

Effects of artisanal practices on the microbiological and physicochemical properties of local pineapple juice

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ABSTRACT: Pineapple, one of the most popular tropical fruits, is consumed in various forms including pasteurized pineapple juice. However, the heat treatment applied during pasteurization has an impact on the microbiological and nutritional qualities of these juices. This study consisted of evaluating the effects of artisanal practices on the microbiological and physicochemical properties of two pasteurized pineapple juice (70, 80 and 90°C for 10, 15 and 20 min). The produced juice had a satisfactory microbiological quality according to KEBS criteria. Physicochemical analyses revealed that the pH was similar for both juices, however the Brix degree of the preheated juice was significantly high ($\approx 17.3 \pm 0.2$) than that of the non-preheated juice ($\approx 15.3 \pm 0.2$). The vitamin C content significantly decreased in the preheated juice (8.81 ± 0.22 mg/100 ml) than in the unheated one (11.45 ± 0.44 mg/100 ml) at the same time and as the time and temperature increased. Artisanal production practices allowed to obtain pineapple juice with a satisfactory microbiological quality but the nutritional quality was compromised.

KEYWORDS: Pineapple juice, pasteurisation, vitamin C, pH, ° Brix.

1 INTRODUCTION

Diet is a major determinant of maintaining the body in a satisfactory state of health. Nutritional recommendations were then formulated to encourage a healthy diet [1]. Various epidemiological studies have shown that low consumption of fruits and vegetables increases the risk of non-transmitted diseases in humans. To prevent these diseases, the World Health Organization recommends a daily consumption of at least 400 g of fruits and vegetables per person [2]. Among the fruits consumed, pineapple holds a special place. Pineapple, one of the most important tropical fruits in the world [3], is highly appreciated because of its flavor, its aroma, its richness in microelements and also its numerous functional properties [4]. Indeed, its richness in soft fibers (cellulose) facilitates intestinal transit and helps fight chronic constipation. The polyphenols it contains, coupled with vitamin C and beta-carotene, give it antioxidant properties [5]. Several sources claim that it has weight-loss properties, hence its appreciation by people wishing to follow a diet in this sense [3], [6]. Fruits are consumed in various forms: fresh or processed, for example sliced, canned, concentrated juice, pulp, dried pieces, pasteurized juice, etc [5]. Pasteurized juice is popular due to its ready availability to consumers [7]. The heat treatment to which pasteurized juices are subjected has the role of eliminating or reducing their microbial load in order to preserve them for a long period [4]. However, negative consequences of pineapple juice have been revealed in numerous research studies focusing mainly on organoleptic and nutritional changes, notably the loss of vitamin C [7], [8], [9] and non-enzymatic browning (Maillard reaction) in the final juice [7]. Mastery of this treatment then proves essential to offer consumers products of satisfactory microbiological and nutritional quality. The production of pasteurized pineapple juice represents a common activity in Togo as in several countries in the sub-region. However, this activity is mainly based on the use of artisanal production processes which often lack adequate control due to the lack of training of the producers [10]. Indeed, a study in reference [11] revealed a lack of standardization of

practices and allowed to distinguish different ways of producing local pineapple juice such as the number of heat treatments applied to the juice and the addition of ingredients (water, sugar). An investigation carried out by some authors in some processing units revealed that one or two heat treatments are applied during juice production [12]. Furthermore, one heat treatment only involved a pasteurization step while two heat treatments involved preheating the unbottled juice followed by the pasteurization step. A control of these production practices carried out by these local processors is then necessary to assess the quality of the juices offered to the population. Therefore, the present study focused on the evaluation of the effects of artisanal production practices on the microbiological and physicochemical properties of local pineapple juice.

2 MATERIALS AND METHODS

2.1 MATERIALS

The pineapple used in this study was of the Brazza variety. These pineapples were obtained from a local supplier in Gbatopé (Prefecture de Zio, Togo).

