.

Acknowledgment: The authors would like to thank the judges and the members of UMR QualiSud (CIRAD, Montpellier) for their technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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