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Effects of Calcium chloride and magnesium sulphate treatments on the shelf-life of climacteric banana and non-climacteric pineapple fruits

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ABSTRACT

Banana (*Musa accuminata*) and pineapple (*Ananas comosus*) fruits were treated by dipping in 50, 100 and 200 mg/l solutions of $CaCl_2$ and $MgSO_4$ to investigate the effects of the divalent cations Ca^{2+} and Mg^{2+} on visually assessed ripening, the quality of pigments, the total lipids content and the water content. Treatments with both salts in the concentration range used retarded the ripening process in bananas and in pineapples. These treatments delayed the chlorophyll a breakdown while the degradation of chlorophyll b was not affected in the peel of the two types of fruits. Treatments with $CaCl_2$ and $MgSO_4$ induced an inhibition of water loss in the peel of banana fruits. In the peel of pineapples, $CaCl_2$ treatments led to a decrease in the water content whereas $MgSO_4$ treatments almost did not affect this parameter. $CaCl_2$ solutions with concentrations up to 100 mg/l induced an increase in total lipids content of the peel of banana fruits while there was no significant change in the content of total lipids in the peel of pineapple fruits. These results indicate that both Ca^{2+} and Mg^{2+} may play a role in stabilizing cell membranes, in maintaining cell wall firmness, and in inhibiting chlorophyll a degrading enzymes in the climacteric banana fruits and in the non-climacteric pineapple fruits as well as in inhibiting respiration and breakdown of membrane lipids in climacteric banana fruits.

Keywords: Carotenoids, chlorophylls, divalent cations, fruit ripening.

RESUME

Les bananes (*Musa accuminata*) et les ananas (*Ananas comosus*) ont été traités par trempage dans des solutions de $CaCl_2$ et $MgSO_4$ de concentrations 50, 100 et 200 mg/l. Les effets des cations divalents Ca^{2+} et Mg^{2+} sur l'aspect visuel du mûrissement, la qualité des pigments, la teneur en lipides totaux et la teneur en eau ont été étudiés. Les traitements avec ces deux sels ont retardé le mûrissement des bananes et celui des ananas. Ces traitements ont provoqué un ralentissement de la dégradation de la chlorophylle a. Ils n'ont pas eu d'effets sur la dégradation de la chlorophylle b dans la peau des fruits. Les traitements avec des solutions de $CaCl_2$ et $MgSO_4$ ont entraîné une diminution des pertes d'eau dans la peau des bananes. On a observé une baisse de la teneur en eau dans la peau des ananas après traitements avec les solutions de $CaCl_2$ alors que la peau des mêmes fruits traités avec les solutions de $MgSO_4$ avait une teneur en eau comparable à celle des témoins. Les solutions de $CaCl_2$ de concentrations inférieures ou égales à 100 mg/l ont provoqué une augmentation de la teneur en lipides totaux dans la peau des bananes. Aucun changement significatif n'a été observé dans la teneur en lipides totaux de la peau des ananas traités avec le $CaCl_2$. Ces résultats sont interprétés en termes des rôles des cations Ca^{2+} et Mg^{2+} dans le ralentissement du processus du mûrissement suite à une stabilisation des membranes cellulaires, à un maintien de la texture dure de la paroi cellulaire et à une inhibition des enzymes du catabolisme de la chlorophylle a aussi bien chez les fruits climactériques que chez les fruits non-climactériques, ainsi qu'à une inhibition de la respiration et de la dégradation des lipides membranaires chez les bananes.

Mots clés: Caroténoïdes, cations divalents, chlorophylles, mûrissement, fruits.

INTRODUCTION

Fruits have traditionally been classified into two groups depending on their respiratory behavior, on the rate of ethylene biosynthesis at the onset of ripening, and on the way they respond to ethylene treatment. Climacteric fruits, such as banana, tomato and mango, are characterized by an increase in respiration and ethylene biosynthesis that both coincide with ripening. Non-climacteric fruits, such as citrus and pineapple, do not exhibit increase in the rate of respiration [1, 2]. However, in mature citrus fruits ethylene is necessary for natural color change even if it may not be the primary inducer [3, 4]. The very low rate of ethylene production in mature, detached citrus fruits is associated with the expression of the System II genes of 1-aminocyclopropane-1-carboxylate synthase and ethylene receptor [5].

