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The effect of the thermal treatment (49 °C - 90 min) coupled with storage at 15 °C on the infection rate, physicochemical and nutritional characteristics of the papaya (*Carica papaya* L. var solo 8).

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Abstract

This study investigated the effects of heat treatment (49 °C - 90 min) coupled with storage at 15 °C on the physicochemical and nutritional characteristics of the papaya. The papaya shows 1/32 of yellow skin was collected and immersed in a water bath at 49 °C for 90 min and then stored at 15 °C. Every four days, we recorded the infection rate, physicochemical and nutritional characteristics of the papaya. The fruits are not infected during the storage period except those stored directly at 28 °C. Mass loss is very high in untreated papayas stored at 28 °C. The loss of firmness is very low (8.64 %) in papayas stored at 15 °C after heat treatment. However, it is 3 and 6 times higher in papayas stored respectively at 15 and 28 °C without prior treatment. The pH increases while the titratable acidity decreases. The levels of sugars are generally increasing. The ascorbic acid content of papayas stored at 15 °C increases during the first four days before decreasing. The storage of papayas at 15 °C, after the heat treatment, improves the organoleptic qualities of fruits. This treatment has extended the fruit shelf life for up to twenty (20) days.

Keywords: thermal treatment, papaya, storage, infection rate, physicochemical and nutritional characteristics

Introduction

Long-distance transport and marketing of papaya have been limited by the perishable nature of this fruit. Papaya fruits suffer from having a brief shelf-life and post-harvest deterioration problems as a result of post-harvest disease, insect infestation, and over-rapid ripening (**Sharmin *et al.*, 2015**). Some chemical treatments applied to fruits to prevent insect attack or prolong their postharvest shelf life are potentially dangerous to human beings. Some physical methods are being extensively studied as substitutes for current chemical methods in commercial use (**Chávez-Sánchez *et al.*, 2013, Djioua *et al.*, 2010)**. Postharvest heat treatments lead to an alteration of gene expression and fruit ripening can sometimes be either delayed or disrupted (**Paull and Chen, 2000**). High-temperature treatments can control insect pests, prevent pathogen infection, induce resistance to chilling injury, slow fruit ripening, and extend postharvest shelf life (**Paull, 1990; Klein and Lurie, 1991**). Application of thermal treatments reduced the softening rate of apples (**Lurie**

and Klein, 1990), tomatoes (**Yoshida *et al*., 1984**), and pears, plums, and avocados (**Klein and Lurie, 1991**). Hot water treatment has been used for fruit preservation in recent years. It has been suggested that heat treatment before storage could decrease pathological and physiological disorders of the fruit by altering its ripening characteristics (**Klein and Lurie, 1992**). It has also been shown in some fruits, such as strawberries, apples, peaches, plums and tomatoes (**Jayyari and Rahemi, 2003**), that proper hot water treatment can make fruit retain a higher firmness during storage, thus keeping the fruit quality. The effect of hot water treatment on fruit firmness associates with the pectin content and the activity of critical enzymes related to cell wall degradation (**Yao *et al.*, 2016**). It is well known that heat treatments offer a viable alternative to the postharvest problems, since they produce a fungicidal and insecticidal action on papaya fruit **(Chávez-Sánchez *et al.*, 2013).** Therefore, the objective of this work is to study the effect of the heat treatment (49 °C - 90 min) coupled with storage at 15 °C on the infection rate, physicochemical and nutritional characteristics of the papaya.

Materials and Methods

1. Collection of fruit and sampling

The papayas (*Carica papaya* L. var solo 8) were harvested from a farm near Tomassé (Azaguié), a village located about 50 km, in the north of Felix Houphouet Boigny Airport, Abidjan, Cote d'Ivoire. The fruit were transported directly to the Laboratory of Food Biochemistry and Tropical Products Technology, Nangui Abrogoua University. The green mature stage (the fruit shows 1/32 of yellow skin) was selected for this study. The fruit were washed with water, sorted according to the shape, the size and the weight, and then immersed in a water bath at 49 °C for 90 min. Withdrawn; they were quickly cooled under a stream of cold water (15 °C). These fruits were packed in boxes then stored immediately at 15 °C. The studies involved 63 boxes, consisting of 21 boxes for heat-treated papayas stored at 15 °C and 42 boxes for untreated papayas stored directly at 15 °C and 28 °C. Every four days, we recorded the infection rate, weight loss, firmness, ascorbic acid content, pH, titratable acidity, reducing and total sugars levels, total soluble solids (TSS) and sensory analysis of the fruit.

