Original Research Article

Evolution of Bioactive Compounds during Storage of poyo Bananas after Treatment with Calcium Chloride

ABSTRACT

Visually, the obvious characteristic of banana ripening is the progressive change of the epicarp from green to yellow according to several stages whose importance contributes to extend the total duration of the process. The present study was carried out with the aim of prolonging the ripening time of bananas while following the behavior of the physiological parameters related to this process. To achieve this objective, after treatment of bananas with calcium chloride, several parameters were evaluated. These were ripening rate, firmness, water content, soluble solids content and pigment content in the peel. Secondly, the evolution of antioxidant parameters such as: lycopenes and β-carotenes in the pulp; total phenols, flavonoids and ascorbic acid contents in the peel and pulp have been assessed. As a result of these analyses, it appeared that the best shelf life was obtained with bananas treated with tween 80 at 2 and 4% Cl_2Ca. The lowest bananas shelf lives were recorded in 6 and 8% Cl_2Ca and in the control. Similarly, the low loss of firmness, low accumulation of total soluble solids, low increase in water content in the pulp were found in the 2 and 4% Cl_2Ca tween 80 treatments. The change in chlorophyll went in a decreasing direction during storage, but almost similarly in all treatments. The increase in lycopene content was effective during this time in a less rapid manner in the 2 and 4% calcium chloride treatments. Variations in β-carotenes, total phenols, and ascorbic acid were also increased throughout the storage period. However, at the end, it was difficult to correlate their evolution to any concentration of calcium chloride used for banana processing. It is also this lack of influence of calcium chloride as a treatment on the significant decrease of flavonoids in the pulp and not significant in the peel that was observed. Furthermore, the contents of the compounds measured in the peel and the pulp were lower in the pulp. It is thus with the bananas treated with tween 80 to 2 and 4% of calcium chloride that the majority of the parameters were influenced in favour of the preservation of bananas.

Keywords: calcium chloride, preservation, ripening, shelf lives, antioxidant compounds

1. INTRODUCTION

Cameroon is a country with high agricultural potential. According to the Ministry of Agriculture and Rural Development, the rural sector represents the first sector of the national economy for its contribution to the gross domestic product (GDP). That is to say 1185 billion FCFA (1/3 of the GDP). In fact, this sector accounts for nearly 62% of the active population. The

banana sector alone contributes in 4.5% to agricultural GDP and mobilizes about 650,000 producers and a little more than 50,000 other intermediaries such as traders, transporters and processors [1]. With a production of nearly 1.8 million tons produced in 2015, Cameroon ranks second in Africa behind Côte d'Ivoire [2]. Moreover, on a national food scale, bananas are the third most important staple food after rice and cassava [3].
In view of nutritional and socio-economic importance of the bananas, its conservation and its preservation of the quality after harvesting in a fresh state, remains a challenge of a certain relevance. The search for new conservation techniques is a constant concern. However, several conservation techniques have already been developed and tested. These include refrigeration and drying [4]; chemical treatments [5,6]; heat treatments [7,8]; and controlled and modified atmosphere packaging [9,10] . Despite this multitude of long-term fruit preservation techniques, the problem still remains at the level of producing countries. Thus, post-harvest losses persist. This could be in the range of 32% for all food categories worldwide [11]. Knowledge of the physical and biochemical changes associated with ripening would be of major benefit in the development of appropriate preservation technology that delays ripening and maintains fruit quality.
The knowledge of the role of calcium chloride in the stabilization of physiological processes of tissues, makes it a preferred compound in the development of preservation methods. The activities of cell wall degrading are induced by increased concentration of ethylene, independently of its effect of ripening [12,13]. There is evidence that calcium inhibits the activities of cell wall hydrolases. Calcium ions delay the senescence by stabilizing cell membrane and increasing the rigidification of monolayers[14]. Some references made here are the works of Aghofack-Nguemezi and Yambou [5], where a soaking of mature green bananas in a calcium chloride solution of concentration 200 mg.l-1 induces a significant retention of water in the peel. Calcium chloride slows the development of physiological disorders and improves fruit quality [15]. However, the excessive absorption of Calcium also, leads to tissue degradation. In fact, the cuticle is the main barrier to the penetration of various solutes [16] and epicuticular waxes are known to reduce cuticular penetration of many solutes [17]. In addition, a surfactant such as tween 80 has properties of modifying energy relationships at interfaces, thereby reducing surface tension [18] and improving leaf uptake of biologically active compounds [19].
Thus, a combination of tween 80 with calcium chloride would contribute more to the preservation of fruit shelf life. The objective of the present study was to extend the shelf life of bananas by highlighting the processes related to ripening.

2. MATERIAL AND METHODS

2.1 Plant material and treatments

The dessert bananas of the poyo variety used were harvested from an orchard in the town of Melong in littoral Cameroon at full green physiological maturity. The present experiment was carried out during the months of February and March 2020, at the University of Dschang, in the laboratory of applied botany. The average temperature and relative humidity conditions were 25.10°C and 74.95% respectively. The fruits were divided into five batches of 60 bananas and treated as follows: the first batch representing the control, was not subjected to any treatment. The remaining four batches (2–5) were soaked for 10 minutes in a 5% V/V solution of tween 80 solution. Then they were soaked 30 minutes in calcium chloride solutions of 2%, 4%, 6% and 8% concentration respectively.

2.2 Green life, firmness, moisture content and soluble solids content

Green life was defined as the time elapsed between the first day of treatment and the day when 100% of the fruit in each lot was at the ripe-yellow stage.
Firmness was assessed by averaging three punctures made at different points on the fruit after peeling using a GY-2 penetrometer following the method of Mehinagic *et al.* [20].
The water content of the banana peel and pulp was determined by the desiccation-based analytical method. 20g of fresh material was oven dried at 90°C until constant weight or total water removal was achieved. Water content expressed as a percentage in this formula:
Water content (WC) = (initial weight - final weight)/(initial weight) x100
The determination of the soluble solids content of banana pulp in °Brix of fruit was performed by refractometry.

2.3 Determination of the pigment content

Pigment contents (chlorophyll a, chlorophyll b and carotenoids) were determined according to the formulas of Lichtenthaler [21]. A 5 g sample of peel was introduced in 15ml of acetone. For two hours, everything was on ice and in the dark. The optical densities of the extracts were determined by spectrophotometry at wavelengths 470nm, 645nm and 662nm.

2.4 Determination of lycopene and β-carotene content

Lycopene and β-carotene contents were determined using the method developed by Nagata and Yamashita [22]. Indeed, a mixture of acetone and hexane 4:6 (v/v) was used as a solvent after grinding the banana pulp. After 2h of extraction in the dark and in ice, the optical densities were determined. The amounts of lycopene and β-carotene were calculated from the following formulas:

Lycopene (mg/100ml) = $-0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$

β-carotene (mg/100ml) = $0.216A_{663} - 1.22A645 - 0.304A_{505} + 0.452A_{453}$

2.5 Determination of total phenols and total flavonoids content.

Total phenols were quantified by the method described by Singleton and Rossi [23] as modified by Nour *et al*. [24]. Phenols and flavonoids were extracted from 5 g of fresh material in 10 ml of 0.5% V/V HCl methanol. The extraction time on the ice in the dark was 45 minutes. For total phenols, 100 µl of supernatant was taken and mixed with 5 ml of distilled water and 500 µl of Folin-Ciocalteau reagent. After 5 minutes, 1.5 ml of 20% sodium carbonate was added. Finally, 2.9 ml of distilled water were added. The solution was incubated for 30 minutes at 40 °C. Optical densities were read at 765 nm and the result was reported in µg of gallic acid equivalent.

