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Microbial and Nutritional Stability of Pineapple Juice during Storage: Effect of Harmonized Thermal Pasteurization Technologies

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Abstract The effect of one or two thermal treatments during pineapple juice production was evaluated on pH, vitamin C and microbiological evolution of 6 categories of juice during 12 months of storage. Three pasteurization temperatures (75 ℃, 80 ℃, 85 ℃) combined with one (1T) or two (2T) thermal treatments defined the juice category. Storage test consisted of green-glass bottled juices packaged in closed boxes, kept at ambient temperature. Analyses were performed each 4 months from production date (0 month). As results, the juices pH was 3.90 - 4.14 after production and no significant variation ($p \geq 0.05$) occurred during storage, except for juices 80°C, 1T and 80°C, 2T at 12 month. The microbiological quality of all juices after production revealed conformity with standards. Enterobacteria and lactic acid bacteria were totally absent all the time. Mesophilic bacteria and yeasts and moulds counts generally decreased in each juice during storage. The initial vitamin C content significantly ($p<0.05$) varied from 4.52 to 23.48 mg/100ml in the juices and so decreased through storage. Juices pasteurized at 75 ℃ contained more initial vitamin C but their content was quickly lost. Vitamin C was more stable in the most thermally treated pineapple juices throughout storage, especially in juice 85 ℃, 2T.

Keywords: *pineapple, thermal treatments, storage ability, safety, vitamin C*

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1. Introduction

Pineapple juice is a low pH fruit juice with sweet taste and beneficial health compounds. These compounds include ascorbic acid, bromelain, carotenoids, phenolic compounds and flavonoids among others [1]. The fresh pineapple juice is a popular product due to its pleasant aroma, flavor and numerous functional properties [2]. Its content in vitamin C is particularly emphasizes and reported to largely vary from 9.2 to 93.8 mg/100mL [3]. But the juice shelf life is restricted by enzyme and microorganism activity [4]. This supposes that it should be adequately processed and stored to preserve its quality from spoilage. Pineapple juice processing represents a common activity in Benin and five categories of technology were identified by Gbaguidi [5] following classification criteria such as the presence of peeling unitary operation, the addition of ingredients (water, sugar, citric acid) and the number of thermal treatments. Considering the later criteria, investigations revealed that one to two thermal treatments are applied in the juice production. Two thermal treatments implied a preheating of the non-bottled juice to at least 70 ℃ followed by a pasteurization step with scales varying from 80 ℃ for 15 min to boiling in the pasteurization water. One thermal treatment implied the pasteurization step only [5]. Thermal pasteurization is considered the most effective technology in inactivating microorganisms and enzymes to extend product shelf life [6]. The consequences on pineapple juice have already been revealed in many research works mainly dealing with organoleptic and nutritional changes in the final juice, specifically the loss of vitamin C. It is also known that besides thermal processing, storage conditions significantly affect the quality of juices [7]. However, there have been to date no works found which evaluated the impact of the number of thermal treatments on pineapple juice quality during storage. Filling this gap will be of great importance to science but especially to Benin processors applying many thermal treatments during pineapple juice pasteurization.

For this purpose, the present study addressed the evolution of the microbial and nutritional quality of pasteurized pineapple juice for 12 months of storage.

2. Material and Methods

2.1. Phase of the Juices Production

Pineapple fruits of “Perolera” cultivar were purchased from fields in the locality of Zè in the southern Benin. They were processed as described in Figure 1, using one or two thermal treatments (1T or 2T) as to fit the number of heating treatments applied to pineapple juice in Benin [5]. The preheating in two thermal treatments technology (2T) was set to 60℃. Three pasteurization temperatures (75℃, 80℃ and 85℃) were considered so that 06 categories of pasteurized pineapple juice were produced in all (see Figure 1). Each production was repeated once using fruits of the same variety. All thermal treatment was performed with a pasteurizer described in photo 1. The temperature of the pasteurization water and the one inside the bottled juices were recorded and controlled using ibutton devices (photo 2). Recorded data from preliminaries revealed that target temperatures in the water were maintained for 5 min on the average in the juice, after the pasteurizer being switched off for water emptying. This procedure was then adopted to consider the 5 min as pasteurization scale time (see Figure 1).

