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Effects of Calcium Chloride Treatment on the Photosynthetic Capacity and Intensity of Banana Fruit during Ripening

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Authors' contributions

This work was carried out in collaboration among three authors. Author PTE performed labour experiment and the statistical analysis. Authors PTE and KJO managed the literature searches, wrote the protocol and the first draft of the manuscript. Author ANJ designed the study and managed the final manuscript. All three authors read and approved the final manuscript.

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ABSTRACT

The general objective of this work was to highlight the physiological phenomenon of photosynthesis that underlies ripening in banana fruits and to follow its bioindicators evolution. To achieve this goal, pigment content, protein content and the amount of oxygen released were determined at different stages of ripening of bananas fruits. The determination of pigment content was carried out by spectrophotometric assay after extraction in pure acetone. Protein content was determined using a known albumin concentration calibration curve. Moreover, photosynthetic intensity was evaluated by measuring the oxygen released by the banana peels by means of an oximeter. Results showed that there was a gradual decrease in pigment levels, while at the same time there were increases in carotenoid and protein levels. Photosynthetic intensity and capacity also decreased significantly. The treatment of bananas with calcium chloride helped to slow the fall of photosynthetic activity. Also, calcium chloride treatment induced a reduction of the decrease in chlorophyll content, photosynthetic intensity and increase in proteins content. Positive correlations

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were found between photosynthetic intensity and levels of chlorophyll a and total chlorophylls. The intensity of photosynthesis was negatively correlated with carotenoid and protein levels. The change in photosynthetic intensity during ripening was proportional to the variation in chlorophyll *a* and total chlorophylls content, but inversely proportional to variations in total carotenoids and proteins contents.

Keywords: Photosynthesis; calcium chloride; pigments; proteins; ripening.

1. INTRODUCTION

The dessert banana is the world's leading fruit product, ahead of grapes and orange [1]. In value, it is the fifth agricultural product of world trade, after coffee, cereals, sugar and cocoa [2]. Banana crops rank 4th among the world's most important food commodities after rice, wheat and milk [3]. Bananas play an important socio-economic role for developing countries in tropical and subtropical areas, particularly in the countries of East, Central and West Africa, East Asia and Central America [4]. World production is around 106 million tons per year for a cultivated area of 10 million hectares [5], 14% of which is for export, the remainder for local consumption or industrial processing [6]. The pulp of the banana represents 60 to 65% of the fruit depending on the variety and its development. Banana is a high energy food (379 kJ per 100 g) and the carbohydrates it contains are easily assimilated. The lipid and protein content is low, but it is rich in fibre. Banana contains amino-acids that are responsible for certain therapeutic effects. It also contains many vitamins, minerals, which makes it interesting nutritionally. The available carbohydrates are present in good quantities [7].

Despite the nutritional and socio-economical importance of bananas, like other fleshy fruits, their post-harvest conservation in the fresh state is a major problem for producers and traders. One of the prerequisites for the development of conservation methods is a better understanding of the components of the physiological mechanisms underlying ripening. Photosynthetic variation is one of them. Indeed, the peel pigmenting equipment of some fruits (examples: banana and tomato) at the mature-green stage resembles that of the green leaves. During the ripening of these fruits, structural and metabolic changes at the level of the peel are observed; chloroplasts are transformed into chromoplasts and there is progressive degradation and disappearance of chlorophylls coupled with the synthesis of new carotenoids [8]. The characteristic colour of each ripe fruit is the result of the colour of the major carotenoids, some of which already existed but were masked in the peel of the mature-green fruit by chlorophylls [9]. So, there would be in the peel of fruits such as banana a variation of photosynthetic activity during ripening.

Most of the existing works on fruit photosynthesis during ripening to date, to our knowledge, involve tomato and mandarin. These studies have shown that although the peel of mature-green fruits is photosynthetically active with a gradual decrease in the intensity of photosynthesis during ripening [10,11], this photosynthesis would not be exactly similar to green leaves [12]. The search for mechanisms that underlie the variability of photosynthetic activity during fruit ripening is therefore an important theme in the contribution to the understanding of the physiological bases of fruit ripening. The work of Aghofack-Nguemezi and Yambou [13] showed that the dipping of mature-green bananas in a solution of calcium chloride at concentration 200 mg.l^{-1}, resulted in a substantial increase in the endogenous content of Ca^{2+} ion and induced a significant retention of water in the peel, thus allowing an extension of the durations of green life and conservation of the fruit. Such a very effective treatment in the slowing down of ripening could make it possible to better evaluate the importance of the variation of the photosynthetic intensity and capacity on the evolution of this process. The main objective of this study was to elucidate the physiological mechanism of photosynthesis that underlies the ripening of banana fruits using changes in photosynthesis bioindicators.