The total flavonoid content was determined by the method developed by Mohommadzedeh *et al.* [25]. 5ml of methanol was introduced into 0.5 ml of methanolic supernatant. 0.1 ml of aluminum nitrate of 10% concentration was added. After 5 minutes, 0.1 ml of 1M potassium acetate aqueous solution and 4.3 ml of methanol were added. After 40 minutes, the mixture was measured with a spectrophotometer by absorbance at 415 nm. Quercetin was used to make the standard calibration curve.

2.6 Determination of ascorbic acid content

Ascorbic acid concentration was determined following the method of Malik and Singh [26]. 20 ml of 6% metaphosphoric acid containing 0.18 g EDTA added 5g of banana pulp or peel. The mixture was then homogenized for 45 min and centrifuged at 4000 rpm for 15 min. 400 µl of the filtrate was taken and mixed with 200 µl of 3% metaphosphoric acid. 1.4 ml of 14% Folin reagent was also added. After 10 minutes, the absorbance of the sample was measured at 760 nm. The ascorbic acid concentration was quantified using an L-ascorbic acid standard curve and expressed as mg ascorbic acid equivalent.

2.4 Statistical Analyses

The obtained data were submitted to the analysis of variance (ANOVA) to check the differences between means and the Tukey test was used in case of need at the *.05* probability threshold for the separation of these means. These statistical analyses were performed using the statistical package IBM SPSS Statistics 20.

3. RESULTS AND DISCUSSION

3.1 Results

3.11 Effect of treatments on Green Life.

As shown in Figure 1, bananas treated with 2% and 4% calcium chloride had the best shelf life. The control bananas and those treated with 6 and 8%, on the other hand, were preserved in a much shorter time. Moreover, these better preservation scores were observed from the Tukey statistical test at the .05 threshold.

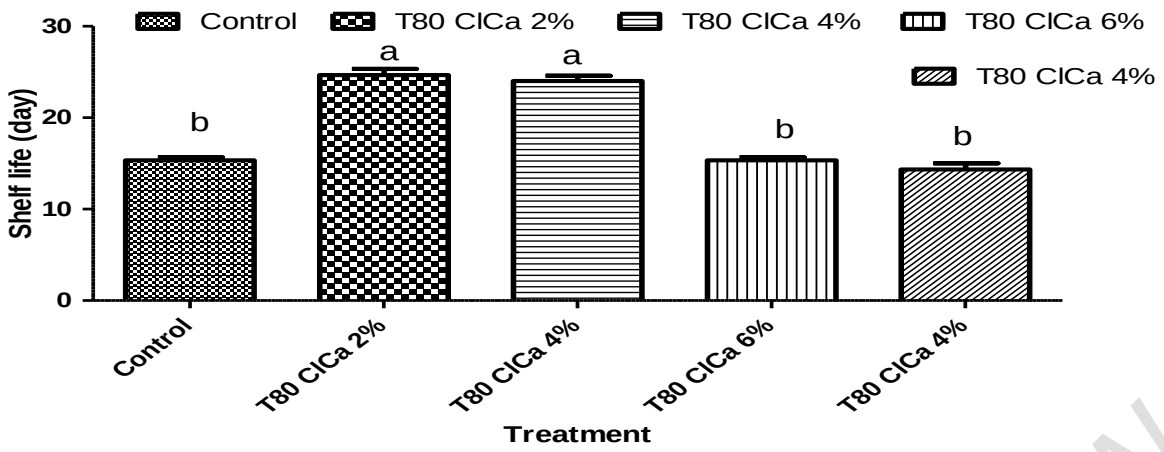

Fig. 1: Evolution of the ripening time according to the treatments

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

3.1.2 Effect of treatments on firmness, water content and soluble solids content

Overall, no decrease in firmness was observed during the first 7 days of the experiment as shown in Fig. 2. However, the loss of firmness from the 14th day was only observed in some treatments. This was the case for the treatments with 6% and 8% tween 80 calcium chloride. Finally, on the 21st day, the rate of decrease in firmness was respected in the batches as on the 14th day. The highest values were always noted in the treatments with tween 80 at 2% and 4%. Moreover, all these decreases were confirmed by the Turkey test at the .05 probability level. However, it should be noted that the firmness was even greater in bananas treated with tween 80 at 4% calcium chloride

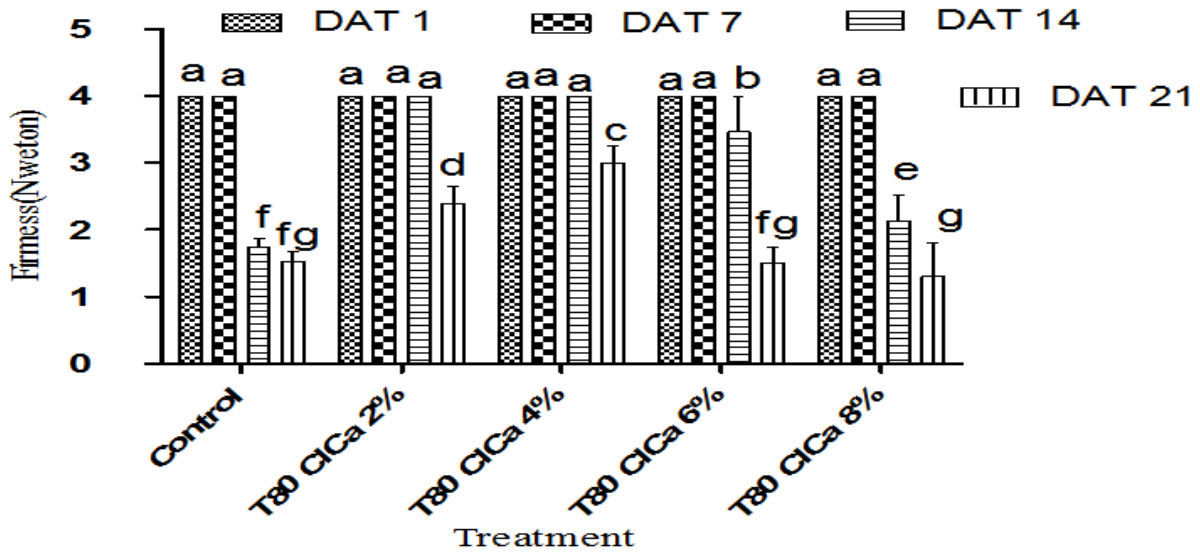

Fig. 2: **Variation of firmness as a function of time**

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

The water content in the peel followed an overall decrease over time in all treatments. The values of this parameter were 80% on the first day for the largest and 61.66% ± 2.88% on the 21st day for the smallest during storage. According to the different treatments, it was noted in the majority that slight differences in water content, and not statistically approved. But

it is worth noting that the decrease in water content had a significant progression over time according to the Tukey test at the .05 threshold (Fig. 3a).
In contrast to the variation of the water content in the peel, that of the pulp had an increase during storage. The smallest value was 60% and the largest was 80% according to Fig. 3b. As in the peel, there was little change in the water content of the pulp. However, the only significant differences according to Tukey's test at the .05 level were found only as a function of time and not between treatments