2.2 METHODS

2.2.1 PRODUCTION OF RAW PINEAPPLE JUICE

Pineapple juice was produced according to the method in reference [13]. After reception, the pineapple was weighed and sorted to eliminate unripe and rotten fruit. After that, the crowns of the fruits were removed, and then followed by their washing. The washed pineapple was peeled and cut into small pieces then pressed using an electric press to obtain raw pineapple juice. This raw juice was filtered to remove the pulp.

2.2.2 PASTEURIZATION OF RAW PINEAPPLE JUICE

Part of the juice obtained was bottled and the other was heated at 100°C for 1 min according to the modified method of reference [13] before filling into the bottle. The both juices were pasteurized at 70°C, 80°C, 90°C for 10, 15 and 20 min. Heated and pasteurized juice (HPJ) and pasteurized juice (PJ) were used for microbiological and physicochemical analyses.

2.2.3 MICROBIOLOGICAL ANALYSES

The microbiological evaluation of the juices was carried out according to the routine standardized methods of the French Association for Standardization based on the KEBS criteria relating to fruits juices (Table 1) [14]. Total mesophilic aerobic flora (TMAF), total coliforms (TC), anaerobic sulfate-reducing germs (ASR) and yeasts and mold (Y&M) by the mass seeding technique. *Staphylococcus aureus* was enumerated by plating following the routine method [15]. The number of colonies was expressed in Colony Forming Unit per ml (CFU/ml) according to the AFNOR standardized formula:

$$N = \frac{\Sigma C}{V(n_1 + 0.1 n_2)d}$$

With:

N: number of Colony Forming Units per ml (CFU/ml), ΣC : sum of colonies counted on all the plates retained, v: Volume of the inoculum, n_1 : number of plates retained at the first dilution, n_2 : number of plates retained at the next dilution, d: dilution rate of the first dilution.

Table 1. Different food microbes enumerated in the chicken sausage samples

| Germ | Reference of the method used | Reactive culture media | Temperature/duration of incubation |
|------------------------------------|------------------------------|-------------------------------|------------------------------------|
| Total aerobic mesophilic (TAM) | NF EN ISO 4833-1 | Plate Count Agar | 30°C/24 -72hours |
| Total coliform (TC) | NF V08-050, 1992 | VRBL | 30°C/24hours |
| Anaerobic Sulphite Reductors (ASR) | NF EN ISO 15213 | Typotone sulfite Neomycine | 37°C/24-48hours |
| <i>Staphylococcus aureus</i> | NF EN ISO 6888-1 | Baird Parker | 37°C/24-48hours |
| Yeast and Molds (Y&M) | NF EN ISO 21517-152 | Sabourand Chloramphenicol + | 30°C/48-72hours |

2.2.4 PHYSICOCHEMICAL ANALYSES

2.2.4.1 DETERMINATION OF THE PH OF PINEAPPLE JUICE

The pH was determined according to the AOAC method [16] using an OAKTON pH 700 pH meter calibrated with pH 7 and 4 buffers. The pH measurement was obtained by immersing the pH meter electrode in a beaker containing the pineapple juice. At each pH determination, rinse the electrode with distilled water.

2.2.4.2 MEASUREMENT OF SOLUBLE DRY MATTER

The soluble dry matter, expressed as Brix degree (°Brix), of the produced pineapple juice was determined by the AOAC method [16] using a PAL-1 digital refractometer (ATAGO brand). A few drops of juice were placed using a Pasteur pipette on the glass part of the refractometer then we pressed the "Start" button on the device. The °Brix value is read directly on the device screen.

2.2.4.3 DETERMINATION OF VITAMIN C

Vitamin C content was measured using the modified method of reference [17]. 10 ml of sample were pipetted into an Erlenmeyer flask, 10 ml of the extraction solution (volume/volume mixture of 3% metaphosphoric acid and 8% acetic acid) was added to the sample. The mixture was homogenized and then stored for 30 min. Finally, the mixture is dosed with a 0.01 N iodine solution (I₂) in the presence of 0.5 ml of 0.5% starch.