2. MATERIALS AND METHODS

2.1 Plant Material and Treatment

The banana fruits used were supplied by the PHP (Plantations de Haut Penja) company located at Njombé, Cameroon. They were harvested at mature green stage. Bananas were washed with water on arrival in Dschang and sorted to remove the injured fruits. After this visual selection, the bananas were divided into two sets of 80 bananas each. The concentration

of calcium chloride (granular form and pure grade) used for the treatment of bananas for this experiment was 200 $mg.l^{-1}$. Bananas of the first set were treated with the calcium chloride solution at a concentration of 200 $mg.l^{-1}$ and the duration of treatment was two days. At the end of this period the bananas were removed from the solution and stored at room temperature. Bananas of the second set also known as a control batch, were dipped only in water for the same period as batch 1 (two days) and after this period the bananas were also removed from the water and disposed on bench without any form of packaging. The mean temperature and relative humidity measured during this conservation test were 24.97°C and 75.28%, respectively.

2.2 Determination of the Pigments Content

2.2.1 Extraction of pigments

To extract the pigments, 18 g of banana peels were removed using a razor blade and introduced into a mortar with 4g of sand. The peel-sand mixture was dry-milled for the purpose of the total destruction of the cell membranes. 4 g of grounded material were taken in a test tube to which10 ml acetone had been added. Then, the test tube was closed and wrapped in aluminium foil. The tubes were shaken well and allowed to stand in ice for one hour before the quantification of pigments contents.

2.2.2 Measurement of pigment contents

The determination of chlorophyll *a*, *b* and total carotenoids contents was made by the method of Lichtenthaler [14]. This choice was based on the fact that the original method was developed for the determination of photosynthetic pigments in green leaves which would be very close to banana peels. This method is based on the absorption of light spectra at different wavelengths by photosynthetic pigments in solution in organic solvents. In the case of this work, the equations used are those corresponding to the extraction with pure acetone (100%).

2.3 Determination of the Protein Content

Total protein content was determined using the method of Cooper [15] with modifications by using the chloroform.

2.3.1 Extraction of proteins

The outermost part of the banana peel was removed with a razor blade and placed in a mortar with 4 g of sand. The mixture was dry milled to completely destroy the cell membranes. Then for 15 g of grounded material, 10 ml of distilled water were added and the mixture was shaken for 5 minutes until the solvent was coloured. Using a nylon touch of very tight mesh that can prevent the passage of debris, the contents of the mortar was filtered into a beaker. To this, a chloroform solution was added to get rid of the impurities and thus increase the yield of proteins in the aqueous phase; then a spoon was used to homogenize the solution for a short time and finally the mixture was allowed to decant and the aqueous upper phase consisting of proteins was removed.

2.3.2 Dosage of proteins

2 ml of aqueous extract of banana peels were taken and introduced into a test tube. In the test tube, 3 ml of Biuret reagent were added to make up the volume at 5 ml; the tube was shaken moderately for homogenization and then incubated in the dark for 20 minutes at 37°C. Finally, spectrophotometer reading was done at 540 nm and the content was spotted from that of the standard range.

2.4 Direct Measurement of Photosynthetic Activity Using an Oximeter

The measurement of photosynthetic intensity was done using a method developed by Schopfer [16]. An oximeter of the type DO6 + was used to measure the photosynthetic activity. The water was boiled for approximately 5min in a water heater and poured into well labelled bottles (100 ml). In the latter, a layer of liquid paraffin was added to the hot water (a few mm thick) to prevent atmospheric oxygen from entering the medium [16]. After the water has cooled to room temperature, 10 ml of sodium bicarbonate solution (source of CO_2) were added to it and also a piece of control banana peel of surface 42,75 cm^2. In another bottle 10ml of sodium bicarbonate solution (source of CO_2) and a piece of treated banana peel of surface 42,75 cm^2 were added. The flasks were placed under a light source (white economic bulb with a capacity of 3 watts) for 15 minutes. There after the flasks were left in open air for a period of 30 minutes. After

this time, the oxygen content was assayed using an oximeter.