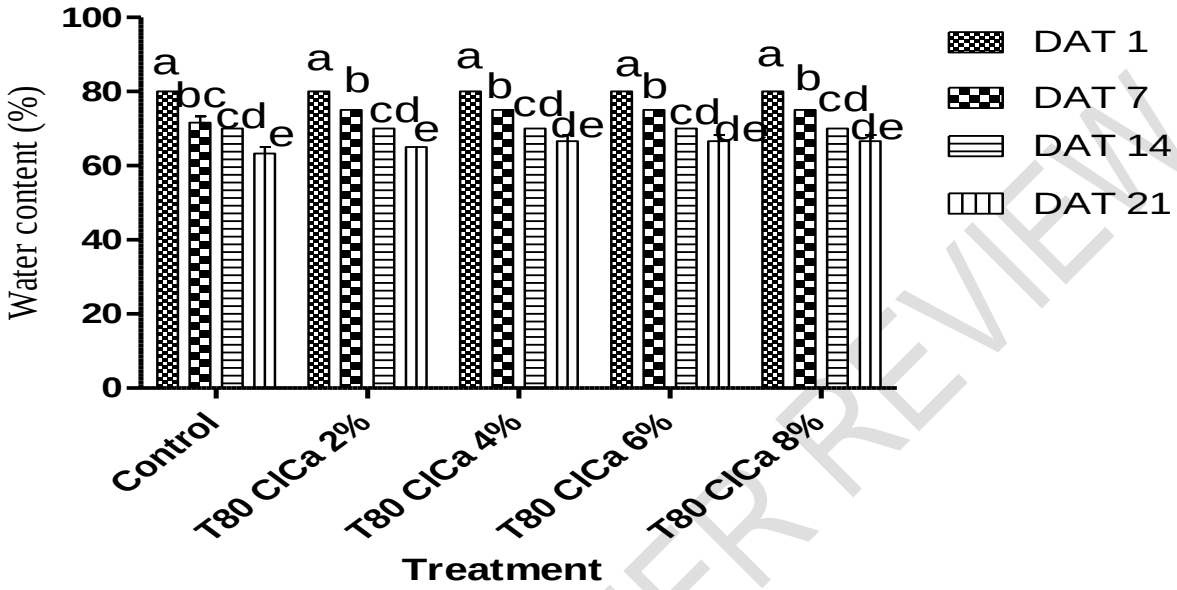

Fig. 3a: Evolution of the water content in the peel

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

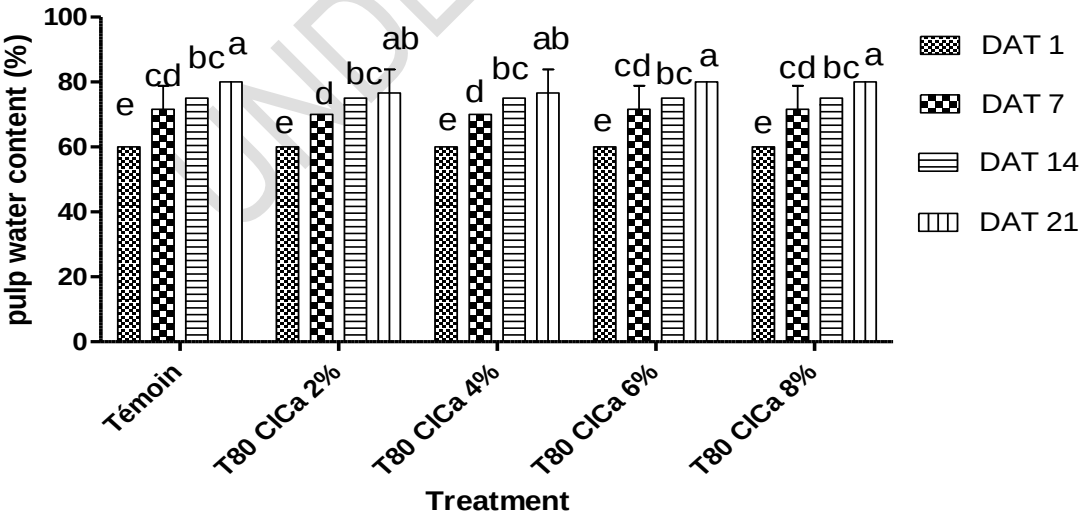

Fig. 3b: Evolution of the water content in the pulp

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

With a very low value of 5.36 °Brix at the beginning of the experiment, the values of total soluble solids had an increasing progression during ripening as a function of time and treatments. Although control bananas and bananas treated with 6% and 8% calcium chloride showed a higher value at day 7, no significant difference was observed at this date. Beyond that, on day 14 of the experiment, the Tukey test at the .05 threshold showed a significant increase in soluble solids content in the control bananas and those treated with 6% and 8% calcium chloride. Moreover, the slight increase in soluble solids content in the 2% and 4% calcium chloride treatments was confirmed on the 21st day of storage (Fig. 4).

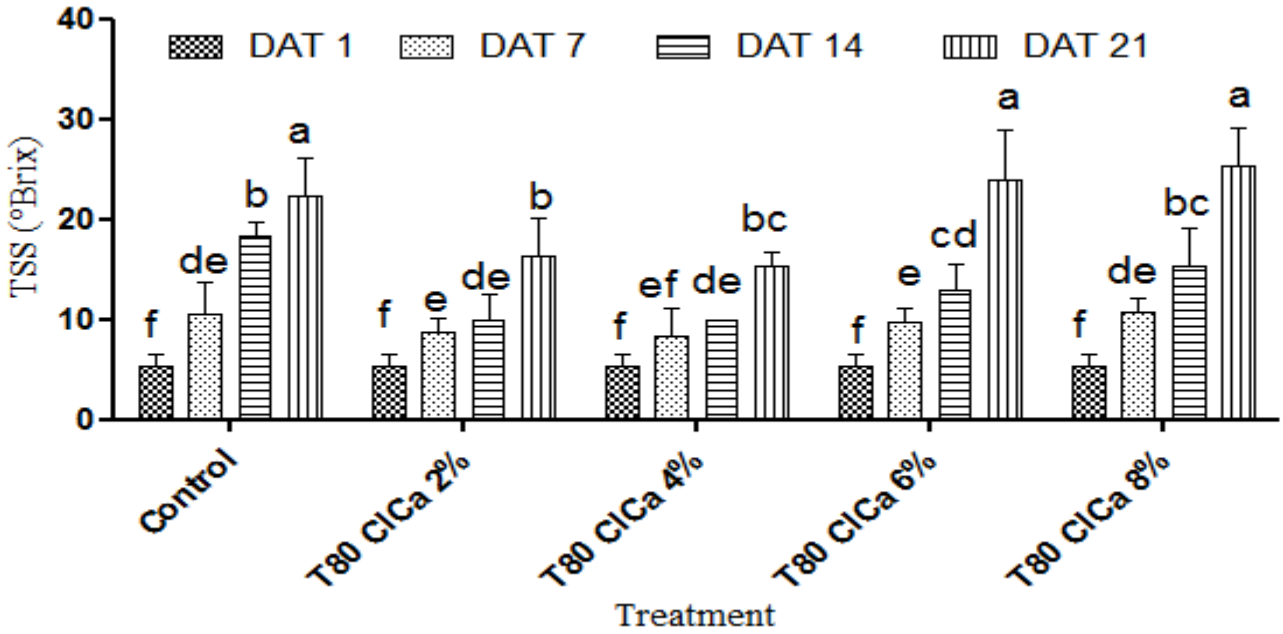

Fig. 4: Evolution of total soluble solids content over time

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

3.1.3 Effect of treatments on the variation of pigments in the pulp

The chlorophyll a content showed lower and lower values with the storage time. The decrease of this parameter was very similar in all treatments as shown in Fig. 5a. Moreover, according to the Tukey test at the .05 threshold, the only significant differences are found in the decrease of chlorophyll a content as a function of time. Thus, even if small differences were observed between treatments, the statistical test did not confirm them.