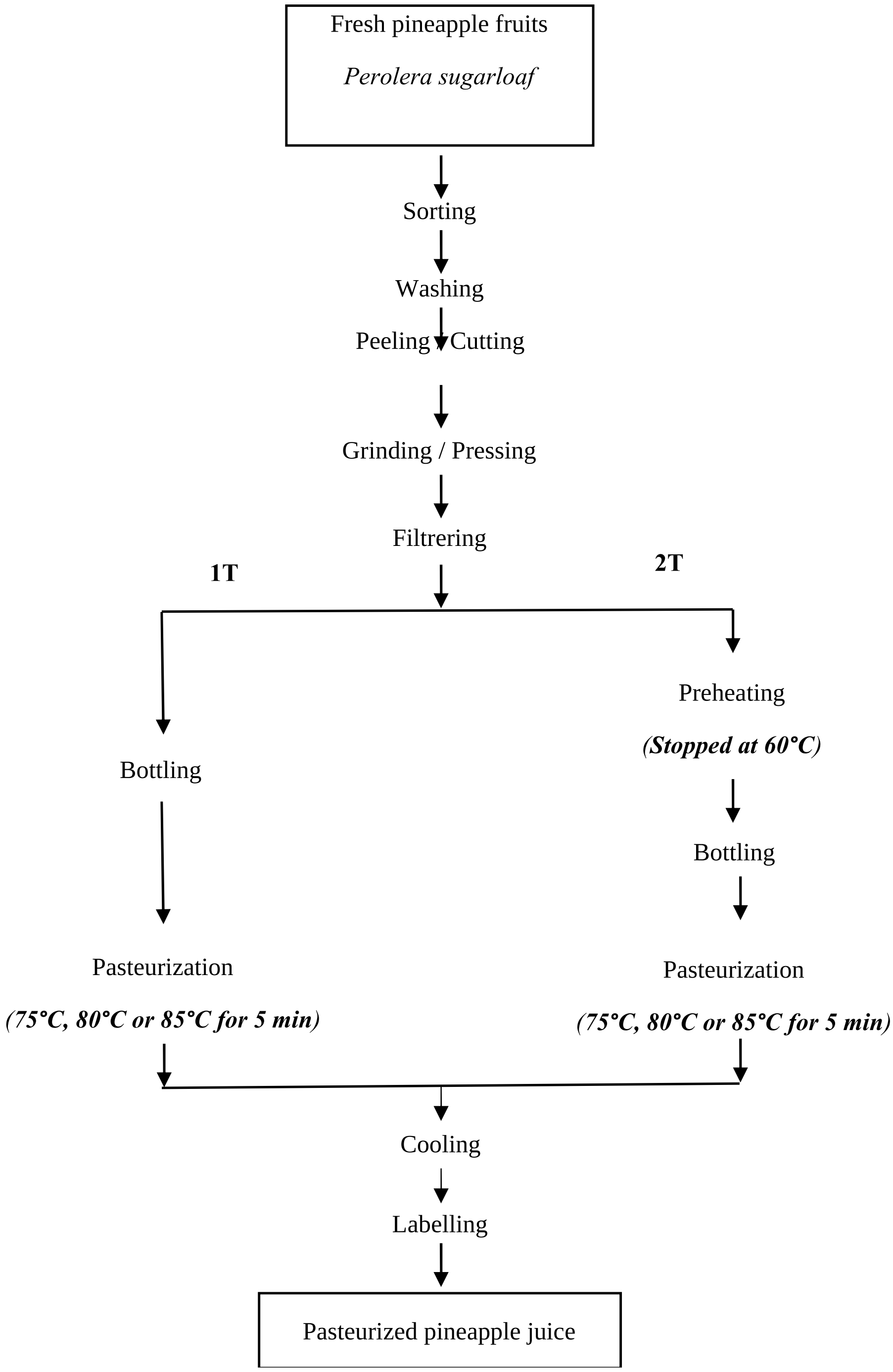

Figure 1. Flow chart for the production of 6 categories of pasteurized pineapple juice

Legend :

1- Needles-thermometer

2- Gaz burners

3- Rotating stirring lever

4- Water level established to 10 cm to the side up

5- Bottled juices inside the pasteurization water

6- Orifice for juice collection or water emptying

7- Gaz source

Photo 1. Pasteurizer charged with bottled juice

Characteristics of the pasteurizer:

Length: 1.65 m

Width: 0.76 m

Height: 0.37 m

Capacity: up to 650 juice bottles of 25 cl

Photo 2. Ibutton device for temperature recording

2.2. Sampling and Storage Design

The pasteurized pineapple juices were pre-packaged in green-glass bottles during production. The bottled juices were sampled after production and analyzed while the rest of each lot of production went through a storage test for 12 months. Some conditions were observed and maintained during storage. As such, the bottled juices were packaged in boxes coded with production details (the date of production). The number of thermal treatment and the temperature of pasteurization). The boxes were stored at ambient temperature (28 - 35℃) in a warehouse until all analysis was performed. Data collected on the juices after production corresponded to the juice's characteristics at 0 month. Further samplings took place at the 4th, 8th and 12th month of storage. All analyses were performed in triplicate with randomly selected bottled juices.

2.3. Analysis of the Pasteurized Juices Quality

2.3.1. Chemical Analysis

The pH of the pasteurized pineapple juices was measured according to the method ISO [8] "Fruits and vegetable products— Determination of pH" using an electronic pH meter (Eutech, Cybernetics, Singapore). Vitamin C content in its reduced form (ascorbic acid) was determined by titrimetic method ISO [9] with 2.6-dichlorophenolindophenol (2.6-DCPIP) dyestuff. Samples were first filtrated, after what a portion of 5 ml was diluted with 5ml of 2% (m/m) oxalic acid and titrate with the dyestuff solution until a salmon pink coloration persisting for at least 5 s, is obtained. The results were expressed as mg/100ml of juice.

2.3.2. Microbiological Analysis

Microbiological analyses were used for the microbial assessment of the juices, from production to the end of storage test. The samples were analyzed in triplicate for aerobic mesophilic total bacterial counts, yeasts and moulds, lactic acid bacteria and enterobacteria following methods ISO [10,11,12], [[13], p. 21] respectively.

2.3.3. Determination of Treatments Efficiency on Vitamin C Preservation During Storage

Vitamin C preservation has been evaluated according to the evolution of its content in each of the 6 categories of pasteurized pineapple juice. To that purpose, vitamin C retention rate corresponding to the percentage of that nutritional substance remaining in each juice at a storage time (*t*), was determined following the formula 1:

$$\mathit{Vitamin\ C\ retention}_t(\%) = \frac{\text{Vitamin C content in a juice at storage time}(t)}{\text{Vitamin C content in the juice at0month}} \times 100 \quad (1)$$

The obtained retention rates were used as response to analyze the nutritional stability of the juices during time and to determine their shelf life.

3. Results and Discussion

3.1. Quality of the Pasteurized Juices after Production

The chemical and microbiological characteristics of the 6 categories of pasteurized pineapple juice were determined after production. According to Table 1, there was no significant difference ($p \geq 0.05$) between the 6 juices pH while vitamin C content significantly varied ($p < 0.05$).

The juices pH was comprised between 3.90 and 4.14. Juice 75 ℃, 1T contained more vitamin C (23.48 mg/100ml) than all the other juices at 0 month. In terms of microbiological quality, lactic acid bacteria and enterobacteria were absent in the 6 juices whereas mesophilic bacteria as well as yeast and moulds were present at counts of 0.39 to 1.35 $\log_{10}$ CFU/ml and 0.65 to 1.88 $\log_{10}$ CFU/ml respectively. The absence of enterobacteria suggests the applied thermal treatments efficacy, but also reflects good manufacturing practices and adequate cleaning procedures. The absence of lactic acid bacteria would be explained by their intolerance to thermal treatments. Indeed, thermal pasteurization of juices is able to destroy them as reported by some authors.