2. Infection rate

For each analysis day, the degree of infection was expressed as the percentage of infected fruit (**Tano *et al*., 2007**)

3. Physico-chemical parameters

3.1. Weight loss

The loss of weight was measured using the method of **Proulx *et al.* (2005)**. Weight loss was determined during the storage period by monitoring the weight of the 12 fruit of each box. Weight loss was expressed as the percentage of the loss of weight with respect to the initial weight and was determined in triplicate.

3.2. Firmness

Using a penetrometer (a device for testing the firmness of the fruit, model FT 327, EFFEGI, Milan, Italy) equipped with stress indicator, the tip of the device is pressed on the middle of the papaya until it penetrates the pulp of the fruit to a depth of 8 mm. The value indicated by the device represents the maximum stress expressed in Newton (N) required for the pulp to be penetrated by the tip of the penetrometer; that value represents the firmness of the fruit (**Tano *et al.*, 2007**).

3.3. Ascorbic acid content

The ascorbic acid (vitamin C) content was determined according to the method described by **Poncracz (1971)** using 2,6-dichlorophenol indolphenol. Ten grams of papaya pulp were ground in 20 mL of metaphosphoric acid/acetic acid (3% metaphosphoric acid – 8 % acetic acid). The ground matter was centrifuged (Centrifuge Jouan Multifunction B4i-BR4i, Germany) at 4000 rpm for 20 min. One milliliter of the supernatant was titrated with 2,6-dichlorophenol indolphenol. The ascorbic acid content was calculated by the following equation: Ascorbic acid content (mg/100 g) = $[(Ve – V0) \times 20 / (Vc – V0) \times 10] / 100$.

Where Ve is the volume of 2,6-dichloropenol indolphenol used to titrate 1 mL of supernatant, V0 is the volume of 2,6-dichlorophenol indolphenol used to titrate 1 mL of metaphosphoric acid/acetic acid, and Vc is the volume of 2,6-dichlorophenol indolphenol used to titrate 1 mL of standard solution of ascorbic acid (1 mg/mL).

3.4. pH and titratable acidity

The pH of the samples was measured with a numerical pH meter (Consort P107, Belgium). Titratable acidity was measured according to the method of **AOAC (2000)**. This measurement was done by titrating against 0.1 N NaOH using 1 % phenolphthalein as indicator.

3.5. Reducing and total sugars

One gram of papaya pulp was ground (Moulinex Masterchef 750, France) in 10 mL of ethanol in order to measure the ethanol-soluble sugars. The mixture was centrifuged (Centrifuge Jouan Multifunction B4i-BR4i, Germany) at 3000 rpm for 30 min. The supernatant was used to determine the reducing sugars according to the method described by **Bernfeld (1955)** using 3,5-dinitrosalicylic acid (DNS). 0.5 mL of DNS was added to 0.1 mL of the supernatant diluted in 0.9 mL of distilled water. The mixture was heated in a water bath at 100 °C for 5 min and let to cool down for 5 min at room temperature (28 ± 2 °C); then, 3.5 mL of distilled water were added. The absorbance was determined by a spectrophotometer (Spectronic Genesys 5, Madison, USA) at 540 nm against the blank containing all the reagents except the supernatant. The determination of the total sugars was performed using the method of **Dubois *et al.* (1956)**. 1 mL of phenol 5 % (w/v) was added to 0.1 mL of the supernatant diluted in 0.9 mL of distilled water. The mixture was homogenized, heated in a water bath at 100 °C for 5 min, and let cool down at room temperature for 5 min. Then 2 mL of concentrated sulfuric acid was added to the mixture. The optical density (O.D) was read at 490 nm against the blank on a spectrophotometer (Spectronic Genesys 5, Madison, USA).

3.6. Total Soluble Solids (°Brix)

The total soluble solids, expressed in °Brix, was measured with a refractometer (model N-20 E, ATAGO, Tokyo, Japan) equipped with a temperature corrector. A drop of papaya juice obtained after grinding was placed on the prism of the refractometer and the total soluble solids was directly read under sun light.

4. Sensory analysis

The sensory evaluation was possible using the method described by **Lateur *et al.* (2001)**. Three slices of very treatment of papaya were served to ten well-trained panelists for evaluation. The evaluated criteria were firmness, crunchiness, sweetness, acidity, skin color, pulp color, juiciness, appearance and Global appreciation of the fruit. A scale of 1 to 5 was used to indicate: 5 = excellent, 4 = good, 3 = average, 2 = bad and 1 = very bad.