Chlorophyll b levels were higher on day 1 compared to the rest of the time in all bananas, both controls and treatments. But apart from this day 1 value, it is difficult to classify the progression of chlorophyll b as a function of time and even as a function of treatments (Fig. 5b). Moreover, the variations in chlorophyll b levels did not show any difference according to the analysis of variances at the .05 probability level.

In both treated and control bananas, carotenoid levels were almost constant over time. If some decreases in carotenoids were observed randomly, it should be mentioned that this was very small. Moreover, according to the analysis of variances, no significant difference was observed between the treatments or as a function of time (Fig. 5c).

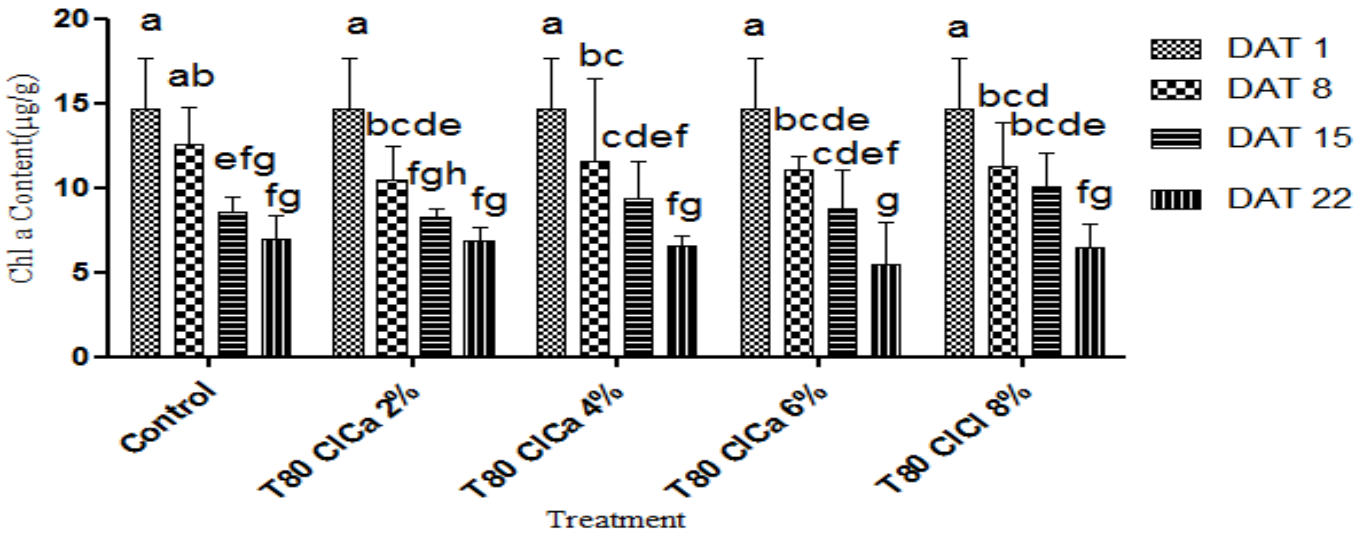

Fig. 5a: Evolution of chlorophyll *a* content

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

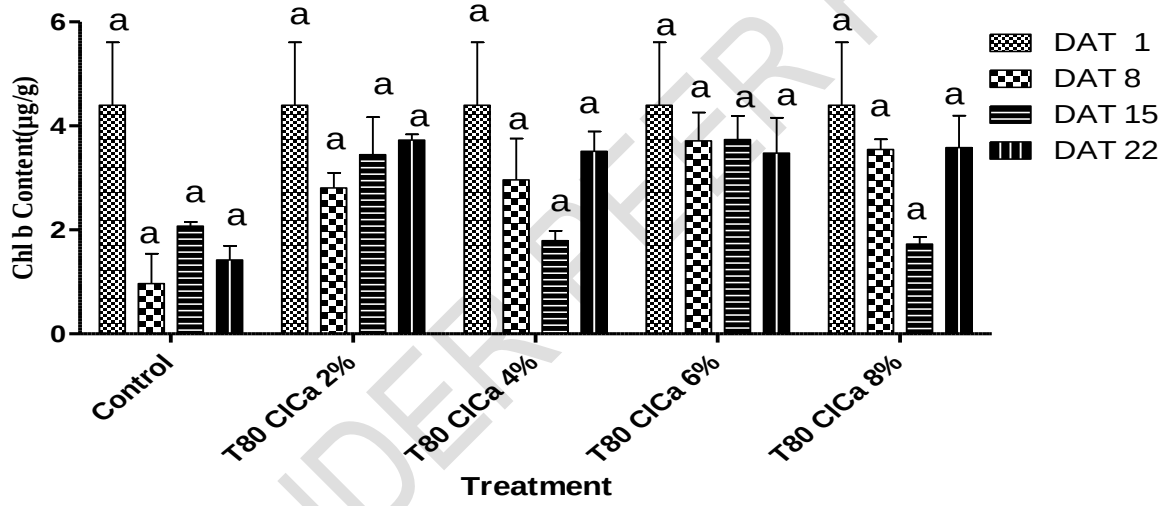

Fig. 5b: Evolution of chlorophyll *b* content

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

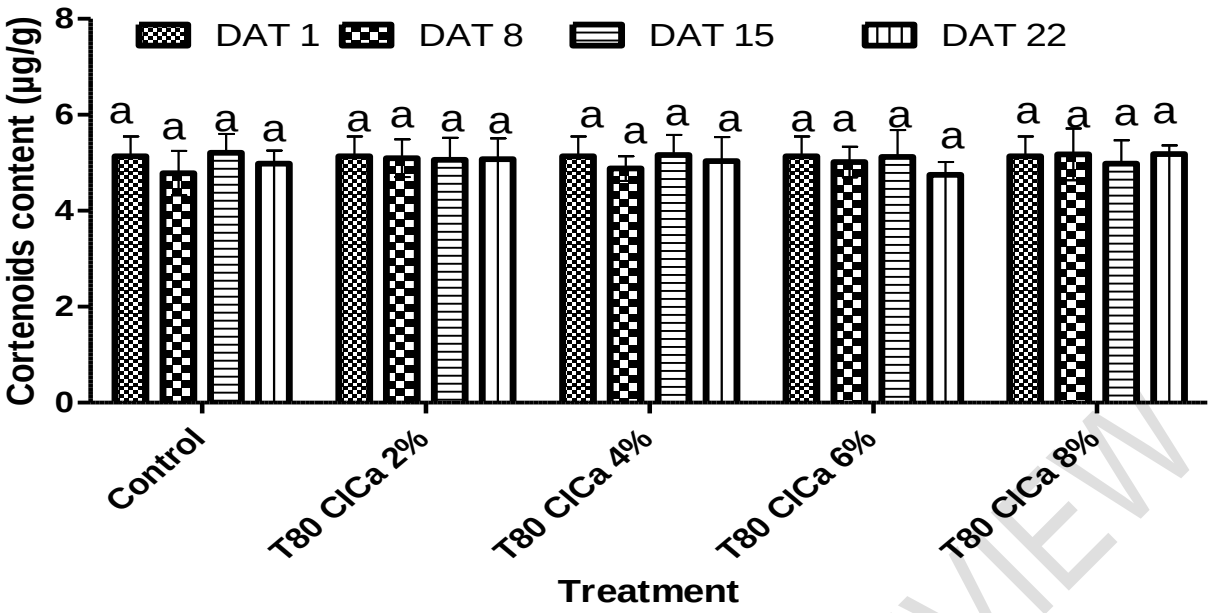

Fig. 5c: Evolution of carotenoids content

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

3.1 4 Effect of treatments on the variation of antioxidant compounds

Lycopene and β-carotene levels showed a similar evolution during storage. All concentrations were increasingly higher with time. Unusually, lycopene levels increased from 0.04 µg/g on day 1 to 0.49 µg/g on day 22, representing the highest value of the different banana lots and belonging to the control bananas (Fig. 6a). Moreover, compared to the others, the lycopene content of the control bananas was significantly high at 22 days after treatment according to the Tukey statistical test. All other treatments showed fairly close values on day 22.