The quantity of vitamin C (Vit C), expressed as mg of ascorbic acid in 100 ml of juice, was determined by the following formula:

$$\text{Vit C (mg / 100 ml)} = \text{Veq} * 8.806 * 10 / V$$

With: Veq: Volume in ml of iodine 0,01 N at the equilibria, V: Volume of sample in ml,

On the other hand, the percentage of preserved vitamin C (%Vit Cp) at each level of heat treatment is determined by the following formula:

$$\% \text{Vit } C_p = \left(\frac{C_f}{C_i} \right) * 100$$

With: C_i: initial Concentration of vitamin C, C_f: final Concentration of vitamin C

2.2.5 DATA ELABORATION

The collected data was elaborated in a Microsoft Excel database, version 2019. The GraphPad prism 8 software was used to carry out statistical analyses of these data. Multiple comparison tests of means were carried out using analysis of variance (ANOVA).

3 RESULTS AND DISCUSSIONS

3.1 MICROBIOLOGICAL QUALITY OF PINEAPPLE JUICE

The microbiological properties of the different categories of pasteurized pineapple juice have been presented in Table 1. The microbiological properties of the different categories of pasteurized pineapple juice determined after production revealed an absence of total coliforms, Anaerobic sulphite Reductors bacteria and *Staphylococcus aureus* in all juices. However, the presence of total mesophilic aerobic flora (TMAF) and yeasts and mould was noted in certain combination of temperature and time of pasteurization. Indeed, the analyses carried out revealed the presence of TMAF during pasteurizations carried out at 70°C for 10, 15 and 20 min and 80°C at 10 and 15 min for the two samples. On the other hand, for the other combined temperature and time of pasteurization, we did not find the TMAF. Compared to yeasts and molds, microbiological analyses revealed a decrease of 85 CFU/ml (70°C-10 min) at a load lower than 1 CFU/ml from 80°C-20 min in PJ and from 4 CFU/ml (70°C-10 min) at a load less than 1 CFU/ml from 70°C-15 min in HPJ. The total absence of total coliforms and ASR in the juices demonstrated the effectiveness of the pasteurization parameters on these groups of germs. However, the total mesophilic aerobic flora, yeasts and molds were observed at certain pasteurization parameters, but their values were well below than those set by the KEBS criteria (10⁵ CFU/ml for TMAF and 100 CFU/ml for yeasts) [14]. That their presence had no importance on consumers health. Finally, a large reduction in the number of colonies of TMAF and that of yeasts and molds of HPJ compared to that of JP was observed. It appears from these analyses that all pasteurized juices had a satisfactory microbiological quality in relation to the criteria in force. These results demonstrated a better effect of the preheating and pasteurization on the microbiological quality of the juices. Concerning the microbiological point of view the combination of preheating and pasteurization is important to obtain the best results, which might be the principal objective of the artisanal producer of juice in developing countries.

3.2 PHYSICOCHEMICAL ANALYSES

3.2.1 PH

The pH values found for both samples are between 4.03 ± 0.02 and 4.06 ± 0.03, but no significant differences were observed. The pH values of our juices were included in the interval of 3.3 – 5.2 which is a reference interval set by the codex [18]. These results corroborate with those obtained by others authors [13] who found pH values varying between 3.90 ± 0.07 and 4.14 ± 0.13 for pasteurized pineapple juice at 75, 80 and 85°C for 5 min. Hydrogen potential is an overall expression of the acidity of a product, a parameter which helps preserve the microbiological quality of drinks by creating an unfavorable environment to bacterial growth and thus extending their shelf life [19]. These pH values from our study confirmed the microbiological results obtained by our pasteurization scales and could be stored at room temperature. Indeed, according to the reference [20], pasteurized fruit juices with a pH < 4.5 are stable at room temperature.

3.2.2 BRUX DEGRE

The soluble dry matter content commonly called Brix degree is an important parameter in food technologies and in particular in fruit juices. The results of Brix degree of the juices are shown in Table 2. The results showed that, the Brix degree of HPJ was significantly high (17.3 ± 0.2) compared to that of PJ (15.4 ± 0.2). This could be due to the phenomenon of evaporation of water contained in the pineapple juice during the preheating phase. This elimination of the water from the solution increases the amount of the dry soluble matter, hence the increase in the °Brix of HPJ compared to PJ. Reference [21] noted the increase in °Brix during the production of concentrated orange juice by osmotic evaporation. These phenomena are therefore observed during the manufacture of concentrated fruit juice [18]. In other hand, the results revealed that neither the time nor the temperature of the pasteurization had a significant effect on the soluble solids content of the juices. This is due the fact that the bottles are hermetically closed, that there is no exchange of material between the content of the bottle and the environment [22]. The Brix values of all the juices produced were greater than 12 which is a fixed reference value [18].