2.5 Statistical Analysis

Data collected on the various parameters (chlorophyll content, carotenoid content, protein content and photosynthetic intensity) were subjected to analysis of variance (ANOVA) in order to see if there were any differences between the means. When the differences were significant, Duncan's multiple comparison test at the *P* = 05 probability threshold was used to determine the level of the significance.

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows very clearly a progressive decrease in chlorophyll *a* content as a function of time. In fact, the content of chlorophyll a ranged between 19.21 ± 3.05 $\mu g.g^{-1}$ on the 1st day and 5.004 ± 0.34 $\mu g.g^{-1}$ on the 22nd day for bananas treated with calcium chloride and between 15.28 ± 3.5$\mu g.g^{-1}$ and 4.5 ± 0.83 $\mu g.g^{-1}$ for untreated bananas. The results obtained with calcium chloride treated bananas showed a slight relative retardation of the decline in chlorophyll *a* content compared to untreated bananas. Statistical analysis revealed that throughout the experiment, there was no significant difference between the peel chlorophyll *a* content of the treated bananas and that of the peel of the control bananas. However, the chlorophyll *a* content in the peel of the control bananas on the first day after the treatment was significantly higher than the levels of chlorophyll *a* measured on the peel of the same type fruit on the 19th and 22nd days. In addition, the peel chlorophyll *a* content of bananas treated on the first day after treatment was significantly higher than those measured in the peel of bananas of the same type on the 7th, 13th, 19th and 22nd days after treatment.

It was difficult to determine the direction of variation of the chlorophyll *b* content. For untreated bananas there was an acceptable decrease in chlorophyll *b* content, with the exception of day 19 where there was a slight increase. The calcium chloride treated bananas also showed a gradual decrease from the first to the 13th day and from the 19th day a slight increase in the chlorophyll *b* content was observed. In most cases, the level of chlorophyll *b* was slightly higher in untreated bananas than in treated bananas except for days 1 and 2. Indeed, to dwell on the numbers, there was an oscillation between 8 ± 2.72$\mu g.g^{-1}$ and 5.55 ± 0.22 $\mu g.g^{-1}$ of chlorophyll *b* for untreated bananas and almost the same with bananas treated with

Table 1. Variation in chlorophyll *a* and *b* contents during banana fruit ripening

Time after treatment in days	Chlorophyll *a* content (µg/g)		Chlorophyll *b* content (µg/g)	
	Control fruits	Treated fruits	Control fruits	Treated fruits
1	15.28 ± 3.5 ab	19.21 ± 3.05 a	7.01 ± 2.33 a	7.13 ± 2.72 a
4	13.85 ± 2.8 abc	15.18 ± 4.98 abc	6.72 ± 2.6 a	6.29 ± 3.12 a
7	12.56 ± 2.67 bcd	13.77 ± 3.6 bcd	5.78 ± 2.11 a	5.59 ± 2.76 a
13	11.88 ± 2.88 bcd	14.54 ± 3.57 abc	5.42 ± 2.54 a	5.11 ± 2.69 a
19	5.05 ± 0.38 e	7.97 ± 2.47 cde	6.33 ± 0.5 a	5.40 ± 1.98 a
22	4.50 ± 0.83 ef	5.004 ± 0.34 e	5.47 ± 0.82 a	6.74 ± 0.33 a

Values followed by the same letters in the same row are not significant

Table 2. Variation in total chlorophylls and total carotenoids contents during the ripening of banana fruit

