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Assessment of Some Ripening Parameters (Antioxidant and Enzymes) during Storage of Banana (*Musa acuminata)* after Treatment with Calcium Chloride

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The valorisation of the large scale production of bananas in the producing countries depends on the safeguarding of the quality during exports throughout the world. The objective of the present study was to extend the shelf life of bananas by elucidating some physical and physiological phenomena that accompany the ripening process. To achieve this, bananas were treated by soaking in solutions of tween 20 for 10 minutes and calcium chloride at 2, 4, 6 and 8% for 30 minutes. The following parameters were evaluated generally in the peel and pulp as a function of time during ripening: green life, firmness of bananas, water content, Brix index, pigments, antioxidant compounds such as ascorbic acid and flavonoids, activity of two enzymes, chlorophyllase and pectin-methylesterase. The green life was 25 days for bananas treated with tween 20 at 4% calcium chloride and almost 15 days for controls. Firmness was lower in bananas treated with tween 20 at 6 and 8% calcium chloride as well as in control bananas. While the Brix index was higher in the latter, photosynthetic pigment contents such as chlorophylls *a* and *b* were lower over time. Lycopene, β-carotene and ascorbic acid contents increased significantly during ripening. Flavonoid content in the skin varied less during the sampling period. However, a regression of the latter was noted in the pulp. The enzymatic activity of both chlorophyllase and pectin-methylesterase was increasing during the whole

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experiment. The effect of the treatments on the majority of the evaluated parameters was elucidated. Although it was not always statistically significant. The treatments with tween 20 at 2 and 4% calcium chloride were the best, certainly because of an adequate integration of calcium chloride in the tissues. On the other hand, the 6 and 8% treatments showed an unexpected result, similar to the control. The integration of a high calcium content would lead to a tearing of the tissues and consequently to a disorganisation of the membrane, resulting in a faster ripening.

Keywords: Banana; calcium chloride; shelf life; ripening; antioxidant; enzyme.

1. INTRODUCTION

The ripening of climacteric fruits like banana is characterized by a sudden increase of the respiratory intensity and an important release of ethylene. This ripening process in bananas at the visual level is materialized by the progressive passage of the green color to the yellow color of the epicarp according to several stages whose importance contributes to shorten or to prolong the total duration of the process [1]. However, at the level of the pulp, the coloration reflects the presence or not of carotenoid pigments precursors of vitamin A [2]. In general, the color of the epicarp, the color of the pulp and the softening of the whole fruit allow consumers to establish a relationship between ripeness and quality [3-5]. On a global scale, banana production is estimated at nearly 134 million tons per year. That is, 79 million tons of dessert bananas and 55million tons of cooking bananas [6] for a cultivated land surface of about 10 million hectares [7]. Dessert bananas represent the most produced and traded fruit in the world with 17 million tons exported ahead of potatoes and citrus [8-10]. It should be noted that, the development of this large production requires the safeguarding of quality during exports around the world. This can be done by monitoring the components associated with ripening which are among others, chlorophylls, carotenoids, bioactive compounds such as antioxidants and enzymes.

Recently, several conservation techniques are being developed and tested. These include refrigeration and drying [11], chemical treatments with agents based on chlorine, peroxy-acetic acid and hydrogen peroxide [12], heat treatments [13,14] and controlled and modified atmosphere packaging[15,16]. However, several limitations have been revealed to some of these methods. For example, the high costs of cold room installations and devices for some heat treatments [17] and the risks of modification of the organoleptic qualities of the fruit following the example of chilling injury in most cases of refrigeration and controlled atmospheres. Moreover, the problem of post-harvest losses persists and these are of the order of 32% for all food categories combined in the world [17]. Therefore, knowledge of the physical and biochemical changes related to ripening would be an important contribution to the development of appropriate preservation technology that would delay ripening and maintain fruit quality.

Many physiological and pathological disorders of fruits are related to the calcium content of the tissues. With reference to other work [18], soaking mature green bananas in a calcium chloride solution of concentration 200 mg l^{-1} resulted in a substantial increase in the endogenous Ca^{2+} ion content and induced significant water retention in the peel. According to other studies, calcium chloride slows the development of physiological disorders and improves fruit quality [19]. However, the excessive absorption of Calcium, leads to lesions which constitute another problem for the fruits. To this end, it should be noted that epicuticular waxes are known to reduce cuticular penetration of many solutes [20]. Therefore, modification of the epicuticular wax without altering its protective properties may allow for increased and more uniform calcium absorption. In addition, surfactants such as tween 20 are known to modify energy relationships at interfaces, thereby reducing surface tension [21] and improving leaf uptake of biologically active compounds [22].

Due to the large local production of bananas, Dschang University, in its mission to provide solutions to the concerns of society, finds a banana conservation trial as an opportunity to be seized.Thus, simultaneous treatments with tween 20 and calcium chloride could provide a more meaningful basis for preserving and improving the quality of fresh fruit during the post-harvest period. The objective of the present study was to develop a method for extending the shelf life of bananas while demonstrating the physical and physiological phenomena that accompany the ripening process.

2. MATERIALS AND METHODS

2.1 Plant Material and Treatments

The dessert bananas of the *poyo* variety used were harvested in an orchard in the town of Melon in West Cameroon at full green physiological maturity. The present experiment was carried out between the months of February and March 2020, at the University of Dschang, West-Cameroon 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 other four batches (2 to 5) were soaked for 10 minutes in a 5% V/V tween 20 solution. Then they were treated by soaking for 30 minutes in calcium chloride solutions of 2%, 4%, 6% and 8% concentration respectively.

2.2 Visual Assessment of Ripening: Green Life

Green life was defined as the time from the first day of treatment to the day when 100% of the fruit in each batch had reached the ripe-yellow stage.

2.3 Measurement of Firmness, Water Content and Total Soluble Solids Content

Firmness was assessed using three punctures made at different points of the fruit after peeling using a GY-2 penetrometer following the method of Mehinagic et al. [23].