To the present study authors' knowledge, there was no specification set for the permissible level of microbes in pasteurized fruit juices in Benin. Hence, some recommended specifications for fruit juices were used to evaluate the juices safety. The mean counts of mesophilic bacteria do not exceed the maximum acceptable limit ($3\log_{10}$ CFU/ml) of bacteria in food products recommended by the International Commission on Microbiological Specification of Foods [14]. They also fit the recommendations for microbiological limits in fruit juices and nectars (< $3\log_{10}$ CFU/g) according to [15] and the microbial standards for any fruit juices sold in Gulf region (< $4\log_{10}$ CFU/ml). The results for yeasts and moulds were lower than the limit (< $2\log_{10}$ CFU/ml) permitted by Gulf region (Gulf Standards, 2000) and the acceptable maximum counts (3 $\log_{10}$ CFU/mL) in fruit juice and nectars stated by Turkish standards [16]. As such, all the 6 juices were highly safe for consumption and the acidic nature of pineapple fruit juice greatly contributed to these results.

3.2. Evolution of the Juice's pH and Microbial Quality During Storage

From production to the 12th month of storage, no significant variation ($p \geq 0.05$) was determined in the pH of the juices, except for 80 ℃, 1T and 80 ℃, 2T (Table 2). Indeed, the pH of these juices were significantly lower at the 12th month (3.52 ±0.12 and 3.44 ±0.12 respectively for 80 ℃, 1T and 80 ℃, 2T) than their value before storage (3.96 ±0.08 and 4.01 ±0.01 respectively). Insignificant pH changes were similarly reported by [4] in thermally pasteurized pineapple juice stored at 4 ±1 ℃ for 13 weeks. Wibowo *et al.* [17] also reported the same trend in thermally pasteurized orange juices stored at 20 ℃, 28 ℃, 35 ℃ and 42 ℃. On the other hand, the significant decrease in the pH of juices 80 ℃, 1T and 80 ℃, 2T might be due to possible biochemical reactions. Such decrease was also found by Lagnika *et al.* [1] in thermally pasteurized pineapple juice stored at room temperature (26 ℃ ± 2 ℃).

Table 1. pH, vitamin C and microbiological counts in the juices at 0 month

Categories of juices		Chemical properties		Microbiological properties	
Temperature	Technology	pH	Vitamin C (mg/100ml)	Mesophilic bacteria ($\log_{10}$ CFU/ml)	Yeasts and moulds ($\log_{10}$ CFU/ml)
75 ℃	1T	3.92 ±0.01 ^{a}	23.48 ±5.37 ^{a}	1.35 ±0.19 ^{a}	1.88 ±0.00 ^{a}
80 ℃	1T	3.96 ±0.08 ^{a}	11.42 ±2.19 ^{ab}	1.29 ±0.07 ^{a}	1.44 ±0.06 ^{a}
85 ℃	1T	4.14 ±0.13 ^{a}	6.30 ±1.49 ^{b}	0.55 ±0.21 ^{a}	1.15 ±0.21 ^{a}
75 ℃	2T	3.90 ±0.07 ^{a}	18.02 ±6.88 ^{ab}	0.54 ±0.87 ^{a}	1.81 ±0.02 ^{a}
80 ℃	2T	4.01 ±0.01 ^{a}	4.52 ±1.12 ^{b}	1.01 ±0.05 ^{a}	1.35 ±0.07 ^{a}
85 ℃	2T	4.13 ±0.14 ^{a}	5.60 ±2.39 ^{b}	0.39 ±0.55 ^{a}	0.65 ±0.92 ^{a}

Means with the same letter in column do not significantly differ at 5% level.

Table 2. Effect of the treatments on the pH of the juices

Temperature	75 ℃	80 ℃	85 ℃	75 ℃	80 ℃	85 ℃
Technology	1T	1T	1T	2T	2T	2T
0 month	3.92 ±0.01 a	3.96 ±0.08 a	4.14 ±0.13 a	3.90 ±0.07 a	4.01 ±0.01 a	4.13 ±0.14 a
4 month	3.78 ±0.06 a	3.72 ±0.00 ab	3.85 ±0.08 a	3.75 ±0.06 a	3.72 ±0.05 ab	3.88 ±0.17 a
8 month	3.84 ±0.13 a	3.96 ±0.11 a	3.93 ±0.02 a	3.72 ±0.02 a	3.91 ±0.10 a	3.96 ±0.02 a
12 month	3.64 ±0.17 a	3.52 ±0.12 b	3.72 ±0.26 a	3.61 ±0.15 a	3.44 ±0.12 b	3.69 ±0.25 a

Means with the same letter in column do not significantly differ at 5% level.