Time after treatment in days	Total chlorophylls content		Total carotenoids content	
	Control fruits	Treated fruits	Control fruits	Treated fruits
1	22.29 ± 6.22 ab	26.35 ± 5 a	4.04 ± 0.7 c	4.30 ± 0.19 c
4	20.58 ±3.44 ab	21.48 ± 6.33 ab	4.24 ± 0.88 c	4.51 ± 0.12 c
7	18.34 ± 3.24 abc	19.36 ± 5.54 abc	4.73 ± 0.39 c	4.71 ± 0.32 c
13	17.31 ± 3.23 abc	19.66 ± 5.32 abc	5.09 ± 0.67 c	6.26 ± 0.57 bc
19	11.39 ± 0.35 c	13.38 ± 3.21 bc	8.50 ± 0.2 a	9.29 ± 0.43 ab
22	9.98 ± 0.16 d	11.74 ± 0.1 c	9.68 ± 0.35 a	9.05 ± 0.1 ab

Values followed by the same letters in the same row are not significant

Table 3. Changes in ratio of chlorophylls a /b and proteins content during banana fruit ripening

Time after treatment in days	Chlorophylls *a*/*b*		Total proteins content	
	Control fruits	Treated fruits	Control fruits	Treated fruits
1	2.17 ± 0.31 abc	2.69 ± 0.28 a	74.46 ± 3,05 hi	38.44 ± 7.43 i
4	2.05 ± 0.55 abc	2.41 ± 0.55 ab	339.24 ± 43 ef	269.75 ± 35.44 f
7	2.17 ± 0.33 abc	2.46 ± 0.33 abc	453.36 ± 65.2 cd	377.01 ± 9.23 de
13	2.18 ± 0.26 abc	2.24 ± 0.49 abc	540.45 ± 26 c	427.95 ± 23.7 de
19	0.79 ± 0.15 c	1.47 ± 0.45 bc	544.62 ± 28.3 c	445.161 ± 13.5 d
22	0.82 ± 0.10 c	0.94 ± 0.24 bc	750.80 ± 67.74 a	640.18 ± 67.56 b

Values followed by the same letters in the same row are not significant

Table 4. Change in the ratio of total chlorophylls on protein contents and photosynthetic intensity during banana fruit ripening

Time after treatment in days	Total chlorophylls / proteins		Time after treatment in days	Photosynthetic intensity (mm^3 of O_2/cm^2/ hour)	
	Control fruits	Treated fruits		Control fruits	Treated fruits
1	0.29 ± 0.09 b	0.68 ± 0.15 a	4	10.01 ± 0.14 a	8.78 ± 0.1 ab
4	0.06 ± 0.02 c	0.079 ± 0.007 c	8	7.15 ± 0.17 bc	6.14 ± 0.1 cd
7	0.04 ± 0.009 c	0.051 ± 0.005 c	11	4.85 ± 0.22 de	5.22 ± 0.12 de
13	0.032 ± 0.008 c	0.04 ± 0.007 c	14	3.37 ± 0.15 efg	4.55 ± 0.5 def
19	0.021 ± 0.009 c	0.03 ± 0.003 c	17	2.69 ± 0.13 fg	3.33 ± 0.12 efg
22	0.016 ± 0.006 c	0.018 ± 0.006 c	20	2.14 ± 0.13 g	2.38 ± 0.15 g

Values followed by the same letters in the same row are not significant

Table 5. Correlation between parameters

Variables	Chl *a*	Chl *b*	Chl *T*	Car	Prot	Chl *a*/*b*	Chl *T*/p
In ph	0.668	0.151	0.626	-0.702	-0.905	0.414	0.649

Chl T: total chlorophylls; Car: carotenoids; prot: proteins; In ph: photosynthetic intensity

calcium chloride. Statistical analysis revealed no significant difference between chlorophyll *b* levels of control bananas and bananas treated with calcium chloride. No significant difference in the variations with time was also observed.

In this study, total chlorophylls content variations were recorded as a function of time elapsed after treatment. The total chlorophylls content decreased progressively as well in the peel of the control fruits as in the peel of the fruits treated with calcium chloride. In fact, the total chlorophylls content was between 22.29 ± 6.22 and 9.98 ± 0.16 $\mu g.g^{-1}$ for untreated bananas and between 26.35 ± 5.0 and 11.74 ± 0.1 $\mu g.g^{-1}$ for bananas treated with calcium chloride. In bananas treated with calcium chloride, there were significant differences between the total chlorophylls content measured on the first day and that measured on the 19^{th} and 22^{nd} days after treatment. Furthermore, in control bananas, a significant difference was observed only between the total chlorophylls content measured on the first day and that measured on the 22^{nd} day. Although the rate of decrease was similar in both control and treated fruits, the total chlorophyll content was in all cases higher in bananas treated with calcium chloride compared to the control bananas. These differences were not significant.