For β-carotene, the increased concentrations went from 0.14 µg/g to 0.46 µg for the highest value and this again representing the control bananas. As a function of treatments, analysis of variance revealed no differences (Figure 6b). However, visually during ripening, the increases in β-carotene content could be observed. But according to the statistical test at the .05 threshold this increase was only significant in the control bananas.

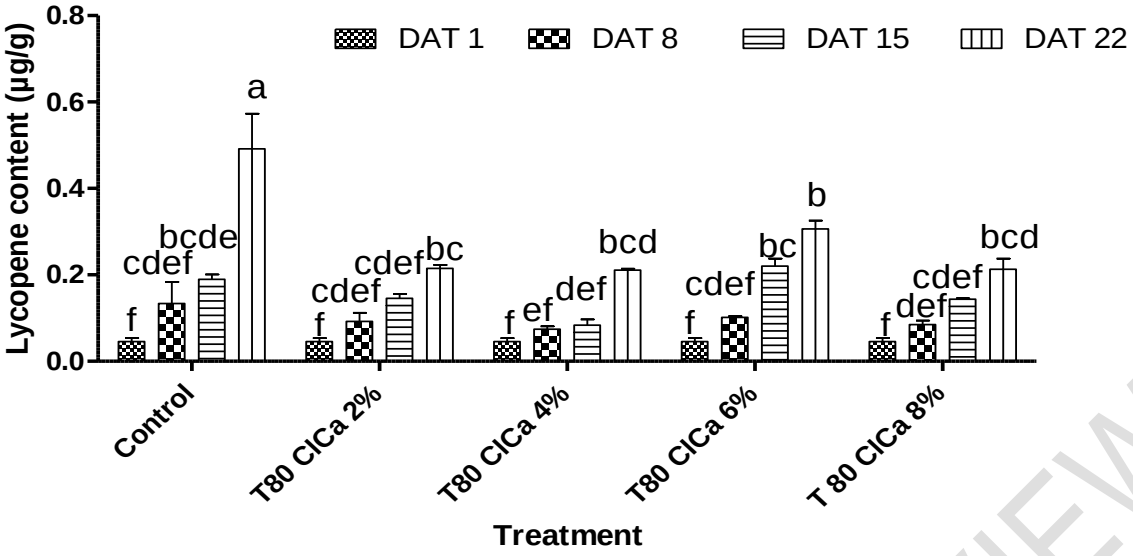

Fig. 6 a: Evolution of lycopene content

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

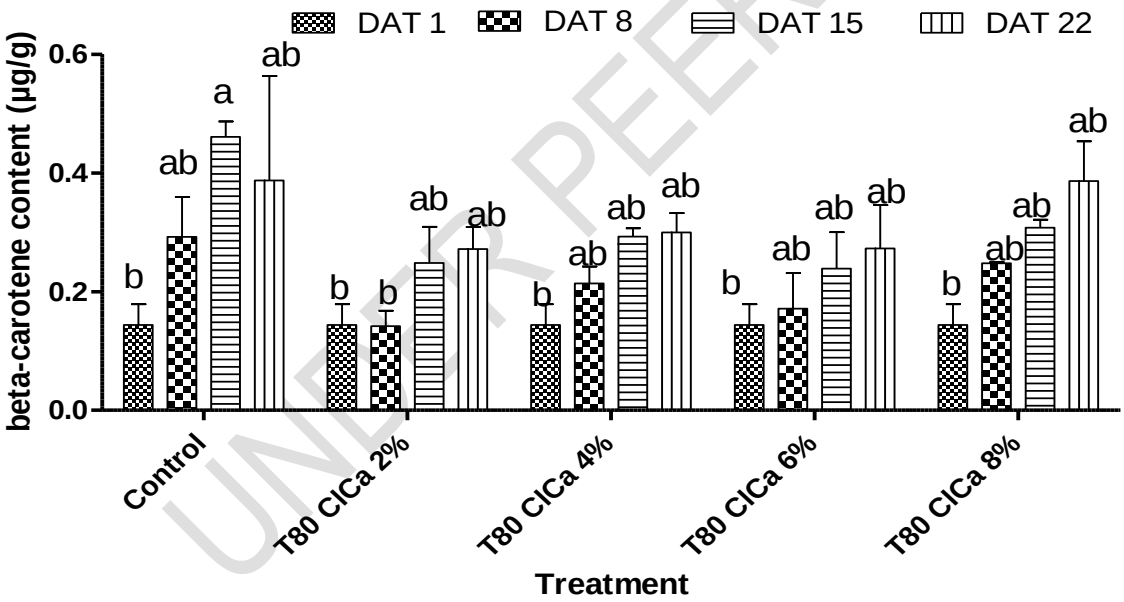

Fig. 6 b: Evolution of β-carotene content

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

In the banana peel, according to Fig. 7a, there is overall a small increase in total phenols content over time. However, depending on the treatments, only slight variations were observed. Apart from day 8 when a significant drop was recorded in the 8% Cl_2Ca treatments, no other significant difference was observed in phenol content either as a function of treatment or time.

Despite some inflections between days 8 and 16, the evolution of total phenol content in the pulp remained elevated (Fig. 7b). In fact, we can conclude levels increase with time. This was statistically confirmed by the Tukey test at the .05 probability level. Even if some significant differences were found here and there, it remains difficult to associate the evolution of the phenol content in the pulp to any calcium chloride treatment. However, in comparison to the values, those in the pulp are lower.