5. Statistical analysis

The statistical analysis was performed on the results using SPSS (version 10.0) software. The comparison of the variables measured during this study was done using the analysis of variance (ANOVA) and Duncan test. The differences were considered significant if $p \leq 0.05$. All the experiments were conducted in triplicate.

Results and Discussion

The fruits are not infected during the storage period except those stored directly at 28 °C, where the infection rate is very high (**Figure 1**). This result shows that storage at 15 °C without or after immersion of the papaya in water at 49 °C for 90 min, completely inhibits infections. These results are in agreement with those of **Baiyewu & Amusa (2005)** and **Djioua (2010)**. These authors have shown that the use of heat treatment or low temperature storage inhibits the growth of microorganisms that can infect fruit during storage. Mass loss is very high in untreated papayas stored at 28 °C. But, low in papayas stored at 15 °C, with or without heat treatment (**Figure 2**). There is evidence that mass loss is the result of fruit respiration. It increases with temperature, which increases transpiration, hence the high mass loss of papayas stored at high temperatures **(Tano *et al.*, 2007)**. The loss of firmness is very low (8.64 %) in papayas stored at 15 °C after heat treatment. However, it is 3 and 6 times higher in papayas stored respectively at 15 and 28 °C without prior treatment **(Figure 3)**. These results show that the heat treatment significantly reduces the loss of the firmness of the papaya. A similar treatment (immersion in water at 49 °C for 75-120 min) reduces the activity of the pectolytic enzymes responsible for the softening of fruits such as mango and papaya (**Bacay-Roldan & Serrano, 2005; Benitez *et al.*, 2006, Djioua *et al.*, 2010**). In addition, the application of heat treatment has made it possible to inhibit the synthesis of ethylene in apple and tomato (**Biggs *et al.*, 1988, Klein, 1989**), reduce the respiratory intensity of the apple (**Klein & Lurie, 1990**), maintain the organoleptic quality of the strawberry (**Garcia *et al.*, 1995**), prolong the life of the avocado (**Bard &**