Carotenoid levels increased with the time of ripening. Overall, carotenoid levels ranged from 4.02 ± 0.7 $\mu g.g^{-1}$ to 9.05 ± 0.35 $\mu g.g^{-1}$ for bananas treated with calcium chloride and 4.3 ± 0.19 $\mu g\ .g^{-1}$ at 9.68 ± 0.1$\mu g.g^{-1}$ in untreated bananas. However, in most cases the carotenoid content remained slightly higher in untreated bananas than in bananas treated with calcium chloride. Moreover, the statistical analysis revealed in both treated and control bananas significant differences between the carotenoid content measured in the peel on the first day and those measured on the 19^{th} and 22^{nd} days after treatment.

Table 3 shows that there was overall a gradual decrease in chlorophyll *a* / chlorophyll *b* ratio during ripening. At all levels, the chl *a* / chl *b* ratio of bananas treated with calcium chloride remained higher than that of untreated bananas. Statistical analysis revealed a significant difference between the chl *a* / chl *b* ratio determined in the peel of the calcium chloride-treated bananas on the first day and those determined on the 19th and 22nd days after treatment. In untreated bananas no significant difference in chl *a* / chl *b* ratios was observed over time.

Also from this Table 3, an increase in protein levels during ripening was observed. Thus, increases from 74 ± 3.05 $\mu g.g^{-1}$ to 750 ± 67.74 $\mu g.g^{-1}$ in the peel of control bananas and from 38.34 ± 7.43 μg^{1} to 640 ± 67.56 $\mu g.g^{-1}$ of protein in the peel of treated bananas during the time of the experiment were observed. However, control bananas always had higher protein content than calcium chloride treated bananas. Statistical analysis also showed that variations with time of protein content were significant in both lots. In addition, there were significant differences in the protein content of the peel of control bananas and the peel of treated bananas on the 13^{th}, 19^{th}, and 22^{nd} days after treatment.

Table 4 shows a gradual decline in the total chlorophyll / protein ratio during ripening. But apart from the first day, the total chlorophyll / protein ratio showed only a very slow decrease over time. However, bananas treated with calcium chloride showed a higher ratio than that obtained with control bananas. Statistically, there was a significant difference between the total chlorophylls / protein ratio in the peel of treated bananas and that determined in the peel of control bananas the first day after treatment. Outside of this day, no other significant difference was observed between the control fruits and the calcium chloride-treated fruits.

The intensity of photosynthesis also decreased over time during ripening in both calcium chloride treated bananas and untreated (control) bananas. At first, this photosynthetic intensity was higher in untreated bananas. From the 11^{th} day, the trend was reversed and the photosynthetic intensity was higher in bananas treated with calcium chloride until the 20^{th} day. Thus, calcium chloride applied to bananas slowed down the loss of photosynthetic activity during ripening. However, apart from changes over time, all other differences observed between the photosynthetic intensity measured in treated bananas and that measured in control bananas were not statistically significant.

The correlation values were above 0.62 for chlorophyll *a*, total chlorophylls, and total ratio chlorophyll / protein that were positively correlated with photosynthetic intensity. Carotenoid contents with a value of -0.702 and protein content with a correlation value of -0.905 were negatively correlated with photosynthetic intensity. Unlike parameters with positive correlations, those with negative correlations were more pronounced. However, although positive, the correlations between chlorophyll b content, the ration chlorophyll a / chlorophyll b and photosynthetic intensity remained below average.

3.2 Discussion

3.2.1 Effects of pigments content on photosynthetic activities

Photosynthetic pigments act like real solar panels. But with the difference that solar panels capture solar energy and transform it into electrical energy while plant chloroplast containing pigments capture the light energy and transform it into chemical energy, in the form of ATP and NADPH. In this way, the increase in the number of sensors would be an asset in the process of transformation of light energy into chemical energy. Overall, a decrease in the total chlorophyll content and an increase in the carotenoid content with the change in peel colour of the banana gradually changing from green to yellow show that chlorophylls are actually responsible for the green colour of the mature fruits and carotenoids responsible for the yellow colour characteristic of ripening. Moreover, during ripening the carotenoids continue to be synthesized. In fact, unlike the gerontoplasts of senescent leaves, the chromoplasts of ripening fruits are still the site of synthesis reactions [8].