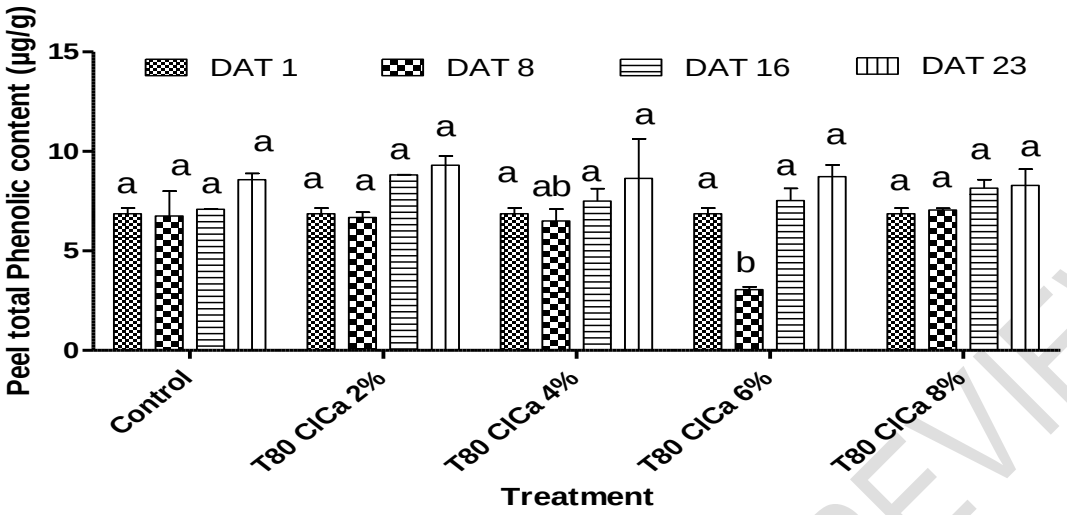

Fig. 7a: Evolution of total phenols content in the peel banana

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

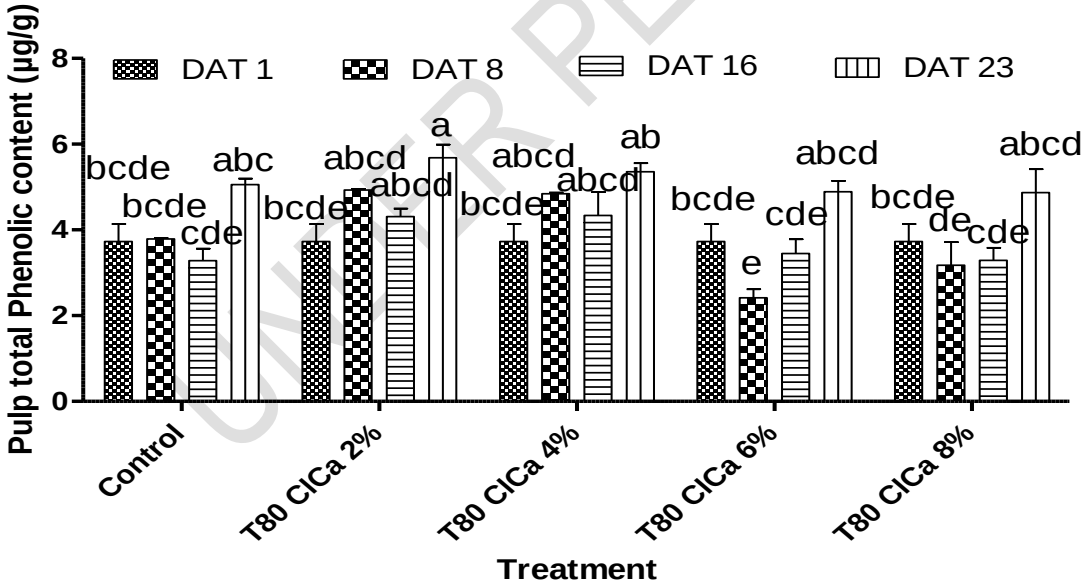

Fig. 7b: Evolution of total phenols in the pulp banana

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

According to Fig. 8a, the flavonoid values remained globally low and the variations were not very observable in the majority of the times. However, in the strict sense of the figures, the direction of variation of the values as a function of time was decreasing without distinction between treatments. However, according to the analysis of variances, no significant difference was found between treatments.
Compared to the flavonoid content in the peel, the flavonoid content in the pulp was higher overall (above 0.1mg/g). Similarly, the progression with time follows a regression as in fig. 8b. However, it is important to mention that according to statistical tests, this regression was in most cases significant. However, the variations in flavonoids could not be corroborated in any case according to the treatments.

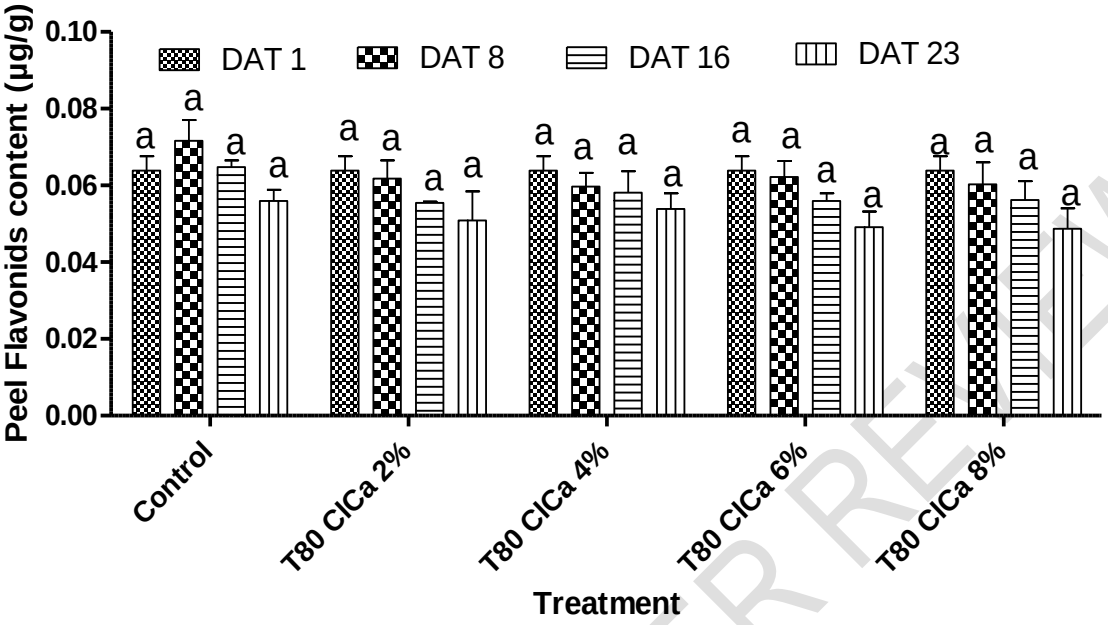

Fig. 8a: Evolution of the flavonoid content in the peel

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

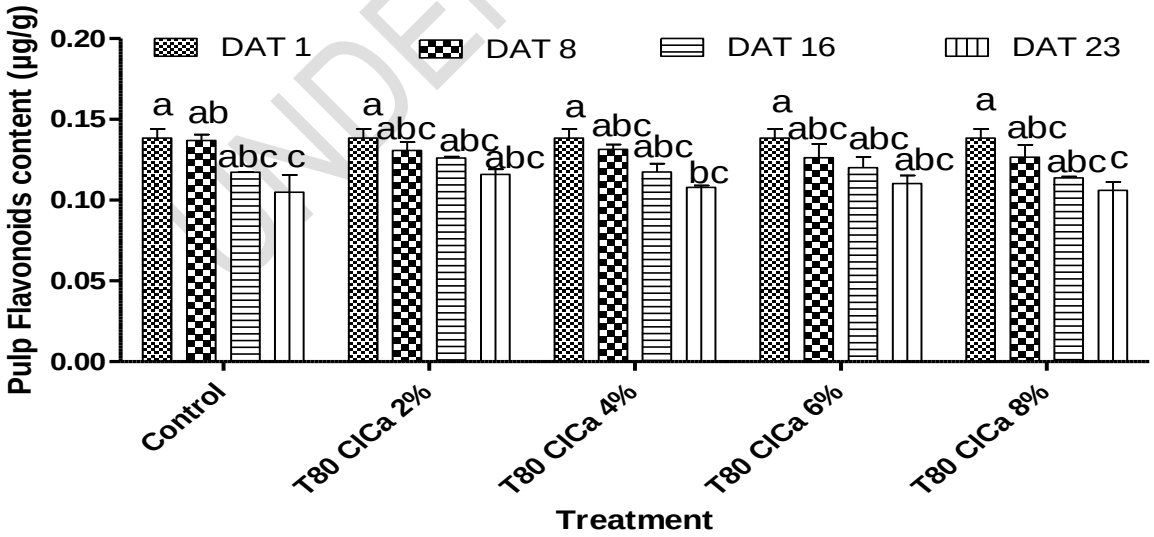

Fig. 8b: Evolution of the flavonoid content in the pulp

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

Apart from the fall observed on the 16th day in all treatments except the T80 ClCa 6%, the content of ascorbic acid in the peel increased globally. In the latter, a stabilization of the contents was observed (Fig. 9a). But also, the ascorbic acid contents were higher in bananas treated with 8% calcium chloride without and also in control bananas. Moreover, this remarkable increase was well observed through the Tukey statistical test on day 23.