One of the metabolic characteristics of ripening of both climacteric and non-climacteric fruits is the softening of tissues, which involves the degradation of polysaccharides in the peel and the pulp [6, 7]. Texture change results from the activities of cell wall hydrolases [8, 9, 10]. The activities of cell wall degrading enzymes are induced by increased concentration of endogenous ethylene, independently of its effect on ripening. There is evidence that calcium inhibits the activities of cell wall hydrolases [7, 11]. The divalent cations Ca^{2+} and Mg^{2+} play many other physiological roles in plant tissues senescence and in the action of phytohormones. Calcium ions delay the senescence by stabilizing cell membrane and increasing the rigidification of monolayers. The Ca^{2+}- mediated cross-linking may occur as bridging between phospholipids, between phospholipids and carboxyl tails of embedded

membrane proteins, and between phospholipids and cytoskeletal. Magnesium ion affects the electrostatic cross-linking between membrane components to a lesser extend than calcium ion [12]. There are also several calcium-pectate interactions, which make the cell wall firmer [13]. Calcium ion acts as second messenger in coupling many stimulus-response systems. It occupies a pivotal position in plant cell signal transduction pathways, some of which are associated with plant growth regulators [14, 15]. While intracellular Ca^{2+} concentration is submicromolar, the concentration of closely related divalent cation Mg^{2+} is millimolar. Despite the concentration difference that would favor Mg^{2+} cellular processes often display an enormous selectivity for Ca^{2+} [15].

Contrarily to the generally known retarding effects of Ca^{2+} on plant leaves senescence [12] and ripening of mango fruits [16], it has recently been reported that treatment by pressure infiltration with 4% $CaCl_2$ reduces the firmness and accelerates the ripening of banana fruits [17]. It is well known that the two different methods of treatment with phytohormones by dipping and by pressure infiltration lead to contrasting results, although pressure infiltration gives uniform distribution of the active substance through the fruit [18, 19]. 4% $CaCl_2$ solution might also be highly concentrated and induced an opposite effect of Ca^{2+}. To the best of our knowledge, no experimental work exists on the influence of Mg^{2+} treatment on the ripening of bananas. Moreover, no study has been done on the effects of Ca^{2+} and Mg^{2+} treatments on the ripening of pineapple, a non-climacteric fruit. The present work was undertaken to examine the effects of treatments by dipping in $CaCl_2$ and $MgSO_4$ solutions at different concentrations lower than 4% on the ripening of banana and pineapple fruits.

MATERIALS AND METHODS

Plant materials

Banana fruits (*Musa accuminata* Colla var. William) were donated by the Cameroon Banana Producing Company (SBM-PH-SPNP) in Loum. All bananas were from second hands of bunches. Pineapple fruits (*Ananas comosus* (L.) Merr. of the Cayenne group) were harvested from a farm in Lowe (Mungo Division). They were of the third class (1.3 to 1.5 kg fresh weight). Bananas and pineapples were dipped in 50 mg/l, 100 mg/l and 200 mg/l $CaCl_2$ or $MgSO_4$ solutions. Untreated fruits were used as control.

Assessment of fruit ripening stages

The different stages of ripeness of fruits (1 to 7) were visually assessed according to Anon [20].

Analysis of pigments

Pigments were extracted from fresh peel of fruits (12 g) with 10 ml of acetone. A spatula of $CaCl_2$ was added to the extract. After homogenization the extract was allowed to stand for 30 min. The green acetone phase was collected and used as total pigment extract. Individual chlorophylls and carotenoids were fractionated on aluminium sheets precoated with silica gel_{60} F_{254} (Merck™, Germany) by thin layer chromatography. The mobile phase was a mixture of benzene, chloroform and acetone (6/3/2, v/v/v).

Determination of water and total lipid content

250 mg of fresh peel of fruits were dried in an oven at 135 °C for 6h. They were then weighted for the determination of dry matter weight. The water content in the peel was calculated using fresh and dry matter weight according to Chapman [21]

Total lipids were extracted from fresh peel of fruits using a 1.5/1/1 (v/v/v) mixture of chloroform, methanol and water. The extract was allowed to stand for 45 min. The chloroform phase was then transferred into a vial of known weight. After complete evaporation of the chloroform the vial was weighed again for the determination of total lipid content.

Statistical analysis

Statistical analyses were performed with the aid of SPSS for MS WINDOWS. Group comparisons were made using ANOVA. A p value of $P<0.05$ was considered as statistically significant.