The water content of the banana peel and pulp was determined by the desiccation method of analysis, where 20g of fresh material was placed in an oven at 90°C until all water was removed.

$$\text{Water content (WC)} = \frac{initial\ weight - final\ weight}{initial\ weight} x100$$

The determination of the soluble solids content of the banana pulp in °Brix of fruit was performed by refractometry.

2.4 Determination of Pigment Content

The content of photosynthetic pigments (chlorophyll *a* and chlorophyll *b*) in the banana peel was determined by the method of Lichtenthaler [24].

In the pulp, the contents of chlorophylls *a* and *b*, lycopene and β-carotene were determined by the method of Nagata and Yamashita [25].

2.5 Determination of Protein Content

The Biuret method developed by Cooper [26] was used for protein determination. Four grams of banana peel were taken and ground with 1 g of sand, 20 ml of distilled water was added and stirred. This was followed by filtering through a very tightly woven nylon cloth. To this solution, chloroform was added in the proportions 1/2 (V/V) and stirred for a short time to get rid of impurities. The supernatant was centrifuged at 4000 rpm for 10 minutes, 2 ml of the supernatant was removed and 3 ml of Biuret reagent was added. The tubes were incubated in the dark for 20 min at 37°C. The absorbance was read with a *Biochrom Libra spectrophotometer, model: S22* at 540 nm. The standard calibration curve from different albumin concentrations was used.

2.6 Determination of Total Flavonoid Content

Using 1 g of fine sand, 5 g of banana pulp or peel was crushed, then 10 ml of methanol containing 0.5% (V/V) hydrochloridric acid was added and the mixture was allowed to stand for 45 minutes at room temperature. The total flavonoid content was determined by the method developed by Mohommadzedeh *et al.* [27] Furthermore, 0.5 ml of the methanolic extract was diluted with methanol (1/10/ v/v), 0.1 ml of 10% concentrated aluminium nitrate, 0.1 ml of 1M potassium acetate aqueous solution and 4.3 ml of methanol were added in order. The mixture was allowed to stand for 40 minutes. The absorbance was measured by spectrophotometer at 415 nm and Quercetin was used to make the standard calibration curve.

2.7 Determination of Ascorbic Acid Content

Ascorbic acid concentration was determined following the method of Malik and Singh [28]. Banana pulp or peel (5 g) was crushed using 1 g of sand and 20 ml of 6% metaphosphoric acid containing 0.18 g of ethylene diamine tetraacetate disodium salt (EDTA) was added. The mixture was then homogenized and centrifuged at 4000 rpm for 15 min. A portion (400 µl) of the supernatant was taken and mixed with 200 µl of 3% metaphosphoric acid, 1.4 ml of

diluted distilled water in which 200 µL of Folin's reagent was also added.

After 10 minutes, the absorbance of the sample was measured at a wavelength of 760 nm using a *Biochrom Libra spectrophotometer, model: S22*. The amount of ascorbic acid was determined from a standard curve of L-ascorbic acid.

2.8 Determination of Chlorophyllase Activity

Banana peel (5 g) was crushed with 1 g of fine sand and 15 ml of acetone was added and left for some time, for total pigment extraction. The residue was rinsed three times in 5 ml of acetone, followed by addition of 5 ml of phosphate buffer at pH 7 with 50 mM potassium chloride (KCl) and 0.24% Triton X-100. The mixture was left for one hour in ice and then centrifuged at 4000rpm for 15min. The supernatant was used as crude enzyme extract. Chlorophyllase activity was determined in the reaction mixture according to the method of Hui-Cong et al. [29], which consisted of adding 200µl of enzyme extract to 1ml of phosphate buffer at pH 7 containing 0.46% Triton X-100 and 1ml of acetone chlorophyll solution of known optical density. The reaction solution was incubated in the water bath at 30°C for 45 minutes. The enzymatic reaction was stopped by adding 5ml of acetone. The remaining undegraded chlorophyll was extracted with 5 ml of hexane. The absorbance was measured at 663 nm. The decrease in absorbance was taken as the unit of enzymatic activity.

2.9 Determination of Pectinmethylesterase (PME) Activity

Pectinmethylesterase activity was determined in banana peel and pulp by the method of Hagerman and Austin [30] as modified by Omar and Aaran [31].

Five grams of peel or pulp and 1 g of sand were crushed and 15 ml of 1M NaCl concentration and 10 g l^{-1} of polyvinypolypyrrolidone (PVPP) were added. The mixture was thoroughly homogenized repeatedly for 4 hours and centrifuged at 4000 rpm for 15min. The pH of the filtrate was adjusted to 7 from 1M NaOH and the extract was used to determine the activity of PME. All steps in the preparation of the extract were performed in ice. Also, the enzyme activity was determined in a mixture consisting of 600 µl of 0.5% pectin, 150 µl of 0.01% Bromothymol Blue in phosphate buffer at pH 7.5, 100 µl of distilled water and 100 µl of the enzyme extract. Lastly, the decrease in optical density was determined by transmittance at 620 nm at the 10-minute time intervals. The result was expressed as the change in optical density (∆ D.O) in time per gram of peel.

2.10 Statistical Analysis

For control and each treatment, the tests were repeated thrice. Data obtained for different parameters (water content, content of pigments, flavonoids, ascorbic acid and activity of enzyme) from repeated experiments were subjected to analysis of variance (ANOVA). When this analysis showed significant differences, means were compared in pairs using Duncan's multiple range test the P = 05 probability level. The statistical package IBM SPSS Statistics 20 was used for this purpose.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Evolution of the green life

The ripening time of the fruits was between 15 and 25 days. As shown in Fig. 1, the green life time is in a curvilinear pattern according to the calcium chloride concentrations with the 2% and 4% treated bananas showing the best shelf life in detriment of the control and 6% and 8% treated bananas. Moreover, these better preservation scores were observed from the Duncan statistical test the P = 05.

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

After one week of storage, no decrease in firmness was recorded. It is from the 14th day that the first loss of firmness was noted. The bananas treated with tween 20 at 4% calcium chloride kept maximum values of firmness which is 4N compared to the rest of the treatments where a statistically confirmed significant decrease was observed. Moreover, on day 21, the firmness of bananas treated with 2% and 4% calcium chloride tween 20 decreased but remained higher than that of the control lots and the 6% and 8% calcium chloride treatments on day 21.