In the pulp of the bananas, the ascorbic acid contents showed an increasing trend with time (Fig. 9b). In contrast to the peel, the levels in the pulp were lower (less than 2 µg/g for the largest). However, all treatments without distinction of calcium chloride concentration were very similar. But according to the Tukey test at the .05 probability level, a significant increase in ascorbic acid was recorded between days 1 and 23 in all treatments.

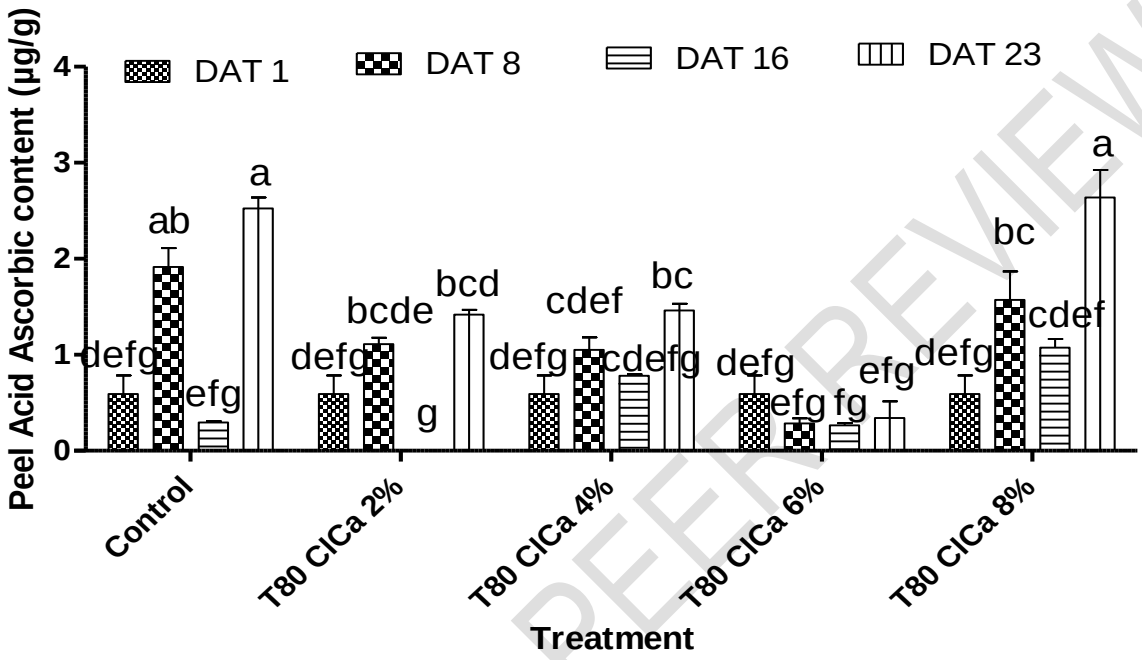

Fig. 9a: Evolution ascorbic acid in the peel

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

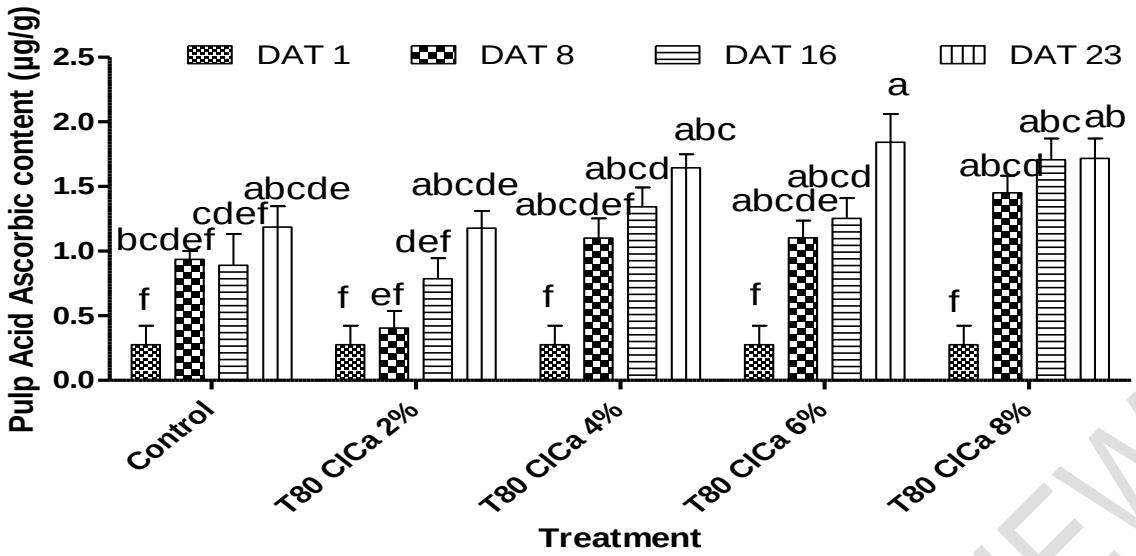

Fig. 9b: Evolution of ascorbic acid in the pulp

Bars for the treatments showing different letters are significantly different (p<.05); DAT: days after treatment T80: tween 80 ; ClCa: calcium chloride

3.2 Discussion

Visually, banana ripening is a process during which the epicarp gradually turns from green to yellow through several stages [27]. Differences in ripening time between treatments ranged from 15 days for control bananas to 27 days for bananas treated with 2 and 4% calcium chloride. The bananas treated with high concentrations of calcium chloride, however, showed an almost similar effect to the control bananas. While increasing calcium content increases the green life of the fruit through the stabilization of physiological processes [28], the opposite result obtained in the present study would be related to the damage orchestrated by the aggressive uptake of Cl_2Ca at the banana cell wall level. Moreover, the work of Bukovac *et al*. [16] showed that excessive calcium uptake causes lesions that are another problem for the fruit. This could also lead to disorders in the membranes which are crucial in stabilizing the structure of living cells.

The progressive decrease in fruit firmness observed in this study corroborates the results obtained by Kouame *et al.* [29] during the ripening of banana fruits. Indeed, the observation of loss of firmness during fruit ripening is related to the action of hydrolyzing enzymes [30] leading to a decrease in polymer levels, and an increase in solids and total soluble sugars levels [31]. The 2 and 4% Cl_2Ca treatments retained greater firmness at the end of the study. This result is similar to that of Aghofack-Nguemezi and Yambou [5] who found a significant loss of firmness in control bananas compared to those treated with 200mg/l Cl_2Ca. Moreover, according to White and Broadly [32], fruit treated with calcium chloride retained greater firmness during ripening. However, a high concentration of Cl_2Ca (6 and 8%), would have a damaging effect on the walls of the fruits during the penetration. According to William *et al.* [28], inappropriate calcium uptake would lead to tissue injury.

Fruits such as bananas contain a large number of water-soluble compounds such as sugars, acids, vitamin C and pectins. The total soluble solids content in this study increased in all bananas during storage. But less rapidly in fruit treated with 2 and 4% Cl_2Ca compared to control bananas and those treated with 6 and 8 Cl_2Ca. This sense of general increase in sugar during ripening is similar to the results on ripening of poyo and plantain bananas obtained by kouame *et al.* [29]. The increase in soluble sugar content is thought to be explained by a degradation of starch and other polysaccharides into soluble sugars in the pulp under the action of conversion enzymes [30,34].