Table 2. Microbiological analysis of pineapple juice

| Criteria of (KEBS) | TMA (UFC/ml) | TC (UFC/ml) | Y&M (UFC/ml) | ASR (UFC/ml) | S. aureus (UFC/ml) |
|---|-------------------|-------------|--------------|--------------|--------------------|
| | 1.10 ⁵ | < 1 | 100 | <1 | NC |
| HPJ (heated and pasteurized juice) | | | | | |
| 70°C-10 min | 520 | <1 | 85 | <1 | <1 |
| 70°C-15 min | 210 | <1 | 51 | <1 | <1 |
| 70°C-20 min | 100 | <1 | <4 | <1 | <1 |
| 80°C-10 min | 150 | <1 | <4 | <1 | <1 |
| 80°C-15 min | 85 | <1 | <4 | <1 | <1 |
| 80°C-20 min | 13 | < 1 | < 1 | < 1 | < 1 |
| 90°C-10 min | <4 | <1 | <1 | <1 | <1 |
| 90°C-15 min | <1 | <1 | <1 | <1 | <1 |
| 90°C-20 min | <1 | <1 | <1 | <1 | <1 |
| PJ (Pasteurized juice) | | | | | |
| 70°C-10 min | 70 | <1 | <4 | <1 | <1 |
| 70°C-15 min | 20 | <1 | <1 | <1 | <1 |
| 70°C-20 min | 7 | <1 | <1 | <1 | <1 |
| 80°C-10 min | 9 | <1 | <1 | <1 | <1 |
| 80°C-15 min | <4 | <1 | <1 | <1 | <1 |
| 80°C-20 min | <1 | < 1 | < 1 | < 1 | < 1 |
| 90°C-10 min | <1 | <1 | <1 | <1 | <1 |
| 90°C-15 min | <1 | <1 | <1 | <1 | <1 |
| 90°C-20 min | <1 | <1 | <1 | <1 | <1 |

Table 3. pH and °Brix of the different samples. Different letters indicate significant differences among samples ($P \leq 0.05$) due to the process. where the letter « a » was attributed to the highest value

| Pasteurization parameters | pH | | °Brix | |
|---------------------------|---------------|---------------|----------------|----------------|
| | PJ | HPJ | PJ | HPJ |
| 70°C-10 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.10 ± 0.12 b | 17.15 ± 0.17 a |
| 70°C-15 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.25 ± 0.06 b | 17.30 ± 0.23 a |
| 70°C-20 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.45 ± 0.06 b | 17.40 ± 0.12 a |
| 80°C-10 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.60 ± 0.12 b | 17.50 ± 0.12 a |
| 80°C-15 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.90 ± 0.12 b | 17.55 ± 0.06 a |
| 80°C-20 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 16.00 ± 0.23 b | 17.95 ± 0.06 a |
| 90°C-10 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.80 ± 0.12 b | 17.85 ± 0.17 a |
| 90°C-15 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.95 ± 0.06 b | 17.95 ± 0.06 a |
| 90°C-20 min | 4.06 ± 0.01 a | 4.06 ± 0.01 a | 15.85 ± 0.17 b | 18.00 ± 0.23 a |