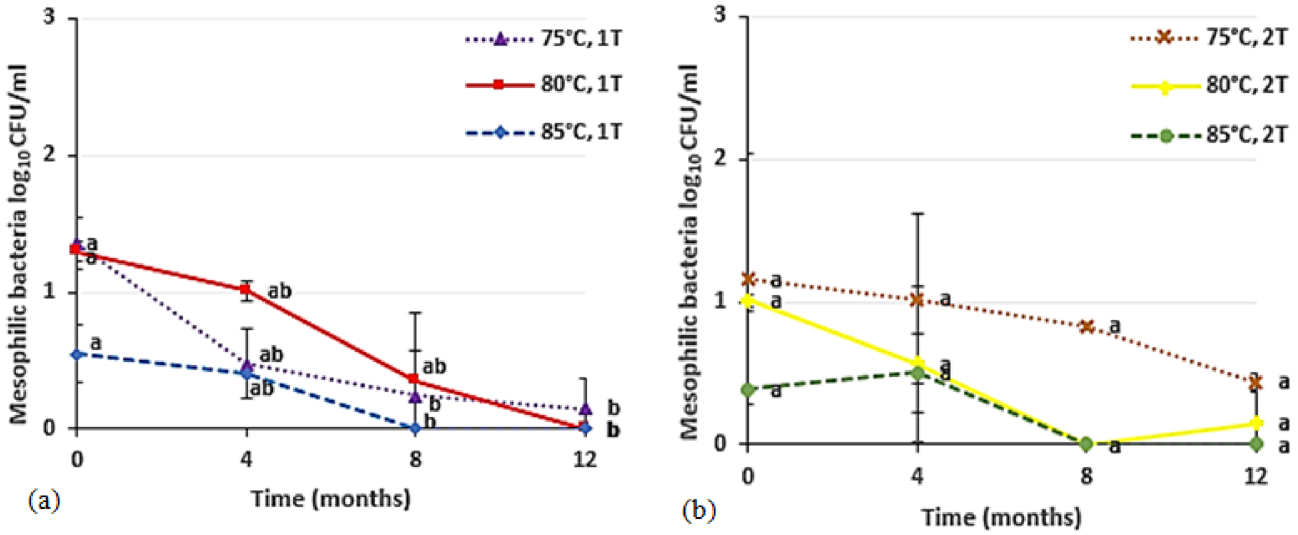

Figure 2. Changes of mesophilic bacteria load in the juices treated once (a) and twice (b) Means with the same letter in function of time do not significantly differ at 5% level

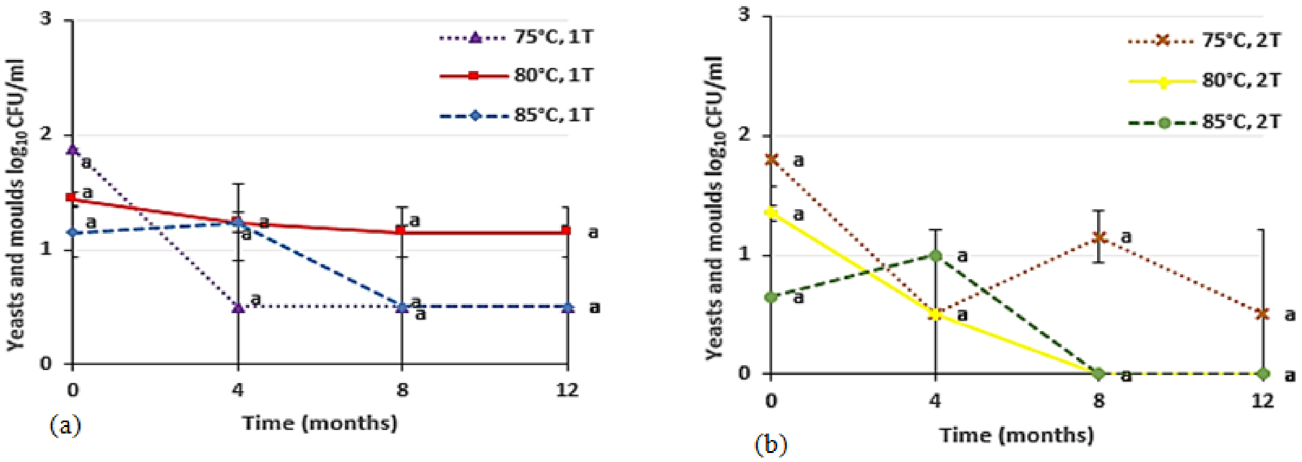

Figure 3. Changes of yeasts and moulds load in the juices treated once (a) and twice (b) Means with the same letter in function of time do not significantly differ at 5% level