During ripening, there was an increase in the carotenoid content, decreases in the chlorophyll a content, total chlorophylls content and photosynthesis intensity. Also for photosynthesis, the pigments are classified into two types: chlorophyll *a* which is the main or active pigment, chlorophyll *b* and carotenoids which are accessory pigments. One might think that despite the fact that the absorption of light energy increases with the increase of the carotenoid

content, the capacity of initiation of the real photochemical act (charge separation) decreases with the decrease of the content of chlorophyll *a* and consequently, the photosynthetic capacity also decreases during ripening of banana fruits.

A approximate ratio of 1 chlorophyll *b* to 3 chlorophylls *a* can generally be measured in green leaves of plants during development [17]. During ripening of the fruits such as tomatoes, the chlorophyll *a* / chlorophyll *b* ratio decreases over time [10]. The observation of a decrease in chlorophyll *a* / chlorophyll *b* ratio with ripening can be due to the fact that chlorophyllase, one of the key enzymes involved in chlorophyll degradation, would preferentially act on chlorophyll *a*. The significant decrease in chlorophyll *a* content, compared with slight declines in chlorophyll *b* content throughout the fruit ripening may be due to the fact that chlorophyll *b* would first be transformed into green intermediates of chlorophyll *a* before complete degradation, as suggested by Matile and Hörtensteiner [8]. Thus, contrary to chlorophyll *a*, chlorophyll *b* cannot be a considered as bioindicator of photosynthetic activity. It has been reported by Aghofack-Nguemezi and Yambou [13] and Aghofack-Nguemezi et al. [18] that Alterations in chlorophyll *b* content were not tightly linked to the visual assessment of fruit ripening and ripening-retarding effects of some treatments. These authors postulated that chlorophyll *a* is a more appropriate biomarker for fruit ripening and related processes than chlorophyll *b*. This could be due to the fact that chlorophyll breakdown pathway in fruits during ripening is obviously similar to the one operating in senescing leaves, where chlorophyll *b* must first be converted into chlorophyllide *a* and then pheophorbide *a* (both being chlorophyll *a* green breakdown intermediates), before its complete degradation in non-green products [8].

Regarding the effect of treating bananas with calcium chloride, the decrease in total chlorophylls content was less rapid in calcium chloride-treated bananas than in control bananas. Chlorophyll *a* also showed less pronounced degradation in treated bananas. Carotenoids, on the other hand, were synthesized more slowly in treated bananas. The ripening process was characterized by the loss of chlorophyll and the development of carotenoids [10]. The inhibition of chlorophyll degradation and carotenoid synthesis by calcium chloride treatment was established in this study. This phenomenon had shown evidence of delayed ripening in bananas treated with calcium chloride compared to control bananas. Moreover, since the degradation of chlorophyll led to the loss of photosynthetic capacity, the treatment of bananas with calcium chloride therefore slowed down the loss of photosynthetic capacity in bananas during ripening.

3.2.2 Effect of variation of protein content on photosynthetic capacity

Living organisms, during growth or senescence are the site of innumerable biochemical reactions. These reactions constitute the metabolism, namely the biosynthesis and catabolism of a large number of biological molecules. They take place under physiological conditions thanks to the presence of enzymes which are specialized proteins in the catalysis of reactions. The peel of the banana is considered to be the site of multiple reactions of catabolism or biosynthesis during ripening. It should be noted that the main proteins during ripening are degradative enzymes such as chlorophyllases, Mg-dechelatase, oxygenases or simply pectinases or depolymerases [8], but also the synthetic enzymes of carotenoids biosynthesis. Due to the fact that these proteins have as substrate chlorophylls which is important in the photosynthetic activity or pectins which are target of softening enzymes, it is therefore clear that the more numerous they are used, the lesser is the photosynthetic activity.