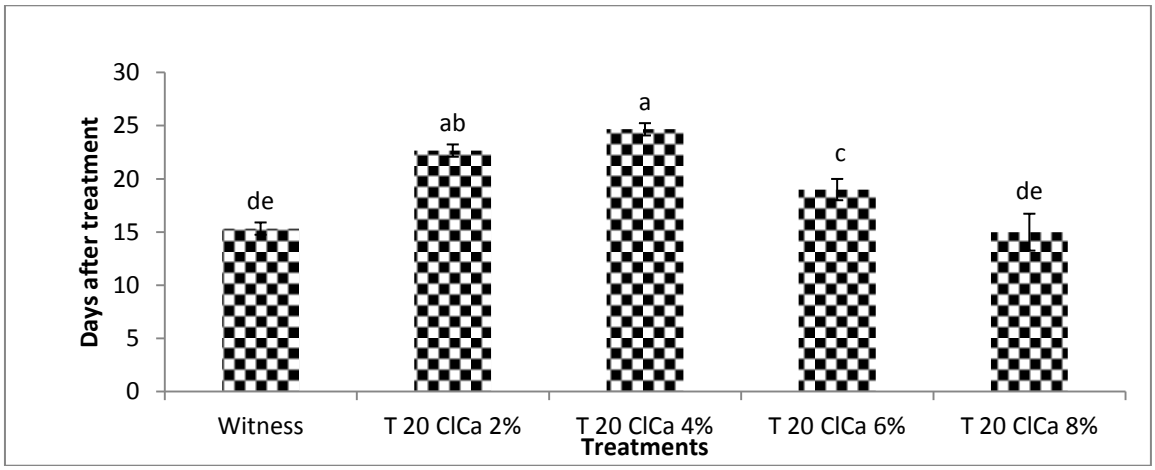

Fig. 1. Variation of ripening time according to treatments

According to Table 1, the water contents evolved in the opposite direction depending on whether they were in the peel or the pulp of the bananas. For the water content in the peel, an overall decrease over time was observed in all treatments. The values were 80% on the first day for the largest and 63.33% ± 2.88% on the 21st day for the smallest during storage. The control bananas showed a lower water content on day 21. However, no significant difference was noted between the other treatments at the end of the experiment

Contrary to the variation of the water content in the peel, the water content in the pulp had an increasing evolution during the storage period. The initial water content was 60% and reached 80% at the end of the experiment for the largest. However, on the 21st day, the treatments T20 $CaCl_2$ 2% and T20 $CaCl_2$ 4% were those that presented lower values of water content. But this difference was only confirmed by Duncan's test at the 5% probability level.

With a very low value of 5.36 at the beginning of the experiment, successively increasing values of soluble solids content as a function of time and treatments were observed. Control bananas and bananas treated with 6% and 8% calcium chloride showed a significantly higher value at day 7. According to Duncan's test at the the P = 05 threshold, this significant increase in soluble solids content in the control bananas and those of the treatments T20 $CaCl_2$ 6% and T20 $CaCl_2$ 8% follows the same logic until day 21 (Table 1).

3.1.3 Effects of treatments on the variation of pigment content in banana peel

Chlorophyll *a* (chl *a*) levels showed lower values more and more as storage time was extended. On day 8, the high chlorophyll *a* content was found in control bananas followed by T20 $CaCl_2$ 2% and T20 $CaCl_2$ 4%. However, on the 15th day except, the T20 $CaCl_2$ 2% shows a high significant value in comparison to T20 $CaCl_2$ 6% and T20 $CaCl_2$ 8%. Moreover, on the 22nd day, this $CaCl_2$ 2% treatment kept the highest value in chl *a*. But according to the analysis of variances no significant difference was observed at this as shown in Table 2.

Chlorophyll *b* (chl *b*) levels were higher on day 1 than the rest of the time in all bananas, both control and treated. But apart from this value on day 1, it is difficult to talk about the direction of progression of chl *b* as a function of time and even as a function of treatments. Moreover, contrary to chl *a*, the variations in chl *b* content showed almost no statistical difference between treatments or over time.

3.1.4 Effects of treatments on the variation of pigment content in banana pulp

Firstly, the concentrations of chlorophylls in banana pulp, whether chl *a* or chl *b*, were revealed only in low quantities, all below 1µg.g^{-1} (Table 3). In the case of chl *a*, overall, a decrease in concentrations was observed over time. However, all treatments then presented non-significantly different contents. Thus, the only significant differences were found as a function of time and almost never as a function of treatment. The only exception was the control bananas at day 15, which had a low level of significance compared to the rest. Concerning chl *b*, the values compared to chl *a* were even smaller. However, there was no real direction of progression of the levels. But from day 15, the chlorophyll b content of T20 $CaCl_2$ 6% was statistically lower than the others.