While a decrease in water content during ripening was observed in the peel, the opposite phenomenon was elucidated in the pulp. This result is similar to the effect of salts and edible oil on calcium and water content during banana ripening, where an increase in water content was observed in the pulp and a decrease in the peel [33]. The ripening process of the fruits is accompanied by the hydrolysis of starch in the pulp into reducing sugars. The latter being molecules with a high osmotic potential. In order to restore the balance between the compartments of the cell, an osmotic migration of water takes place from the peel to the pulp. This is due to the high concentration of reducing sugars with greater osmotic power [23,35,36]. Indeed, an increase in peel dry matter is related to the fact that the pulp is enriched in water following the

migration of water from the peel to establish the balance of the ratio of dry matter of the pulp to the peel [37]. However, the water contents of bananas in these works (60 to 80% in the pulp and 80 to 62% in the peel) remained lower than those found by Aghofack-Nguemezi and Dassie [33]. Indeed, the latter used bananas harvested at ¾ of maturity whereas here we are dealing with fully ripe bananas.

Overall, a decrease in total chlorophyll content and a stabilization of carotenoid content were noted. In the case of chlorophylls, a similar result was reported by Youmbi *et al.* [38] on morphological and biochemical changes during development and ripening of *Spondias cytherea* fruits. A similar result was also obtained by Kouete *et al*., [39] with the preservation process of Cameroon mangoes. The coloring of the banana peel gradually changed from green to yellow. Chlorophylls are actually responsible for the green color of mature fruits and carotenoids responsible for the yellow color characteristic of ripening. The evolution of the pigments is linked to the ripening process of the fruits which is translated externally by the progressive loss of the green color of the peel in favor of a brighter color which varies according to the fruits [40]. This new color is linked to the presence of pre-existing carotenoids or newly synthesized ones [5]. In previous studies, a decrease in carotenoid content was observed in bananas up to a certain level before a further increase [41]. Thus, during ripening, the synthesis of new carotenoids would be coupled to its own degradation. This hypothesis would explain the stabilization of carotenoid levels in this work. Random variations in chlorophyll b showed no statistical difference. This could be due to the fact that chlorophyll b is first transformed into green intermediates of chlorophyll a before its complete degradation as suggested by Matile and Hörtensteiner [42]. Furthermore, based on its behavior, chlorophyll b cannot be used as a biological indicator of plant cell activity [41,43].

Lycopene and β-carotene contents were increasingly higher with time. This result is similar with that of Kouete *et al.*, [39] who presented an increase in β-carotenes during ripening in mangoes of the improved variety from Cameroon. According to Kouame *et al.* [29], at the end of ripening, the initially white pulp of Poyo banana turns orange-yellow. This change in pulp color reflects the presence of carotenoid pigments that are precursors of vitamin A [44]. In comparison, the lycopene content of control bananas is significantly elevated at day 22. The increase in carotenoid pigments is a function of ripening. The control bananas had a normal ripening process with a normal accumulation of carotenoids. Therefore, the injuries caused by Cl_2Ca at 6 and 8% would have damaged the structure of the cells and consequently altered the functioning during the synthesis of new carotenoids.

The total phenol contents, in general, both in the peel and in the pulp were increasing during ripening. This increase agrees with the result obtained in previous studies on the evaluation of bioactive compounds during tomato ripening [24,45]. According to these authors, the content of total phenols in tomatoes increases until the ripening stage, depending on the variety. The last stage of ripening is considered as the beginning of senescence during which the fruit uses its antioxidant potential to counter the effect of free radicals responsible for aging. In a preventive way therefore, during normal ripening, it would have an accumulation of total phenols. In fact, phenolic compounds are the most active antioxidants present in fruits and vegetables [46]. Their antioxidant function results from the combination of chelation and free radical scavenging properties as inhibitors of oxidases and other enzymes [47,48]. However, in comparison to the total phenol values, those in the pulp are lower. In fact, extracts from banana peels have a phenol content almost double that of extracts from the pulp [49]. Regarding the effects of calcium chloride, even if some significant differences were found here and there, it is difficult to associate the evolution of phenol contents to any concentration of calcium chloride.

Flavonoids play a very important role in the control and regulation of plants during periods of stress caused by environmental factors or by other living beings [50-53]. In relation to these works, the obtained flavonoid values presented small decreases in the majority of the times. Except during tomato ripening, there is an increase until a certain stage of ripening before an almost stabilization of flavonoid values [24,54]. In the same vein the evolution of flavonoid content during ripening even only in one species does not occur with the same speed. This evolution is a function of the variety. This is the case of three varieties "Cheramy" "Admiro" "Komet" which presented at any time different speeds of variation [24]. Therefore, the results related to the direction of progression of flavonoid contents in this work would be specifically associated with the species *Musa accumunata* and particularly with the variety poyo. Then the weak decrease of flavonoids would be related to its use in the reactions against the free radicals of oxygen. Moreover, flavonoids are considered as potential wellness-promoting molecules because of their antioxidant, anti-cancer, anti-diabetic and cardiovascular protective properties [55-57]. Also, the decrease in flavonoids can be correlated to the dilution of the medium with the increase in water content at the pulp level.

Ascorbate is able to react directly with superoxide anion and singlet oxygen to reduce lipid peroxidation and protein and DNA damage [58]. Also, ascorbic acid plays an indirect antioxidant role by recycling carotenoids and vitamin E [59]. During the present study on the preservation of bananas, an increase in ascorbic acid content was recorded as a function of time. Only in the pulp the values were slightly lower than in the peel. The trend of ascorbic acid contents progressions in this study agrees with that of Nour *et al.* [24]. According to their results, ascorbic acid accumulation occurs at all stages of ripening in tomato. In climacteric fruits, a peak in respiration is observed during ripening. In fact, the increase in ascorbic acid levels during this process is related to the enhancement of respiration processes [60]. However, some studies have concluded that ascorbic acid levels are constant during the first stages of fruit ripening, with only a slight increase observed near the end of ripening [61]. In addition, according to, Nour *et al*. [62] the accumulation of vitamin C is effective until a certain stage of ripening from which the value decreases significantly. In view of the multiple results obtained here and there, it is necessary to postulate the hypothesis that the physiological functions of vitamin C

are dependent on both environmental and varietal conditions. Indeed, in the work of Nour *et al.* [24] alone, the three tomato varieties show a different rate of progression. With reference to the treatments, in the majority of cases, ascorbic acid levels were higher in control bananas. In the latter, the ripening was also faster. In relation to the physiological role of ascorbic acid during ripening, it would be understood that the concentrations of 2 and 4% Cl_2Ca influenced the increase in vitamin C content.

4. CONCLUSION

A new preservation trial of bananas using calcium chloride and a surfactant, tween 80 to extend shelf life, was the focus of this experiment. In particular, direct ripening components such as ripening rate, firmness, total soluble solids, chlorophyll loss and water contents were evaluated in the first part. In the second part, the experimentation was focused on the analysis of parameters with antioxidant potential. Thus, the best preservation time was obtained in bananas treated with tween 80 at 2 and 4% Cl_2Ca. The treatments with 6 and 8% Cl_2Ca behaved like the control. This difference in storage time was generally observed in the parameters evaluated. This is the case for the slower accumulation of total soluble solids, the loss of firmness, and the slower increase in water content in bananas treated with 2 and 4% Cl_2Ca. For compounds with antioxidant potential, in most cases the small differences found were not significant according to the statistical test depending on the treatments.

AUTHORS' CONTRIBUTIONS

This work was done in collaboration between all authors. Author TPE, BDR and ANJ carried out the laboratory experiment and the statistical analysis. Authors KJO, CNN and DR managed the bibliographic research, wrote the protocol and the first version of the manuscript. The protocol and the first draft of the manuscriptt. The TPE author designed the study and managed the final manuscript. Authors read and approved the final manuscript.

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