According to pasteurized juices microbial stability, neither lactic acid bacteria nor enterobacteria were detected during the storage. A general decrease of mesophilic bacteria and yeasts and moulds counts was noticed in each of the juice as illustrated by Figure 2 and Figure 3. Then, it could be considered that the applied thermal treatments were such enough to produce an unfavorable environment to the microorganisms and prevent the juices spoilage. Indeed, after thermal treatments, fruit juices with pH lower than 4.5 are stable at ambient temperature [18].

Mesophilic bacteria decrease was significant ($p<0.05$) only in the juices 75℃, 1T, 80℃, 1T and 85℃, 1T produced with one thermal treatment throughout the storage (Figure 2.a). The counts actually vary from 1.35 $\log_{10}$ CFU/ml at 0 month to 0.15 $\log_{10}$ CFU/ml at the 12^{th} month in the juice 75℃, 1T while there was no more mesophilic bacteria present in juices 80℃, 1T and 85℃, 1T after the 12 months.

In terms of yeasts and moulds, there were no significant changes in all the 6 categories of juice from production to the 12^{th} month of storage. Starting from the 8^{th} month, there was no more microorganism present in juice 85℃, 2T.

3.3. Nutritional Quality Change during Storage

Ascorbic acid is considered an indicator of the nutritional quality of juices [19], and was determined in this study as a function of storage time. All categories of pasteurized pineapple juice showed decrease in their vitamin C content throughout the storage (Figure 4). This could be attributed to the fact that ascorbic acid is readily oxidized and lost during staying of juices, at rates depending on storage conditions [20]. The storage conditions could be temperature, oxygen and light exposure as reported by the authors. The effects of light exposure could be neglected in the present study as the juices in green-glass bottles were packaged in boxes to create dark condition, what might limit contact with light. Storage temperature here is room temperature with values varying between 28℃ and 35℃, producing therefore a difference of 7℃ which might potentially favor vitamin C degradation in the juices. Indeed, a study has shown that an increase of storage temperature by 10℃ caused a distinct decrease in vitamin C [21]. As to oxygen, the degradation of ascorbic acid can be attributed largely to the contribution of oxygen that was dissolved in the juices and headspace oxygen in the bottles, in the early stages of storage. In fact, air is naturally present in the intercellular spaces of fruits, and it is mixed into the juice during operations like maceration, homogenization and extraction because cells are crushed [22]. In addition, oxygen could be considered the principal gas present during bottling process [23]. The non-dissolved part of that oxygen stays in the headspace of the bottle [22]. Ascorbic acid easily oxidized in the presence of oxygen by both enzymatic and non-enzymatic catalyst [24].