Table 1. Evolution of firmness, water content and total soluble solids

par	DAT	Control	T20 $CaCl_2$ 2%	T20 $CaCl_2$4%	T20 $CaCl_2$ 6%	T20 $CaCl_2$8%
Fir	1	$4±0^{a}$	$4±0^{a}$	$4±0^{a}$	$4±0^{a}$	$4±0^{a}$
(N)	7	$4±0^{a}$	$4±0^{a}$	$4±0^{a}$	$4±0^{a}$	$4±0^{a}$
	14	$1.73±0.05^{e}$	$3.56±0.15^{b}$	$4±0^{a}$	$2.43±0.05^{c}$	$2,13±0.15^{d}$
	21	$1.53±0.05^{f}$	$2.1±0.1^{d}$	$3.66±0.11^{b}$	$1,60±0.17^{ef}$	$1.23±0.15^{g}$
wc pe	1	$80±0^{a}$	$80±0^{a}$	$80±0^{a}$	$80±0^{a}$	$80±0^{a}$
(%)	7	$71.66±2.88^{def}$	$76.66±2.88^{abc}$	$78.33±2.88^{ab}$	$80±0^{a}$	$76.66±2.88^{ghi}$
	14	$70±0^{efg}$	$75±0^{bcd}$	$73.33±2.88^{cde}$	$73.33±2.88^{cde}$	$75±0^{bcd}$
	21	$63.33±2.88^{h}$	$68.33±2.88^{fg}$	$66.66±2.88^{gh}$	$66.66±2.88^{gh}$	$66.66±2.88^{gh}$
wc pu	1	$60±0^{f}$	$60±0^{f}$	$60±0^{f}$	$60±0^{f}$	$60±0^{f}$
(%)	7	$71.66±2.88^{cde}$	$68.33±2.88^{e}$	$68.33±2.88^{e}$	$70±0^{de}$	$73.33±2.88^{cd}$
	14	$75±0^{bc}$	$71.66±2.88^{cde}$	$71.66±2.88^{cde}$	$73.33±2.88^{cd}$	$75±0^{bc}$
	21	$80±0^{a}$	$78.33±2.88^{ab}$	$78.33±2.88^{ab}$	$80±0^{a}$	80±0a
TSS	1	$5.36±0.47^{h}$	$5.36±0.47^{h}$	$5.36±0.47^{h}$	$5.36±0.47^{h}$	$5.36±0.47^{h}$
(°Brix)	7	$10.50±1.32^{de}$	$7.66±0.57^{fg}$	$7.33±1.52^{gh}$	$9.33±1.52^{def}$	$9.66±1.52^{de}$
	14	$18.40±1.51^{b}$	$10.66±1.52^{d}$	$8.66±0.57^{efg}$	$14.66±0.57^{c}$	$15.33±1.52^{c}$
	21	$22.33±1.51^{a}$	$15.33±1.52^{c}$	$16±1.52^{c}$	$22.66±0.57^{a}$	$23.66±1.52^{a}$

Values follow by the same letter(s) are not statistically different at a threshold of 5%. Pe, peel ; pu, pulp ; wc, water content ; TSS, total soluble solids; DAT, days after treatment

Table 2. Evolution of pigment content in banana peel

para	DAT	Control	T20 $CaCl_2$ 2%	T20 $CaCl_2$4%	T20 $CaCl_2$ 6%	T20 $CaCl_2$8%
Chl *a*	1	$14.65±1.22^{a}$	$14.65±1.22^{a}$	$14.65±1.22^{a}$	$14.65±1.22^{a}$	$14.65±1.22^{a}$
	8	$12.60±0.86^{b}$	$11.13±0.81^{c}$	$9.91±1.07^{cd}$	$9.58±0.7^{de}$	$9.45±0.80^{def}$
(μgg^{-1})	15	$8.54±0.37^{defg}$	$9.55±1.40^{de}$	$8.22±0.49^{efg}$	$7.89±0.13^{fgh}$	$7.60±0.38^{ghi}$
	22	$6.92±0.56^{ghi}$	$7.19±0.84^{ghi}$	$6.37±0.58^{hi}$	$6.04±0.21^{i}$	$6.51±0.44^{hi}$
Chl b	1	$4.39±2.09^{a}$	$4.39±2.09^{a}$	$4.39±2.09^{a}$	$4.39±2.09^{a}$	$4.39±2.09^{a}$
(μgg^{-1})	8	$0.96±0.99^{c}$	$2.97±0.84^{abc}$	$2.70±1.19^{abc}$	$2.70±1.19^{abc}$	$3.0±0.50^{abc}$
	15	$2.07±0.13^{abc}$	$2.70±0.72^{abc}$	$2.24±0.08^{abc}$	$2.24±0.08^{abc}$	$3.44±1.24^{ab}$
	22	$1.42±0.47^{bc}$	$2.68±0.33^{abc}$	$3.30±0.61^{abc}$	$2.96±0.61^{abc}$	$3.72±0.18^{ab}$

Values follow by the same letter(s) are not statistically different at a threshold of 5%. DAT, days after treatment chla, chlorophyll a; chlb, chlorophyll b

Table 3. Evolution of pigment content in banana pulp

Par	DAT	Control	T20 $CaCl_2$ 2%	T20 $CaCl_2$4%	T20 $CaCl_2$ 6%	T20 $CaCl_2$8%
Chl a	1	$0.67±0.28^{a}$	$0.67±0.28^{a}$	$0.67±0.28^{a}$	$0.67±0.28^{a}$	$0.67±0.28^{a}$
(μgg^{-1})	8	$0.52±0.10^{ab}$	$0.50±0.22^{abc}$	$0.50±0.09^{abc}$	$0.38±0.02^{abcde}$	$0.42±0.07^{abcd}$
	15	$0.007±0.12^{f}$	$0.09±0.04^{ghi}$	$0.28±0.01^{bcde}$	$0.29±0.06^{bcde}$	$0.26±0.07^{bcde}$
	22	$0.16±0.17^{cdef}$	$0.06±0.04^{ef}$	$0.13±0.03^{def}$	$0.10±0.05^{def}$	$0.15±0.23^{def}$
Chl b	1	$0.29±0.12^{abc}$	$0.29±0.12^{abc}$	$0.29±0.12^{abc}$	$0.29±0.12^{abc}$	$0.29±0.12^{abc}$
(μgg^{-1})	8	$0.23±0.13^{abc}$	$0.28±0.07^{abc}$	$0.15±0.04^{bc}$	$0.38±0.12^{ab}$	$0.32±0.14^{abc}$
	15	$0.01±0.03^{c}$	$0.47±0.19^{a}$	$0.18±0.03^{abc}$	$0.04±0.11^{fgh}$	$0.36±0.11^{ab}$
	22	$0.01±0.27^{c}$	$0.15±0.26^{bc}$	$0.19±0.045^{abc}$	$0.08±0.08^{efgh}$	$0.26±0.40^{abc}$
Lyco	1	$0.04±0.01^{fg}$	$0.04±0.01^{fg}$	$0.04±0.01^{fg}$	$0.04±0.01^{fg}$	$0.04±0.01^{fg}$
(μgg^{-1})	8	$0.13±0.08^{cdef}$	$0.11±0.04^{ef}$	$0.02±0.01^{g}$	$0.09±0.028^{efg}$	$0.07±0.01^{fg}$
	15	$0.18±0.019^{bcde}$	$0.18±0.01^{bcde}$	$0.19±0.02^{bcde}$	$0.13±0.03^{def}$	$0.13±0.06^{cdef}$
	22	$0.49±0.14^{a}$	$0.24±0.08^{bc}$	$0.27±0.00^{b}$	$0.22±0.01^{bcd}$	$0.22±0.04^{bcd}$
β-car	1	$0.14±0.06^{c}$	$0.14±0.0^{c}$	$0.14±0.06^{c}$	$0.14±0.06^{c}$	$0.14±0.06^{c}$
(μgg^{-1})	8	$0.29±0.11^{abc}$	$0.18±0.03^{bc}$	$0.24±0.04^{abc}$	$0.14±0.11^{c}$	$0.17±0.12^{bc}$
	15	$0.46±0.04^{a}$	$0.14±0.12^{c}$	$0.22±0.02^{abc}$	$0.40±0.06^{abc}$	$0.17±0.16^{bc}$
	22	$0.38±0.30^{abc}$	$0.35±0.25^{abc}$	$0.29±0.02^{abc}$	$0.42±0.07^{ab}$	$0.31±0.28^{abc}$