RESULTS

The visual assessment of stages of ripeness showed that salt-treated bananas and pineapples were still respectively at stages 2 and 4 of ripening scale while untreated fruits were at stage 7. Thin layer chromatography of pigment extract from peel of fruits revealed that both treated bananas and pineapples contained chlorophyll a and carotenoids while untreated fruits contained only carotenoids. It is noteworthy that the peel of green unripe fruits contained chla, chlb and corotenoids (Fig. 1 & 2).

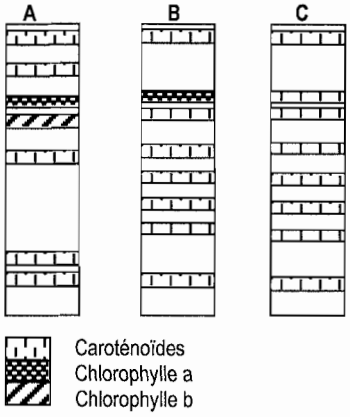

Figure 1: Chromatograms of pigments extracted from the peel of banana fruits. A= control fruits at stage 1 of ripening; B = treated fruits at stage 2 ; C = control fruits at stage 7.

Measurement of the water content in the peel of fruits showed that untreated bananas contained significantly less amount of water than $CaCl_2$-treated fruits whereas the reverse relationship was observed in the peel of pineapple fruits. The water contents were 89%, 89% and 89% in the peel of bananas treated with 50, 100 and 200 mg/l $CaCl_2$ solutions while the peel of control fruits contained 81% (Fig. 3). The water content in the peel of untreated pineapple fruits was 88% while the peel of pineapples treated with 50, 100 and 200 mg/l $CaCl_2$ solutions contained respectively 85%, 85% and 86% water (Fig. 3).

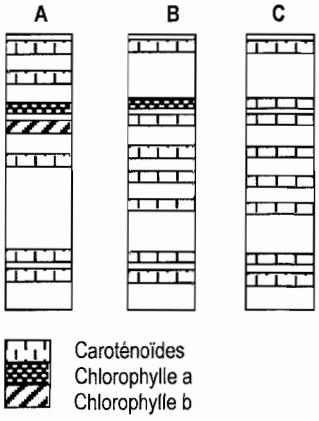

Figure 2: Chromatograms of pigments extracted from the peel of pineapple fruits. A = control fruits at stage 1 of ripening; B = treated fruits at stage 2 ; C = control fruits at stage 7.

No significant differences were observed between the effects of different $CaCl_2$ and $MgSO_4$ concentrations used. The effects of $MgSO_4$ treatments on water content in the peel of bananas followed similar trends as observed with $CaCl_2$ treatments (Fig. 4). However, there were no significant differences between the water contents in the peel of pineapples treated with 50, 100 and 200 mg/l $MgSO_4$ solutions, and in the peel of control fruits. Furthermore, no dose-dependent effect of $MgSO_4$ treatments on water content in the peel of pineapples could be observed (Fig. 4).

The determination of total lipid content in the peel of fruits showed that treatment of bananas with 50 and 100 mg/l $CaCl_2$ solutions resulted in an increase of respectively 32% and 40% while treatment with 200 mg/l $CaCl_2$ solution did not affect this parameter. No significant change was observed in the total lipid content in the peel of pineapples after treatments with different solutions of $CaCl_2$ (Fig. 5).

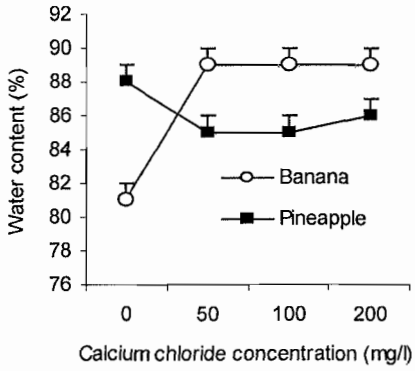

Figure 3: Water content in the peel of banana and pineapple fruits during ripening after treatment with different concentrations of $CaCl_2$.