Kaiser, 1996), slow tomato ripening (**Lurie & Sabehat, 1997**), decrease the production of ethylene and Khaki respiratory intensity (**Luo, 2006**). It is recognized that respiration and ethylene synthesis favor metabolic reactions such as degradation reactions of fruit cell wall polysaccharides by pectolytic enzymes (**Fisher & Bennett, 1991, Fils-Lycaon & Buret, 1991; Ketsa & Daengkanit, 1999**). Therefore, if the heat treatment inhibits ethylenic synthesis, reduces the respiratory activity of the fruits, it is obvious that this treatment also inhibits the enzymatic degradation of the cell wall and in turn the loss of firmness of the fruit. This explains a minimal loss (8.64 %) of the firmness of the papaya which has undergone heat treatment. The pH increases while the titratable acidity decreases when papaya is kept regardless of the storage temperature **(Table 1)**. Each observation is a mean ± SD of three replicate experiments (n = 3). The values followed by the same letter in the same column and on the same line are not significantly different ($p \leq 0.05$) according to the Duncan test. However, there is no significant difference between the different pH values, as is the titratable acidity values of heat treated and non heat treated fruits. The heat treatment does not influence the pH and acidity of the fruit. These results are identical to the results obtained by **Yao *et al.* (2011)**, which confirms that the heat does not influence the pH and the acidity of the fruit. Similarly, heat treatment of mango (immersion in water at 50 °C for 75 min) did not affect the pH and acidity of the fruit **(Djioua *et al.*, 2009)**. The levels of sugars (total soluble solids, reducing sugars, and total sugars) are generally increasing (**Table 2**). However, they are lower in papayas that have undergone heat treatment. This heat treatment therefore had a denaturing effect on the enzymes responsible for the synthesis of these compounds. Their synthesis is consequently delayed, which explains their low rate in the fruits which have undergone the heat treatment. These results are confirmed by the work of **Schirra *et al.* (2000)**; **Bacay-Roldan & Serrano (2005).** They showed that immersing the papaya in water at 49 °C for 120 min reduces xylanal and polygalacturonase activity by inhibiting the synthesis of these enzymes. Moreover, during the degradation of the cell wall of fruits by hydrolytic enzymes, there is release of simple sugars. The synthesis of the enzymes responsible for the degradation of the polysaccharides of the cell wall of the fruit being inhibited by heat, there is therefore a slowdown in their activity, hence the low sugar content in the papayas that have undergone the heat treatment. As for the total sugar levels, it increases during the first four days, then decreases by losing about 20% of its value of the day 4, during ripening of the fruit. This decrease in the amount of total sugars is due to increased consumption of sucrose during climacteric respiration **(Gomez *et al*., 2002)**. The ascorbic acid content of papayas stored at 15 °C increases during the first four days before decreasing. However, it drops drastically when papayas are stored at 28 or 15 °C after immersion in water at 49 °C for 90 min **(Figure 4)**. These results are consistent with the work of **Walker & McKersie (1993)**. They showed that the vitamin C content of tomato increases when it is exposed to a low temperature. Similarly, fruits with advanced maturity have a lower vitamin C content **(Yahia *et al.*, 2001)**, which explains the drop in vitamin C content after four days of storage at 15 ° C. In contrast, high temperatures lead to a degradation of vitamin C levels in fruits, because this compound is thermolabile **(Lester, 2004, Richardson *et al*., 2004, Gautier *et al.*, 2008)**. This degradation is linked to oxidation of vitamin C **(Torres *et al*., 2006)**, which explains the drastic loss of vitamin C content in papayas stored at 28 and 15 °C after immersion in water at 49 °C for 90 min. The storage of papayas at 15 °C, after the heat treatment, improves the organoleptic qualities of fruits such as the firmness, the color of the pulp and the skin of the fruit **(Figure 5)**. In addition, they eliminate infections and minimize weight loss. The appearance and overall appreciation of the fruit are also improved by these treatments. However, these organoleptic qualities depreciate considerably during storage at 28 °C of the fruits. This result suggests that high temperature thus favors enzymatic reactions, loss of firmness, multiplication of pathogens (fungal and bacterial), loss of mass etc. As a result, it causes the fruits to soften and ripen rapidly, resulting in a rapid aging of the fruits, hence the depreciation of their quality over time (**Djioua, 2010**). As a result, heat treatments have been used in post-harvest in recent years, not only to control insects and other pathogens (fungal and bacterial), but also to inhibit post-harvest enzymatic activity to delay softening and the ripening of the fruit. They are now mandatory when exporting certain fruits to certain countries. These treatments are called quarantine treatments (**Klein & Lurie, 1992**). They aim to ensure the fruits the optimal market quality (**Djioua, 2010**). In addition, the high temperature improves the sweet taste of the fruit. It would be the result of cell wall degradation of fruits releasing simple sugars such as galactose and mannose.