Values follow by the same letter(s) are not statistically different at a threshold of 5%. DAT, days after treatment chla, chlorophyll a; chlb, chlorophyll b; β-car, β-carotene; lyco, lycopene

Lycopene and β-carotene levels showed a similar evolution during storage. Unusually, lycopene levels increased from 0.04 µg g^{-1} on day 1 to 0.49 µg g^{-1} on day 22, representing the highest value of the different banana lots and belonging to the control bananas. Moreover, compared to the others, the lycopene content of the control bananas was significantly high at this date. All other treatments showed fairly close values. The concentrations of β-carotene also, having increased, were located between 0.14 µg g^{-1} to 0.46 µg g^{-1} for the greatest value and this, still representing the control bananas. According to the analysis of variance, no difference was observed in the means of β-carotene between the different treatments (Table 3). Thus, significant increases in β-carotene content according to Duncan's test at the 5% threshold were only observed as a function of time and treatment.

3.1.5 Evolution of total flavonoid and ascorbic acid contents

According to Table 4, the flavonoid values obtained in peel ranged from 0.05±0.00 to 0.07±0.00 mg.g^{-1}. These values were overall low with minimal fluctuation in the majority of times. However, in the strict sense of the numbers, it is difficult to note the direction of variation of the values with time. Moreover, according to the analysis of variances, no decrease or increase was really significant. Compared to the flavonoid content in the peel, the flavonoid content in the pulp was higher overall (above 0.1mg.g^{-1}). A regression of values in all treatments was statistically observed. However, the variations in flavonoids were not different according to the treatments.

During the preservation of bananas, both in the peel and in the pulp, an increase in ascorbic acid content was observed as a function of time. In the peel, although the increase was at all times, it was not similar in all groups. In fact, the highest levels were obtained from day 8 to day 23 in T20 $CaCl_2$ 8% bananas. Moreover, this remarkable increase was well observed from the Duncan statistical test since day 16.

For the banana pulp, the ascorbic acid levels in contrast to the peel were somewhat lower. The highest values in the pulp were observed in bananas treated with 6% and 8% calcium chloride (Table 4). Except that this difference was approved by analysis of variances only for the 6% T20 $CaCl_2$.

3.1.6 Protein evolution and enzymatic activity

The protein content recorded during the storage of bananas increased with time in both control and treated bananas. From day 8 of the experiment, protein levels were higher in T20 $CaCl_2$ 6% and T20 $CaCl_2$ 8% bananas compared to the rest. On day 22, it was confirmed that the latter plus the control bananas kept a higher content compared to the 2% and 4% treatment (Table 5). Moreover, the Duncan's analysis test was used to confirm the superiority of the protein contents in the controls and the 6% and 8% calcium chloride treatments.

Table 4. Variation of flavonoid and ascorbic acid contents during ripening

Par	DAT	Control	T20 $CaCl_2$ 2%	T20 $CaCl_2$4%	T20 $CaCl_2$ 6%	T20 $CaCl_2$ 8%
Fl pe	1	$0.06±0.00^{abcd}$	$0.06±0.00^{abcd}$	$0.06±0.00^{abcd}$	$0.06±0.00^{abcd}$	$0.06±0.00^{abcd}$
	8	$0.07±0.00^{a}$	$0.06±0.00^{ab}$	$0.06±0.00^{abc}$	$0.06±0.00^{abc}$	$0.06±0.00^{bcd}$
	16	$0.06±0.00^{bcde}$	$0.06±0.00^{bcde}$	$0.05±0.00^{cdefg}$	$0.05±0.00^{cdefg}$	$0.05±0.00^{bcdef}$
	23	$0.07±0.00^{a}$	$0.07±0.00^{a}$	$0.07±0.00^{a}$	$0.06±0.00^{bcd}$	$0.06±0.00^{bcd}$
Fla	1	$0.13±0.00^{ab}$	$0.13±0.00^{ab}$	$0.13±0.00^{ab}$	$0.13±0.00^{ab}$	$0.13±0.00^{ab}$
pu	8	$0.13±0.00^{ab}$	$0.13±0.00^{ab}$	$0.14±0.00^{a}$	$0.12±0.01^{bc}$	$0.13±0.00^{ab}$
	16	$0.11±0.00^{cd}$	$0.11±0.00^{cd}$	$0.11±0.00^{cd}$	$0.11±0.00^{cd}$	$0.12±0.00^{bc}$
	23	$0.10±0.01^{d}$	$0.11±0.00^{cd}$	$0.10±0.00^{cd}$	$0.10±0.00^{cd}$	$0.11±0.00^{cd}$
AA	1	$0.59±0.33^{hi}$	$0.59±0.33^{hi}$	$0.59±0.33^{hi}$	$0.59±0.33^{hi}$	$0.59±0.33^{hi}$
pe	8	$0.29±0.02^{i}$	$1.03±0.03^{fgh}$	$0.85±0.02^{gh}$	$1.40±0.02^{ef}$	$1.53±0.02^{de}$
	16	$1.91±0.34^{cd}$	$1.23±0.22^{efg}$	$1.88±0.23^{cd}$	$1.86±0.45^{cd}$	$2.72±0.12^{b}$
	23	$2.5±0.19^{b}$	$1.94±0.19^{cd}$	$2.30±0.13^{bc}$	$2.44±0.35^{bc}$	$3.01±0.17^{a}$
AA	1	$0.25±0.28^{f}$	$0.25±0.28^{f}$	$0.25±0.28^{f}$	$0.25±0.28^{f}$	$0.25±0.28^{f}$
pu	8	$0.93±0.10^{bcde}$	$0.59±0.17^{ef}$	$0.69±0.11^{def}$	$0.93±0.50^{bcde}$	$0.62±0.34^{def}$
	16	$0.88±0.41^{bcde}$	$0.70±024^{def}$	$0.95±0.04^{bcde}$	$1.46±0.27^{ab}$	$1.01±0.26^{bcde}$
	23	$1.18±0.27^{abcd}$	$0.85±0.12^{cde}$	$1.14±0.53^{bcde}$	$1.69±0.12^{a}$	$1.31±0.44^{abc}$

