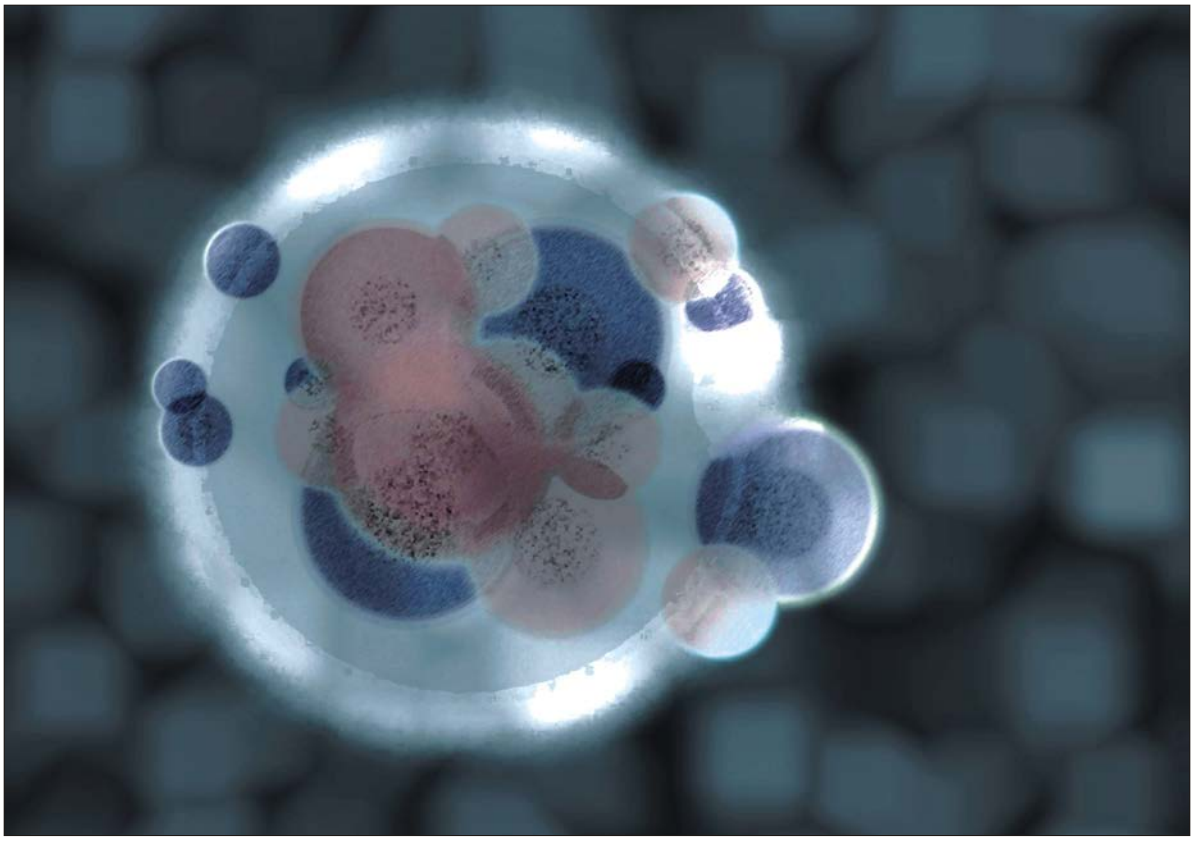

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Postharvest Biochemistry of the Plantain (*Musa paradisiacal* L.)