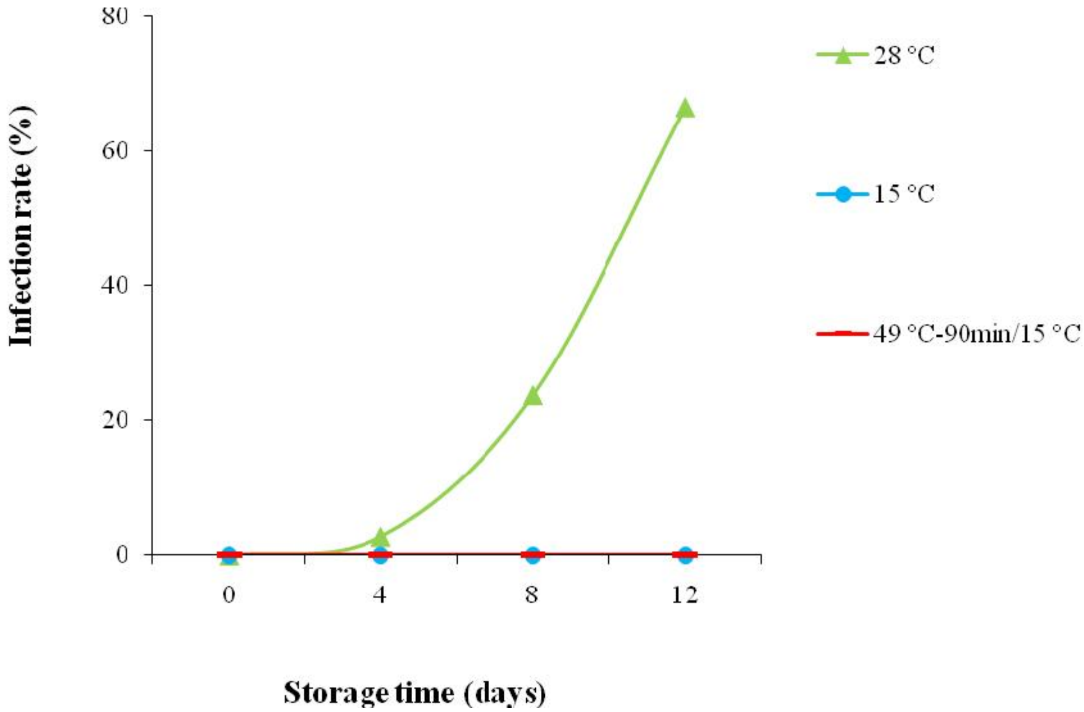

Figure 1: Effect of heat treatment on the evolution of the infection rate of the papaya. 28 ° C: papaya stored at 28 ° C, 15 ° C: papaya stored at 15 ° C and 49 ° C-90 min / 15 ° C: papaya stored at 15 ° C after immersion in water at 49 ° C.

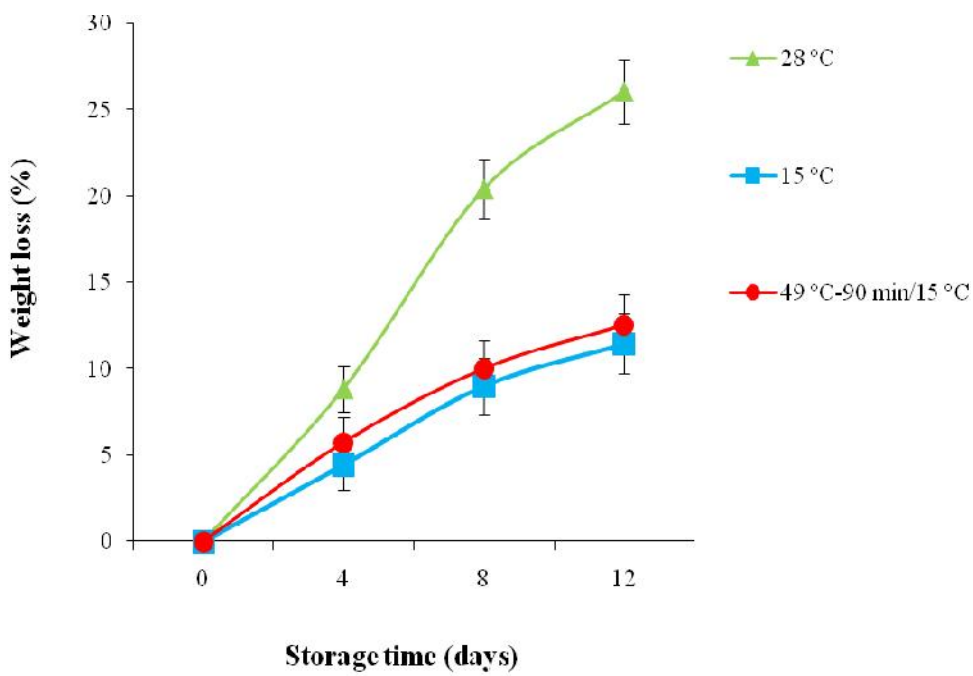

Figure 2: Effect of heat treatment on the weight loss of papaya. 28 °C: papaya stored at 28 °C, 15 °C: papaya stored at 15 °C and 49 °C-90 min / 15 °C: papaya stored at 15 °C after immersion in water at 49 °C.