Values follow by the same letter(s) are not statistically different at a threshold of 5%. DAT, days after treatment Fla, flavonoids ; AA, acorbic acid; pe, peel; pu, pulp

Table 5. Variation of enzyme activities during ripening

Par	DAT	Control	T20 $CaCl_2$ 2%	T20 $CaCl_2$ 4%	T20 $CaCl_2$ 6%	T20 $CaCl_2$8%
Prot	1	3.73±0.62^{g}	3.73±0.62^{g}	3.73±0.62^{g}	3.73±0.62^{g}	3.73±0.62^{g}
(µg/g)	8	7.42±0.15^{ef}	7.34±0.32^{ef}	6.15±0.48^{f}	8.34±0.98^{de}	9.66±0.24^{cd}
	15	9.80±1.12^{cd}	14.13±0.80^{b}	9.02±1.36^{cde}	10.27±1.01^{c}	10.26±0.51^{c}
	22	17.34±1.74^{a}	13.90±0.91^{b}	14.98±0.94^{b}	17.60±1.49^{a}	18.22±1.76^{a}
Chlase	1	0.06±0.0^{e}	0.06±0.0^{e}	0.06±0.0^{e}	0.06±0.0^{e}	0.06±0.0^{e}
	8	0.20±0.0^{cd}	0.20±0.0^{cd}	0.20±0.0^{cd}	0.20±0.0^{bcd}	0.19±0.0^{d}
	16	0.21±0.00^{a}	0.21±0.0^{abc}	0.21±0.0^{ab}	0.21±0.0^{a}	0.21±0.0^{a}
	23	0.21±0.0^{ab}	0.21±0.0^{abc}	0.19±0.01^{d}	0.21±0.0^{a}	0.21±0.0^{a}
PME pe	1	0.01±0.01^{ef}	0.01±0.01^{ef}	0.01±0.01^{ef}	0.01±0.01^{ef}	0.01±0.01^{ef}
	8	0.04±0.03^{de}	0.05±0.01^{cde}	0.03±0.01^{def}	0.05±0.01^{cde}	0.04±0.02^{def}
	16	0.07±0.01^{bcd}	0.10±0.013^{b}	0.09±0.01^{b}	0.08±0.04^{bc}	0.10±0.03^{b}
	23	0.04±0.00^{def}	0.005±0.004^{f}	0.008±0.001^{f}	0.26±0.00^{a}	0.25±0.005^{a}
PME Pu	1	0.10±0.03^{gh}	0.10±0.03^{gh}	0.10±0.03^{gh}	0.10±0.03^{gh}	0.10±0.03^{gh}
	8	0.17±0.00^{ef}	0.23±0.00^{abc}	0.18±0.01^{cde}	0.20±0.01^{bcde}	0.12±0.06^{fg}
	16	0.12±0.03^{fg}	0.18±0.01^{de}	0.10±0.03^{gh}	0.12±0.05^{fg}	0.05±0.00^{h}
	23	0.23±0.00^{abc}	0.26±0.02^{a}	0.25±0.00^{ab}	0.24±0.01^{abc}	0.16±0.01^{ef}

Values follow by the same letter(s) are not statistically different at a threshold of 5%. DAT, days after treatment prot, proteins ; chlase, chlorophyllase; PME, pectinemethylesterase ; pe, peel; pu, pulp

Chlorophyllase activity showed an overall increase in all treatments including the control bananas. Compared to day 1, this activity tripled on day 8 of the experiment and kept increasing until day 16 in all treatments. In all bananas, both control and treated, the activity of this enzyme reached a maximum value on day 16 of the experiment (Table 5.). Thus, the activity of chlorophyllase had a significant increase between day 1 and the rest of the time, and also between day 8 and 16. However, between days 16 and 23, T20 $CaCl_2$4% showed a significant decrease in chlorophyllase activity according to Duncan's test at the *p=05* probability threshold; all others were constant.

Pectinemethylesterase activity assessed in the peel and pulp of bananas in most of the time increased during ripening. In the peel in almost all treatments and even in controls, pectinemethylesterase activity increased very slightly on day 8 of the experiment. On the other hand, on day 16, all bananas, without exception of treatments, showed a significant increase in this activity compared to day 1. After day 16, the 6% and 8% tween 20 treatments maintained their increased activity and even significantly so. Moreover, it should be noted that those treatments that continued to increase their activity on day 23 were the ones that had a greater activity at all times, to the detriment of the others, and very often significantly so. Thus, in the control bananas as well as in all the other bananas that decreased in activity on the 23rd day, like the 2 and 4% tween 20, the maximum activity seems to be reached on the 16th day in banana pulp, as shown in Table 5. Apart from the drop in activity observed on day 16, the direction of progression of activity was strictly increasing. Beyond day 16, pectinemethylesterase activity joined the positive progression and significantly on day 23. However, the highest activities were recorded in T20 $CaCl_2$ 2% and T20 $CaCl_2$ 4%. This difference was only significant in relation to the T20 $CaCl_2$ 8%.