3.2.3 VITAMIN C

Figure 1 illustrated the data regarding the concentration of vitamin C. The vitamin C concentration of fresh pineapple juice was 34.75 ± 0.54 mg/100 ml. The vitamin C content in this study was lower than that measured by other authors, who reported that the content of vitamin C in pineapple was 49.38 ± 1.87 mg/100 ml [23] and 45.3 mg/100g [24]. This difference could be due to several factors such as the maturity of the pineapple, the variety, the climate, the geographical area, etc [25]. The concentration of the vitamin C in PJ was significantly high (20.69 ± 0.62) than in HPJ (18.05 ± 0.62) after their pasteurization at 70°C for 10 min. This difference might be associated to the preheating effect, which significantly decrease the concentration of the vitamin. According to the microbiological results, this treatment is not necessary in manner to limit the reduction of the vitamin C, which is an important parameter for the appreciation of the heat treatment effect of nutritional properties. In all samples, the results showed that at the same time of treatment, the vitamin C content significantly decreased in both juice as the processing temperature increased. At 70°C there was not a significant difference as the treatment time increased for PJ

but a significant difference was observed at 20 min for HPJ. At high temperature, 80°C and 90°C, the results highlighted that the vitamin C content significantly decreased in increasing the treatment time almost in all samples. These results showed a significant decrease in the concentration of ascorbic acid contained in the juices as a function of the increase in temperature or pasteurization time. Reference [13] also found that the concentration of vitamin C in pineapple juices pasteurized at 75, 80 and 85°C for 5 min varied between 4.52 ± 1.12 and 23.48 ± 5.37 mg/100 ml. This decrease could be explained by the fact that vitamin C is a thermolabile compound [7], [8].

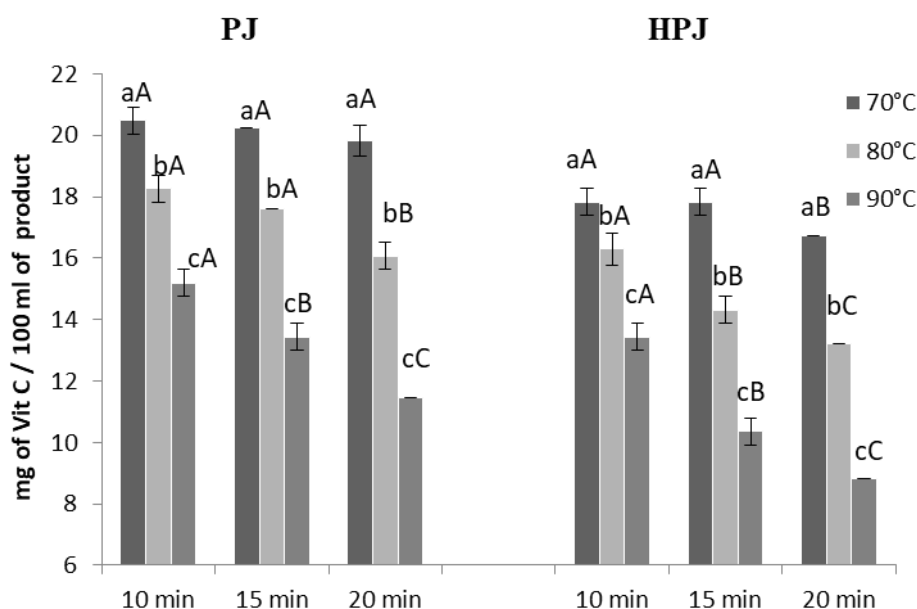


Fig. 1. Effect of thermal treatment on the vitamin C concentration. Different letters indicate significant differences among samples ($P \leq 0.05$), where the small letters indicate the effect of temperature and the big letters indicate the effect of time of the treatment. The letters "a" and "A" were attributed to the highest value

Figure 2 showed the results of preserved vitamin C. The results revealed a very considerable amount of vitamin C was destroyed. The treatment which allowed to preserved more than 50% of vitamin C 70°C at all times for the both samples except in the case of HPJ at 20 min, and also at 80°C for 10 min. The lowest quantity of preserved vitamin C was observed at 90° per 20 min in HPJ ($\approx 25\%$) and the highest amount was revealed in PJ at 70°C for 10 min ($\approx 58\%$). These results confirmed that the preheating might significantly affect the quality of the final product. This information might be useful in the developing of the new product in manner to define the best treatment to apply for preserving more nutritional thermolabile component. Vitamin C content is a key parameter for assessing the nutritional quality and potential health benefits from fruit juices [26]. Thus, this preheating would further reduce the nutritional quality of the juices. However, it should be noted that, despite this degradation, the quantities of vitamin C present in the different juices are all greater than 2 mg/100 ml, the minimum quantity set by the AFNOR standard relating to the commercial juice [27]. Considering the microbiological results in both samples and the data on vitamin C destruction, we might suggest than the preheating can be removed from the process, because it affects significantly the nutritional quality of the juice. The pasteurization of the bottled juice without preheating allows to reach the commercial criteria in microbiological and preserved more nutritional components.