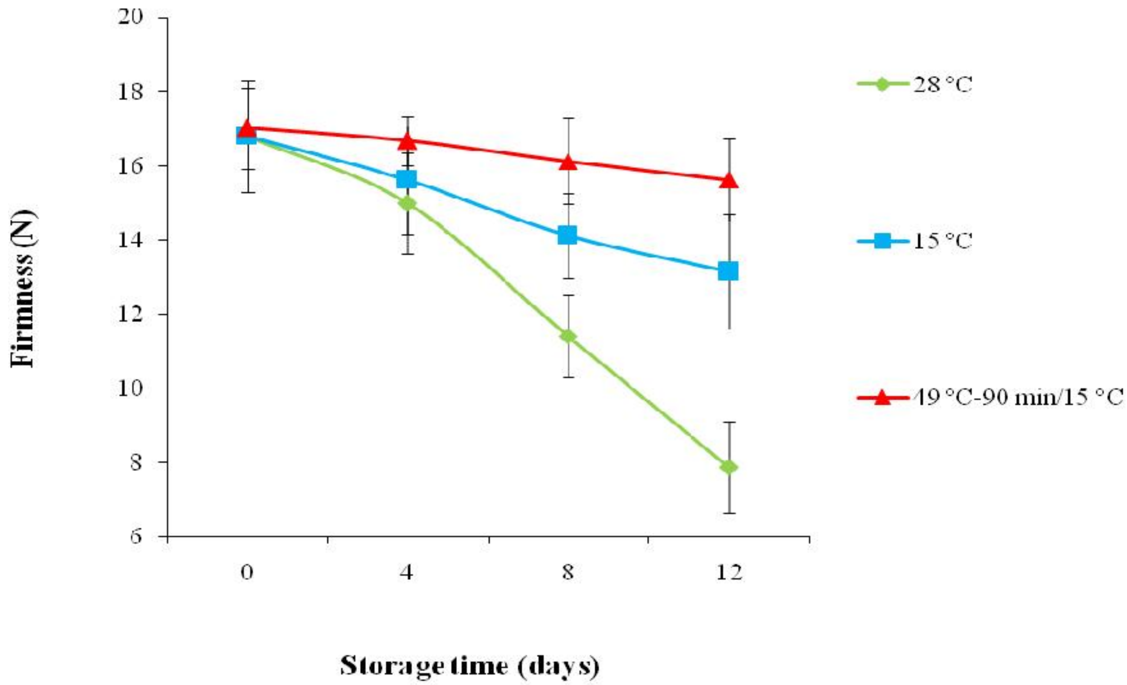

Figure 3: Effect of heat treatment on the firmness of the papaya. 28 ° C: papaya stored at 28 °C, 15 °C: papaya stored at 15 °C and 49 °C-90 min / 15 °C: papaya stored at 15 °C after immersion in water at 49 °C for 90 min.

Table 1: Effect of heat treatment on the pH and titratable acidity of the papaya picked at the mature green stage. 28 °C: papaya stored at 28 ° C, 15 °C: papaya stored at 15 °C and 49 °C – 90 min / 15 °C: papaya stored at 15 °C after immersion in water at 49 °C for 90 min.

	pH			**Titratable acidity (%)**		
Day	**28 °C**	**15 °C**	**49 °C - 90 min/15 °C**	**28 °C**	**15 °C**	**49 °C - 90 min/15 °C**
0	5.60 ± 0.00^{E}	5.60 ± 0.00^{E}	5.40 ± 0.048^{E}	0.018 ± 0.002^{A}	0.018 ± 0.002^{A}	0.017 ± 0.002^{A}
4	5.65 ± 0.05^{F}	5.73 ± 0.05^{F}	5.42 ± 0.051^{F}	0.017 ± 0.004^{B}	0.017 ± 0.002^{B}	0.015 ± 0.004^{B}
8	5.71 ± 0.05^{G}	5.81± 0.03^{G}	5.74 ± 0.055^{G}	0.016 ± 0.003^{I}	0.016 ± 0.000^{I}	0.015 ± 0.003^{I}
12	5.81 ± 0.07^{H}	5.82 ± 0.00^{H}	5.62 ± 0.066^{H}	0.015 ± 0.002^{J}	0.010 ± 0.000^{J}	0.011 ± 0.001^{J}

Each observation is a mean ± SD of three replicate experiments (n = 3). The values followed by the same letter in the same column and on the same line are not significantly different (p≤0.05) according to the Duncan test.

Table 2: Effect of heat treatment on the sugar content of the papaya picked at the mature green stage. 28 °C: papaya stored at 28 ° C, 15 °C: papaya stored at 15 °C and 49 °C - 90 min / 15 °C: papaya stored at 15 °C after immersion in water at 49 °C for 90 min.