3.2 DISCUSSION

Variability in green life of bananas under actual storage conditions was observed. Differences in green life between treatments were found from 15 days for control bananas to 25 days for bananas treated with 4% calcium chloride. Bananas treated with high concentrations of calcium chloride, however, showed an almost similar effect to control bananas. While increased calcium content improves the green life of the fruit through stabilization of physiological processes [32], the opposite result obtained in the present study is thought to be related to damage orchestrated by the aggressive penetration of $CaCl_2$ into the banana cell walls. Moreover, the work of Bukovac et al. [33] 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 is in agreement with the

results of Kouame et al. [34] during the ripening of banana fruits. Indeed, the observation of loss of fruit firmness is related to the action of enzymes such as pectinases and polygalacturonases that have a hydrolyzing action on protopectic materials into soluble pectins [35]. This leads to a decrease in starch, protopectin, cellulose, hemicellulose, and an increase in solids and total soluble sugars [35]. In this study, some treatments, especially those with 2 and 4% $CaCl_2$, retained greater firmness at the end of the study. This result is similar to that of Aghofack-Nguemezi and Yambou [18] who found a significant loss of firmness in control bananas compared to those treated with 200mg l^{-1} $CaCl_2$. Moreover, according to White and Broadly [36], calcium chloride-treated fruit retained greater firmness during ripening. However, a high concentration of $CaCl_2$ (6 and 8%), would have a damaging effect on the fruit walls during penetration. This would lead to tissue tearing and membrane disorganization resulting in physiological changes leading to accelerated ripening of bananas. Moreover, according to William et al. [32], inappropriate calcium uptake would lead to tissue injury.

Total soluble solids content increased in all bananas during storage. However, it was slower in fruit treated with 2 and 4% $CaCl_2$ than in control bananas and those treated with 6 and 8% $CaCl_2$. This general sense of increase in total soluble solids during ripening correlates with the results on ripening of poyo and plantain bananas obtained by kouame et al. [34] The increase in soluble and reducing sugars in all treatments would be explained by a degradation of starch and other polysaccharides into soluble sugars in the pulp by the action of conversion enzymes [37,38].

The water contents evolved in opposite directions for the peel and the pulp. While a decrease in water content during ripening was observed in the peel, the opposite phenomenon was observed in the pulp. This result is similar to that of Aghofack-Nguemezi and Dassie [39] in a study on the influence of salts and edible oil on calcium and water content during ripening of banana. These authors observed an increase of water in the pulp and a decrease in the peel. The ripening process in fruits is a process that is accompanied by the hydrolysis of starch in the pulp into reducing sugars. The latter are molecules with a high osmotic potential. In the role of restoring the equilibrium between the compartments of the cell, there is therefore an osmotic migration of water from the peel to the pulp because of the high concentration of reducing sugars with a greater osmotic power [39-41]. However, the water content of bananas in these studies (60 to 80% in the pulp and 80 to 62% in the peel) remained lower than those found by Aghofack-Nguemezi and Dassie [39]. Indeed, the latter used bananas harvested at ¾ of ripeness whereas here we are dealing with fully ripe bananas.

A significant decrease in chlorophyll a content was observed. Compared to the latter, the chlorophyll b contents were so low and also decreased throughout the experiment. In fact, an approximate ratio of 1 chlorophyll b to 3 chlorophylls a, may generally be measured in green leaves of plants during their development [42]. A possible explanation could be that chlorophyllase, one of the key enzymes involved in the degradation of chlorophylls has a preferential action for chlorophyll a. Overall, a decrease in total chlorophyll content was noted. This is a similar result to that reported by Youmbi et al. [43] on morphological and biochemical changes during development and ripening of Spondias cytherea fruits. A similar result was obtained by Kouete et al. [44] with the preservation process of mangoes in Cameroon. The peel coloration of bananas gradually changed from green to yellow. Chlorophylls are actually responsible for the green colour of mature fruits. The evolution of the pigments is linked to the ripening process of the fruits, which is reflected externally by the progressive loss of the green coloration of the peel in favour of a brighter coloration that varies according to the fruits [45,46]. Particularly with regard to the treatments applied, on the 8th day of storage, the 8% $CaCl_2$ treatment showed lower chlorophyll a contents than all the others. The $CaCl_2$ in its normal integration at the cell level would prevent the degradation of photosynthetic pigments. The degradation of pigments is already linked to ripening and $CaCl_2$ stabilises the integrity of cell membranes. It is then possible to conclude that $CaCl_2$ is an obstacle to the decrease of chlorophyll a contents. The variations in chlorophyll b levels showed little or no statistical difference between treatments or over time. 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 [47]. Also, chlorophyll b alone cannot be a bio indicator of plant cell activity [46,48].

Chlorophyll concentrations in banana pulp were revealed only in small quantities. The pulp of banana before treatment has a white colour. This shows that it is low in chlorophylls. Chlorophylls are responsible for the green colour of plant tissue [47]. During ripening, the chlorophyll content decreased and got closer to 0 and the lycopene and β-carotene contents showed an increase during storage. This result is similar to that of Kouete et al. [44] who presented a decrease in chlorophylls and an increase in β-carotenes during ripening in mangoes of the improved variety from Cameroon. According to Kouame et al. [34], at the end of ripening, the initially white pulp of Poyo banana turns orange-yellow. This change in pulp colour reflects the presence of carotenoid pigments that are precursors of vitamin A [2]. In comparison, the lycopene content of control bananas was significantly elevated at day 22. The increase in carotenoid pigments is a function of ripening. The control bananas had a natural ripening with a normal accumulation of carotenoids. Therefore, the injuries caused by $CaCl_2$ at 6 and 8% would have damaged the structure of the cells and consequently altered the normal functioning in the synthesis of new carotenoids.