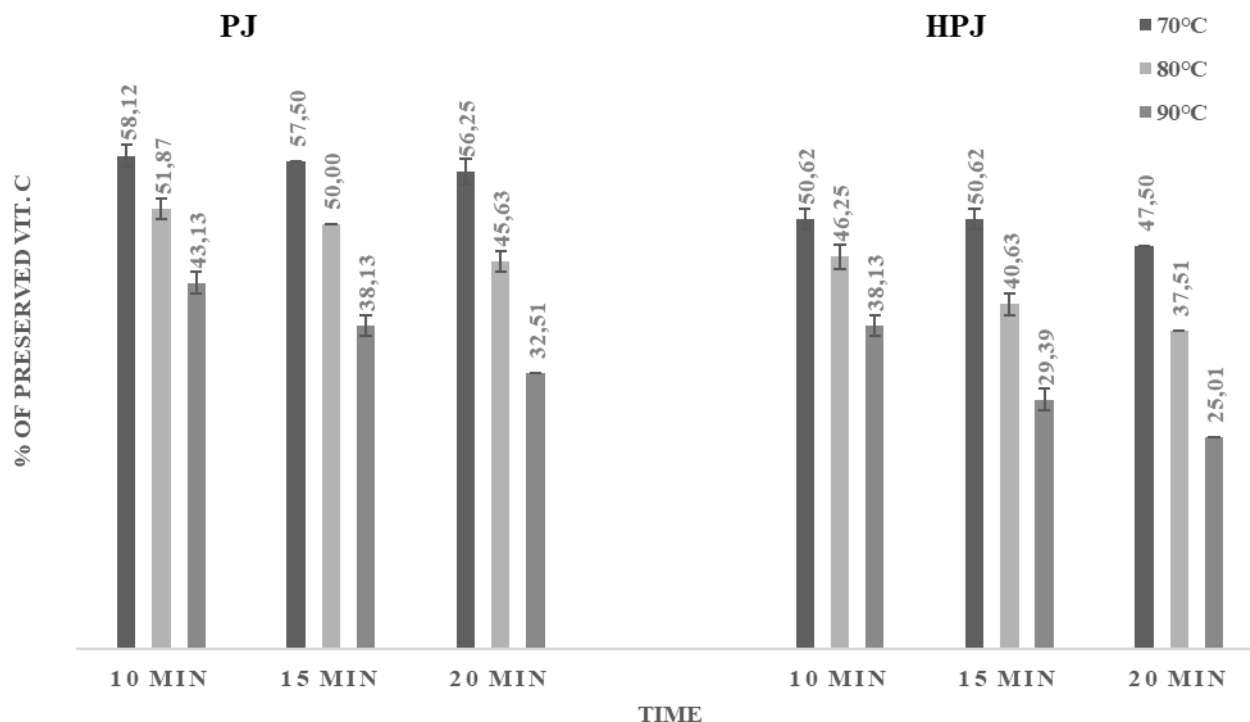


Fig. 2. Percentage of the preserved vitamin C in different pineapple juice

4 CONCLUSION

The quality of pineapple juice is largely influenced by the technology used during processing. Even if the preheating operation and pasteurization conditions (temperature and time) explored in this study guaranteed the microbiological quality of the juices, they had a negative impact on its nutritional value. Given the microbiological results obtained, non-preheated pineapple juices have of satisfactory microbiological quality compared to the criteria that is used for juice quality, the preheating phase could be removed from the production processes in order to avoid a large loss of nutrients.

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