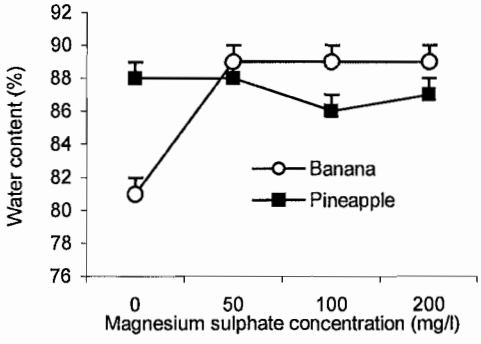

Figure 4 : Water content in the peel of banana and pineapple fruits during ripening after treatment with different concentrations of $MgSO_4$.

DISCUSSION AND CONCLUSIONS

The results showed that treatments with Ca^{2+} and Mg^{2+} salts in the concentration range from 50 mg/l to 200mg/l retarded the visually assessed ripening. At the metabolic level the chlb breakdown was not affected by these treatments whereas there was a delay in chla degradation and water loss, and an increase in the total lipid content in the peel. Ca^{2+} and Mg^{2+} cations would have stabilized cell membranes and maintained the cell wall firm by mediating cross-linking between their components [12, 13]. These cations are also known to play an important role in the inhibition of cell degrading enzymes [11]. The pigment composition in the peel of green unripe fruits and in green plant leaves is almost the same with chla, chlb and carotenoids being the major components. The yellowing of senescent leaves is due to unmasking and partial retention of carotenoids [22]. During fruit ripening there is a

degradation of chlorophyll, retention of pre-existing carotenoids and new synthesis of other yellow pigments [23, 24, 25]. Isolation, functional expression and characterization of cDNA encoding chlorophyllase in citrus fruits have been reported [26]. Chlorophyllase is an enzyme catalyzing the first step of chlorophyll breakdown pathway. Chlorophyllase, Mg-chelatase, methyl esterase, peroxidase, lipoxygenase and phenol oxidase have been suggested as catalysts for chlorophyll bleaching in senescing tissues [7, 22]. Indeed, lipoxygenase and peroxidase are believed to be Ca^{2+} stimulated [12, 27]. The detailed mechanisms whereby $CaCl_2$ and $MgSO_4$ treatments delayed the degradation of chla but not that of chlb in the peel of ripening banana and pineapple fruits are still to be elucidated. Since chla is predominantly found in the reaction center while chlb is exclusively an antenna pigment of the photosystems [28, 29], there might be a specific Ca^{2+} and Mg^{2+} independent destruction of the antenna zone. Moreover, some experimental indications exist on the conversion of chlb to chla prior to chlorophyll degradation in de-greening plant leaves [30].

$CaCl_2$ and $MgSO_4$ treatments induced an inhibition of water loss in the peel of banana fruits, indicating that Ca^{2+} and Mg^{2+} inhibited water loss through transpiration by stabilizing cell membranes and rendering cell wall firmer [12]. Furthermore, the inhibition of respiration by Ca^{2+} that delays the transformation of starch into reducing sugars could retard the increase of osmotic potential in the pulp and thus inhibit the movement of water from peel to pulp [31, 32]. The peel of $CaCl_2$ treated pineapples contained less water than the peel of untreated fruits and there was no significant effect of $MgSO_4$ treatments on pineapples. This could be due to the fact that in non-climacteric fruits, such as pineapple, there is no measurable increase in the rate of respiration at onset of ripening [1, 2]. Further studies should clarify the metabolic basis of these differential effects of $CaCl_2$ and $MgSO_4$ treatments on the water content in the peel of bananas and pineapples.

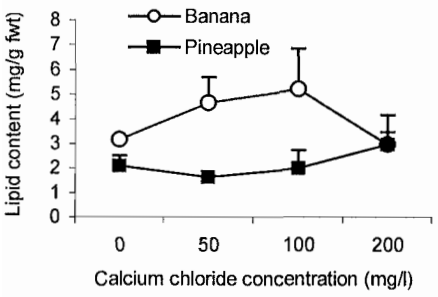

Figure 5 : Lipid content in the peel of banana and pineapple fruits during ripening after treatment with different concentrations of $CaCl_2$.

Treatments with $CaCl_2$ solutions up to 100 mg/l led to an increase in the content of total lipids in the peel of bananas while there was no significant effect of salt treatments on this parameter in peel of pineapples. These results could be related to the difference in the climacteric and non-climacteric nature of both fruits. Since most of plant lipids are constituents of membranes and are important in the modulation of many physiological processes including senescence [33, 34], a new synthesis or an inhibition of their breakdown may support the delaying effects of salt treatments on fruit ripening.

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