Ascorbic acid is mainly used at the cellular level as an electron donor in its ionized form (ascorbate). The latter being able to react directly with superoxide anion and singlet oxygen, thus reducing lipid peroxidation and damage to proteins and DNA [49]. In the present study on the preservation of bananas, both in the peel and in the pulp, an increase in ascorbic acid contents as a function of time was observed. Only, in the pulp the values were a little lower compared to the values recorded in the peel. The trend of ascorbic acid content progressions in this study agrees with that of Nour et al. [50] 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 [51]. However, some results have concluded instead that ascorbic acid values are constant during the first phases of fruit ripening and a slight increase is only observable at the end of ripening [52].In addition, according to, Nour et al. [53] 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 would be dependent on both environmental and varietal conditions and the type of fruit. Indeed, in the work of Nour et al. [50] alone, the three tomato varieties show different rates of progression. In relation to the low ascorbic acid content obtained in the peel, it should be correlated to its function of recycling carotenoids and vitamin E which are antioxidants [54]. Indeed, extracts from banana peels have an antioxidant content almost double that of extracts from the pulp [55]. With reference to the treatments, in most cases, ascorbic acid levels were higher in bananas treated with 6% and 8% calcium chloride and also in control bananas. Ripening was also faster in bananas with 6 and 8% $ClCa_2$. In relation to the physiological role of ascorbic acid during ripening, it would be understood that the 2% and 4% $ClCa_2$ concentrations influenced the slow increase in vitamin C content.

The recorded protein contents of the bananas had an increasing progression with time in both the controls and the treated bananas. Indeed, it should be noted that the main proteins during ripening are degradation enzymes such as chlorophyllases, Mg-dechelatases, oxygenases, pectinases or depolymerases [47]. According to Buchanan-Wollaston [56], leaf senescence induces the degradation of chlorophylls, nucleic acids and proteins and their transport to other parts of the plant. But this hypothesis is based exclusively on gerontoplasts, which have an essentially catabolic activity. In contrast, the particularity of chromoplasts is the incorporation of new sets of proteins that have the function of synthesis of secondary carotenoids and their incorporation into fibrillar and globular structures [57]. This would justify the increase in protein levels in the fruits. Thus, this increase could be explained in two ways: the most acceptable hypothesis would be that the proteins responsible for carotenoid synthesis and those involved in the degradation of chlorophylls and pecto-cellulosic compounds continued to be synthesized during ripening. The other hypothesis would be that the protein assay method used would recognise a molecule as a protein if it contained at least four peptide bonds. Meanwhile it would appear that during protein degradation, there is a breakage at the level of peptide bonds which can nevertheless produce fragments with four or more peptide bonds. However, protein levels were higher in bananas treated with tween 20 at 8% $CaCl_2$ compared to the rest. In the 6 and 8% $CaCl_2$ treatments, ripening-related processes were accelerated. As

always, it is likely that the penetration of $CaCl_2$ caused injury to the fruit. This affected the normal functioning of the cell by altering the structure of the membranes and consequently altering its protective properties.

The first step of chlorophyll degradation corresponds to the hydrolysis of the phytyl ester to give the chlorophyllide. This step is carried out by the chlorophyllase. It is an enzyme responsible for both the formation of the phytyl ester and its hydrolysis. This enzyme thus has a biosynthetic and degrading role [58]. This step is crucial in the regulation of chlorophyll catabolism in fruits [59]. During storage of bananas, chlorophyllase activity showed an overall increase in all treatments. Thus in all bananas, this activity had a significant increase from day 1 to day 16. However, between day 16 and day 23, a stabilisation in the values was observed. This result corroborates those obtained by Minamide and Ogata [60] in tomato and by Wang et al. [61] with lychee. Indeed, the increase in chlorophyllase activity is associated with the decrease in pigment content. According to the results of previous work, the decrease in chlorophyll content in fruits during ripening is related to the increase in chlorophyllase activity [62,63]. However, this observation in fruits is different from that in senescent leaves. In the latter, chlorophyllase activity decreases during senescence [64]. Therefore, chlorophyllase activity in senescing leaves is not directly responsible for the loss of chlorophyll. In relation to the treatments, no difference was observed between the treated and control bananas.

Pectin plays an important role in fruit consistency and structural changes during ripening and storage. Tissue softening is attributed to enzymatic degradation (pectinemethylesterase) and solubilisation of protopectin [65]. In this experiment, the progression of pectinemethylesterase (PME) activity, assessed in banana peel and pulp, increased with ripening. This result is similar to that of Patil and Magar [66]. According to the latter, the activity of PME is significantly higher in a ripe banana than in a green mature banana in previous studies. Moreover, several authors think that the activity of PME increases during the ripening of the fruit. It has even been established that fruit softening is related to the increase of the said activity [67-69]. This is explained by a process of solubilisation, followed by depolymerisation and de-esterification of pectic polysaccharides during the ripening of fruits such as olive [70], and banana [71]. In relation to the treatment with tween 20 combined with $CaCl_2$, low PME activity was found in bananas treated with 2 and 4% $CaCl_2$ on day 23. Since ripening was slower and even loss of firmness in these bananas, it can be concluded that PME activity increases with ripening. Therefore, $CaCl_2$ at 2 and 4% would slow down the activity of PME.

4. CONCLUSION

Extending the green life of bananas with the use of tween 20 and different concentrations of calcium chloride was the focus of this work. The variations of $CaCl_2$ concentrations allowed the elucidation of some physiological mechanisms underlying ripening. Indeed, during the ripening process, the photosynthetic pigments in the peel as well as in the pulp decreased in content. The contents of antioxidant compounds such as ascorbic acid, lycopenes and β-carotenes increased with time. Also, the activity of two enzymes, namely chlorphyllase and pectin-methylesterase, changed positively during ripening. Banana treatments with 2 and 4% $CaCl_2$ and tween 20 showed better shelf life with higher firmness at the end and higher moisture content. The 6 and 8% $CaCl_2$ treatments had rapid ripening and almost similar to the control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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