	Total soluble solids (°Brix)			**Reducing sugars (g/100g)**			**Total sugars (g/100g)**		
Days	**28 °C**	**15 °C**	**49 °C - 90 min/15 °C**	**28 °C**	**15 °C**	**49 °C-90 min/15 °C**	**28 °C**	**15 °C**	**49 °C - 90 min/15 °C**
0	8.13 ± 0.208^{A}	8.13 ± 0.500^{A}	8.12 ± 0.300^{A}	2.34 ± 0.142^{E}	2.29 ± 1.249^{E}	2.31 ± 1.249^{E}	3.41 ± 0.930^{E}	3.41 ± 0.930^{E}	3.41 ± 0.430^{E}
4	8.83 ± 0.208^{K}	8.50 ± 0.208^{J}	8.21 ± 0.104^{B}	2.57 ± 0.504^{G}	2.43 ± 1.017^{G}	2.33 ± 1.017^{F}	7.21 ± 3.196^{G}	4.45 ± 1.170^{H}	3.97 ± 0.396^{F}
8	9.45 ± 0.135^{M}	9.17 ± 0.153^{L}	8.64 ± 0.132^{C}	2.79 ± 1.249^{H}	2.59 ± 0.160^{J}	2.44 ± 0.160^{I}	5.68 ± 0.680^{H}	4.31 ± 0.996^{I}	3.85 ± 1.090^{J}
12	9.93 ± 0.115^{N}	9.63 ± 0.153^{O}	9.14 ± 0.161^{P}	2.87 ± 0.647^{K}	2.64 ± 0.017^{M}	2.49 ± 0.017^{N}	4.98 ± 0.935^{K}	3.82 ± 0.405^{L}	3.72 ± 0.215^{M}

Each observation is a mean ± SD of three replicate experiments (n = 3). The Values followed by the same letter in the column and on the same line are not significantly different ($p \leq 0.05$) according to the Duncan test.

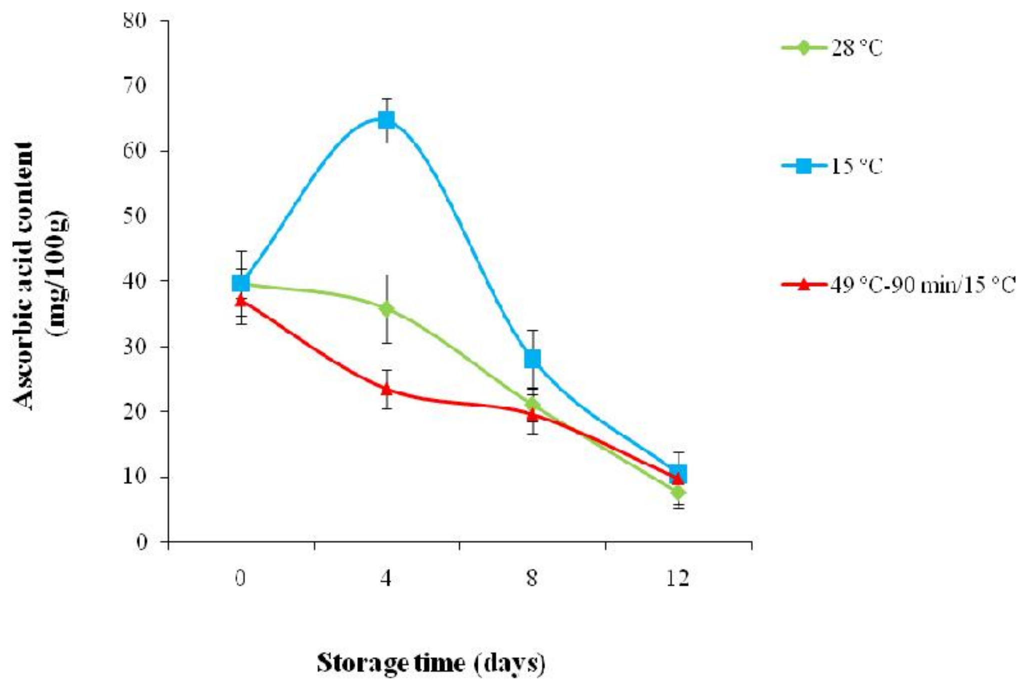

Figure 4: Effect of heat treatment on the ascorbic acid content of the papaya.
28 °C: papaya stored at 28 °C, 15 °C: papaya stored at 15 °C, 49 °C-90 min / 15 °C: papaya stored at 15 °C after immersion in water at 49 °C.