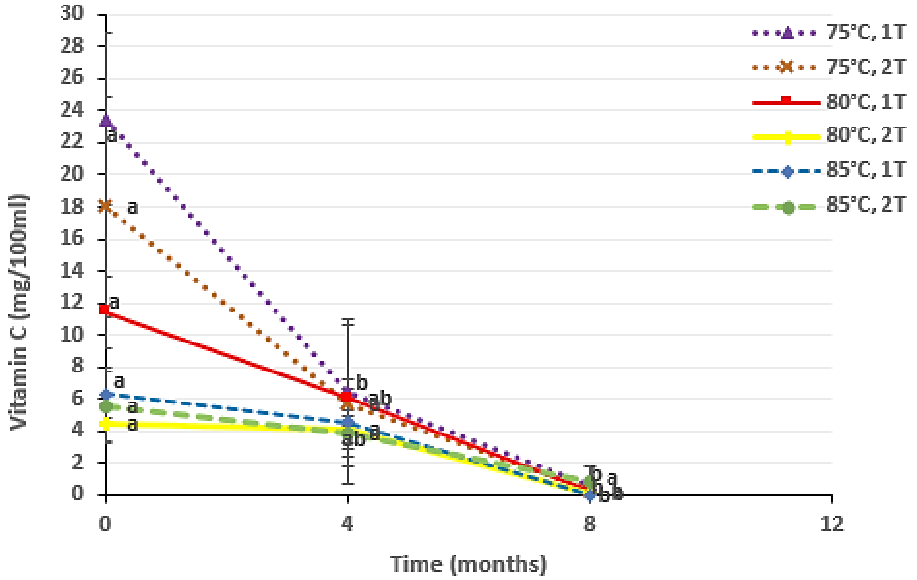

Figure 4. Evolution of vitamin C content in each category of juices (Means with the same letter in function of time do not significantly differ at 5% level)

The decrease in vitamin C of juices obtained with only one thermal treatment (75 ℃, 1T - 80 ℃, 1T - 85 ℃, 1T) was significant since the 4th month ($p<0.05$). As a matter of act, the mean vitamin C content in these juices respectively fell from 23.48 to 6.38 mg/100ml, 11.42 to 6.08 mg/100ml and 6.30 to 4.57 mg/100ml at 4 months. The amount of vitamin C in the juice 80 ℃, 2T was significantly low at the 8th month while no significant decrease ($p \geq 0.05$) was observed in the juices 75°C, 2T and 85 ℃, 2T. The maximal vitamin C content in the juices at 8 month was 0.81 mg/100ml (85 ℃, 2T) while almost no vitamin C was found in the juice 85 ℃, 1T. With consideration to this observation, no vitamin C was further determined after 8 month of storage.

3.4. Efficiency of the Treatments on Vitamin C Preservation during Storage

The percentage of vitamin C still present in each category of pasteurized pineapple juice was calculated from 0 to 4 and 8 months. The results obtained were gathered in Table 3. No significant difference ($p \geq 0.05$) was noticed between the juices vitamin C retention rates after 4 or 8 months of storage. This could be attributed to the great and non-uniform variation of vitamin C content in the juices during time, which ways out more clearly through the standard deviations obtained.

The juices obtained with only one thermal treatment preserved an average of 25.6 to 70.6% of vitamin C during their staying to the 4th month, whereas the juices with two thermal treatments retained more (28.4 to 90.0%). A mean of 11.6% of initial vitamin C content remained in juice 85 ℃, 2T after 8 months while almost no content was found in juice 85 ℃, 1T.

Shaw [25] revealed that juices with 50% retention of their initial vitamin C are considered as at the end of their shelf life. In line with this retention rate, the results for the stability study test are illustrated in Figure 5 (a, b, c and d). From this figure, a shelf life of 2.43 months was deduced for pineapple juices treated at 75 ℃, and 3.79 months for juices 80 ℃, 1T and 85 ℃, 1T. However, a shelf life of 4.31 months is found for juice 80 ℃, 2T and 4.88 months for 85 ℃, 2T. It can be retained that vitamin C was more stable in the most treated pineapple juices (higher pasteurization temperature or two thermal treatments) stored at ambient temperature.

Table 3. Mean values of vitamin C retention rates in the juices during storage (%)

Temperature ℃	75	80	85	75	80	85
Technology	1T	1T	1T	2T	2T	2T
After 4 months	25.6±13.66 [a]	55.2±20.45 [a]	70.6±17.01 [a]	28.4±16.54 [a]	90.0±3.60 [a]	73.3±21.88 [a]
After 8 months	2.4±0.47 [a]	2.6±3.74 [a]	0.0 [a]	1.9±0.76 [a]	3.2±3.28 [a]	11.6±12.77 [a]

Means with the same letter in row do not significantly differ at 5% level.