Buchanan-Wollaston [19] noted in his previous work that leaf senescence induced the degradation of chlorophylls, nucleic acids and proteins and their transport in other parts of plants. In contrast to gerontoplasts, which have essentially catabolic activity, the particularity of chromoplasts is the incorporation of new sets of proteins whose function is the synthesis of secondary carotenoids and their incorporation into fibrillar and globullary structures [20]. The increase in protein content during the ripening of banana fruits as observed in the present study is in fact contrary to the results of Buchanan-Wollaston [19] mentioned above. This increase in the protein content could be explained in two ways: the first and most acceptable hypothesis is that the proteins responsible for the synthesis of carotenoids and those involved in the degradation of chlorophylls and pectocellulosic compounds continued to be synthesized during ripening. On the other hand, this could be due to

depolymerisation of certain protein molecules associated with the pigments.

3.2.3 Effect of pigment / protein ratios on photosynthetic capacity

The relationship between photosynthesis activity and light energy uptake increases in higher plants when illuminated simultaneously with radiation of different wavelengths. This shows that the photosystems consist of different pigment / protein complexes. In fact, the chlorophyll / protein complexes are the main actors involved in the achievement of photosynthesis [21]. In a photosystem, a single molecule of pigment namely chlorophyll *a* is able to transform light energy into chemical energy and it is in the reaction centre. This reaction centre consists of chlorophyll *a* combined with one or more protein (s). This pigment is of great interest for photosynthesis because it is alone in the reaction centre and it is also involved in the collection of energy at the antenna [17].

With the increase in protein content and decrease in chlorophyll levels, the ratio of pigments to protein decreased during ripening of bananas. The possible meaning is that ripening would disrupt the pigment-protein complexes that are indispensable for functioning of a photosystem and therefore, there would be a gradual decline in photosynthetic capacity during ripening. Moreover, the treatment of bananas with calcium chloride made it possible to reduce the rate of disorganization of the complexes, which is compatible with the results of the effect of the treatment of bananas with calcium chloride on chlorophyll and protein contents and thus confirms that the treatment of bananas with calcium chloride helped to slow down the loss of photosynthetic capacity.

3.2.4 Effect of calcium chloride treatment on photosynthetic activity

During the process of photosynthesis, the biomass (energy reserve) is synthesized from CO_2 and water with release of oxygen under the action of solar energy. This oxygen molecule is a direct and indisputable indicator of photosynthetic activity. The photosynthetic activity follows the kinetics of the appearance of this oxygen over time. Thus, if there is a decrease in this appearance, the photosynthetic intensity decreases.

In this study, photosynthetic intensity decreased significantly with ripening in bananas. Indeed Carrara et al. [11] in a previous work found that photosynthetic intensity decreased with the ripening of mature green fruits. In addition, the decrease in photosynthetic intensity in calcium chloride-treated bananas during ripening was mostly slowed down compared to control bananas. It is therefore clear that calcium chloride would have reduced the rapid decline of photosynthetic intensity. Moreover, in a direct way, it is normal to say that the treatment of bananas made it possible to prolong the duration of photosynthesis, thus to improve the shelf life of bananas. Of course, with a higher chlorophyll *a* content that is involved in the separation of charges at the reaction centre during photosynthesis, the photosynthetic intensity cannot be less in calcium chloride-treated bananas than untreated bananas. Finally, the treatment of bananas with calcium chloride retarded the degradation of chlorophylls *a* and *b* and slowed the synthesis of proteins and the disorganization of the pigment-protein complexes. This would have slowed down the loss of photosynthetic capacity. Therefore, in combination with the effect of calcium chloride treatment on the photosynthetic capacity and intensity of ripening banana fruits, it is evident that the photosynthetic capacity and intensity evolved in an opposite direction to ripening of bananas.

4. CONCLUSION

The present study focused on the influence of calcium chloride treatment on the photosynthetic capacity and intensity of banana fruit during ripening. The approach used to achieve the objectives was based on the determination of pigment and protein contents, and the measurement of the photosynthetic intensity. The photosynthetic capacity decreased progressively during the ripening of banana fruits. This was strongly materialized with chlorophyll *a* degradation as compared to chlorophyll *b*. Unlike chlorophylls, protein and carotenoid levels increased during ripening of bananas. The treatment of banana fruit with calcium chloride had the effect of slowing the degradation of chlorophyll *a*, the biosynthesis of proteins and carotenoids and the fall in photosynthetic intensity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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