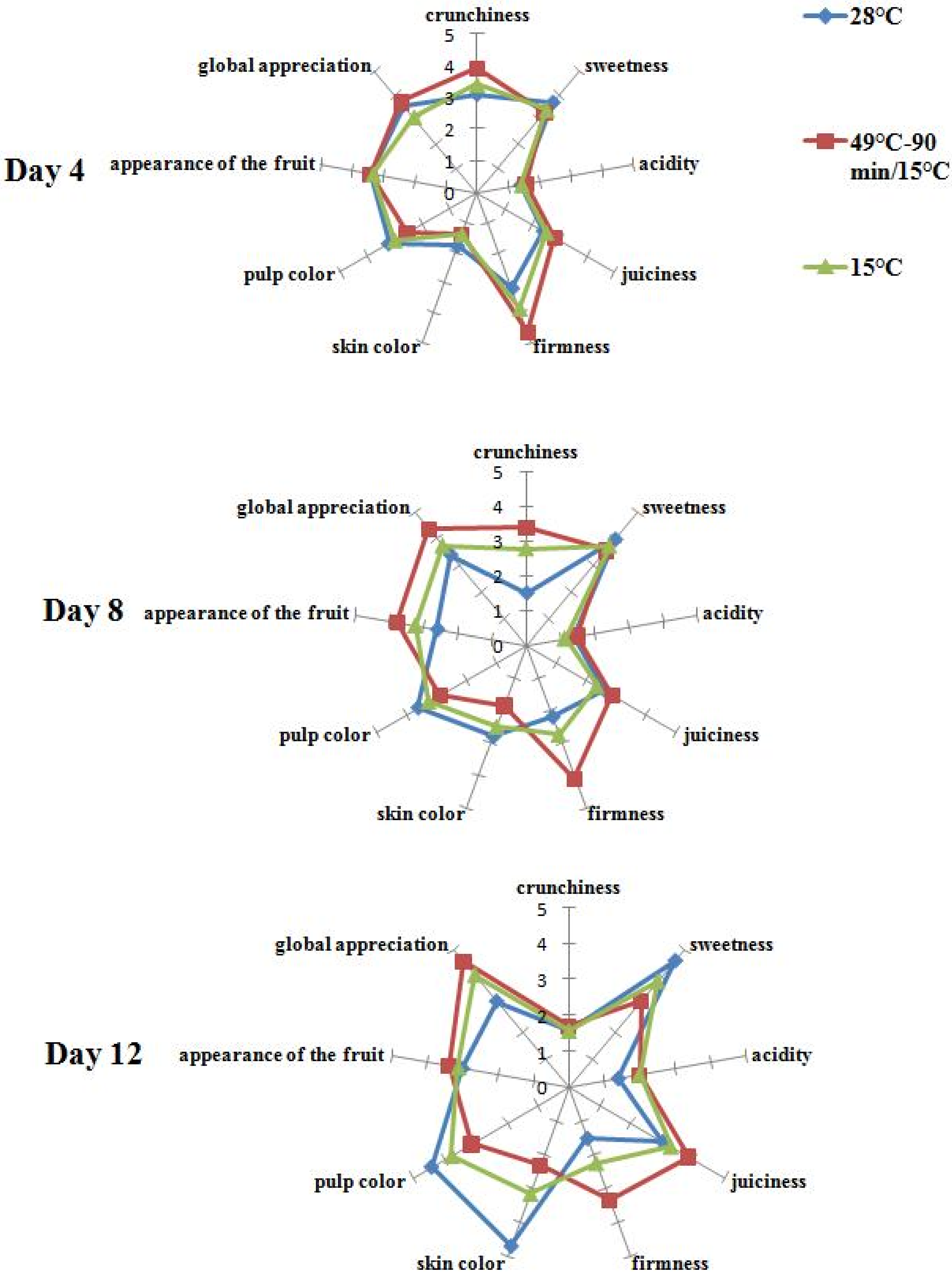

Figure 5: Effect of heat treatment on the evolution of some organoleptic parameters of the papaya.

Conclusion

Heat treatment of papayas in water at 49 °C for 90 min coupled with storage at 15 °C has greatly reduced the loss of firmness, 6 and 3 times the loss of firmness of the papaya when stored at room temperature 28 °C and 15 °C respectively without heat treatment. This treatment also greatly improved the organoleptic quality of the fruits, namely the skin and pulp color and the overall appearance. However, the level of sugar is low in fruits that have undergone this treatment compared to fruits stored at room temperature (28 °C) and at 15 °C without heat treatment. However, it has extended fruit life for up to twenty (20) days, allowing them to reach normal sugar levels. This treatment can therefore be proposed as an alternative and effective method to better preserve the papayas picked at the mature green stage. As a result, this treatment could be applied industrially and thus contribute to the marketing of papaya on the local and international market.

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