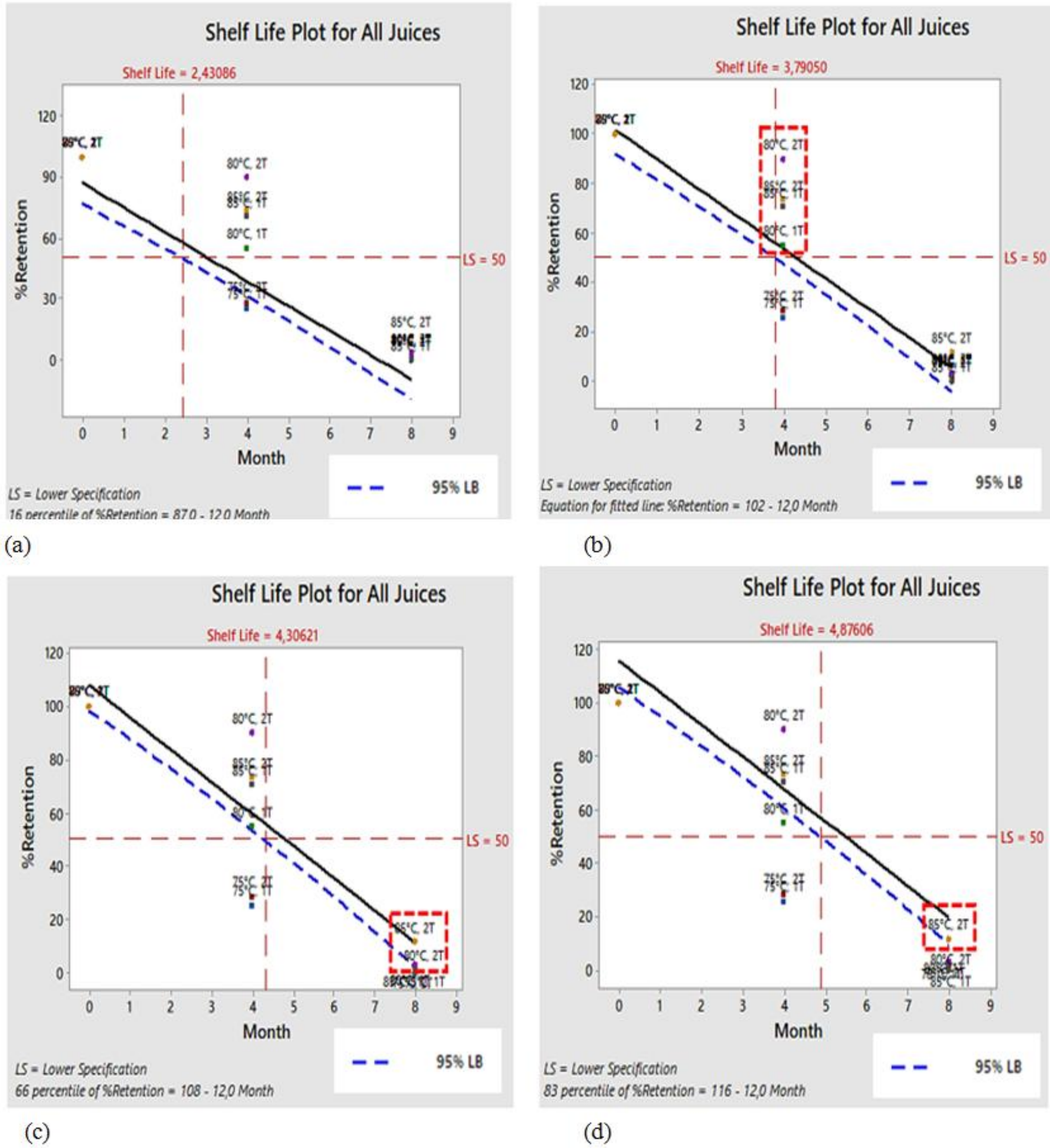

Figure 5. Shelf life when 50% vitamin C remain in at least 5 juices (a), 3 juices (b), 2 juices (c) and 1 juice (d)

4. Conclusion

The study revealed that one or two thermal treatments in pineapple juice production, with pasteurization temperature of 75 ℃ to 85 ℃, guaranty the safety of the juice. The low pH in all juices although it did not significantly decrease during storage, was sufficient to preserve from spoilage till 12 months. Vitamin C was generally affected in the juices throughout the storage, decreasing following different rates. The juices pasteurized at 75 ℃ contained more vitamin C after production but their content was quickly lost as storage time advanced. As suggestion, it would be interesting to deepen analyses on the tested juice categories through other chemical and biochemical changes investigation, to better understand the microbial stability observed. Furthermore, reducing time intervals between sampling during storage might give more clarification.

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