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Abstract: ACC oxidase activity (ethylene production) in the peel and pulp was monitored over the ripening stages of the plantain (*Musa paradisiacal* L.). The activity of the enzyme in the peel increased from the hard green stage (0.36 ng ethylene h^{-1} g^{-1} Fw) and reached a maximum at the more green than yellow stage (21.99 ng ethylene h^{-1} g^{-1} Fw). There after the activity of the enzyme declined rapidly to 1.20 ng ethylene h^{-1} g^{-1} FW at the yellow with few brown patches stage. No enzyme activity was detected at the yellow with large brown patches stage. Similar results were obtained for the pulp. The enzyme was inhibited by sodium dithionite and sodium metabisulphite, 31.8 and 25.0%, respectively. The activity of β-D-galactosidase, β-D-glucosidase and N-acetyl-β-D-glucosaminidase was determined at the unripe, ripe and over-ripe stages of ripening in the plantain. β-D-galactosidase and β-D-glucosidase activity was greater in the pulp than in the peel when p-nitrophenyl-β-D-galactopyranoside and p-nitrophenyl-β-D-glucopyranoside were used as respective enzyme substrates. The reverse was true for N-acetyl-β-D-glucosaminidase (using p-nitrophenyl-N-acetyl-β-D-glucoaminiside as substrate); where more activity was detected in the peel than in the pulp at all the ripening stages. When o-nitrophenyl-β-D-glactopyranoside was used as the substrate for β-D-galactosidase more activity was detected in the peel than in the pulp. The cell wall degrading enzyme polygalacturonase showed more activity in the pulp than in the peel for all the ripening stages, with the activity increasing, going from the unripe stage (1.03×10^{-4} μmole galacturonate min^{-1} g^{-1} Fw) through to the over-ripe stage (4.5×10^{-4} μmole galacturonate min^{-1} g^{-1} Fw). Postharvest technology was performed on the plantain, at controlled atmosphere (CA) and regular atmosphere (RA). The production of ethylene under CA conditions (using the diffusion channel system) was not detected when compared to fruit kept at RA. The use of postharvest technology resulted in the enhancement of the shelf-life of the plantain when stored at CA.

Key words: Plantain, ethylene, ACC oxidase, glycosidases, postharvest technology

INTRODUCTION

Plantain is one of the world's most important crops and it is part of the staple diet in sub Saharan Africa, the West Indies, Asia, the Pacific, Central and South America. Worldwide, plantain is cultivated in the humid tropics (Price, 1995; Chandler, 1995).

Plantain, can be eaten either in the unripe, ripe or overripe stage; it is either boiled, fried, roasted, steamed, baked or grilled. In East Africa, a beverage which is high in vitamins A, C, B and which is therefore of some significance in nutrition is made from plantain. This plantain beverage can be given to children and even babies. Other products derived from plantain are chips and flour (both made from unripe fruits). The green unripe plantains are peeled, sun-dried, ground and usually sieved to produce the flour (Thompson, 1995). Plantain contains little fat and protein but it is a fairly good source of vitamins A, B and C as well as carbohydrate. The high carbohydrate and low fat content of plantain makes it of use in low fat diets, which could promote weight loss in obese individuals. Plantain is a vital crop for many tropical countries, as it is one of the sources of starch which can be easily produced, since it requires relatively little land preparation, care and maintenance. The cultivation of plantain has a special importance, not only because it is part of the diet, but also in view of its contribution as a source of income, the establishment of employment and poverty alleviation (Chandler, 1995; Turner, 2001).

The post harvest life of plantain has three stages; (i) the preclimacteric phase or green life (ii) the ripening phase or climacteric and (iii) the senescent phase (John and Marchal, 1995). The preclimacteric and senescent phases are of relatively low metabolic activity compared to the ripening phase. The end of the preclimacteric phase marks the beginning of the ripening phase, which is

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associated with enhanced evolution of ethylene and textural changes due to softening (Thompson and Burden, 1995).

Ethylene, a gas under physiological conditions has been known since the beginning of the past century to be used by plants as a signaling molecule for regulating a variety of developmental processes and stress responses (Abeles *et al*., 1992; Dupille *et al*., 1993).

Ethylene is therefore considered a plant hormone because of its dramatic effects on plants (Taiz and Zeiger, 2002).The phytohormone ethylene, plays important roles throughout the plant life cycle, these include seedling growth, leaf abscission, flower senescence, fruit ripening, nodulation, wound and pathogen responses as well as environmental stresses such as heat, cold, flooding and drought (Yang and Hoffman, 1984; Liang *et al*., 1996; Bleecker and Kende, 2000; Chang and Bleecker, 2004). Ethylene is biosynthesized in plants from the amino acid methionine (Met), which is converted to S-adenosyl methionine (SAM) by the enzyme S- adenosylmethionine transferase. SAM is converted by the enzyme 1-amino cyclopropane-1-carboxylic acid synthase (ACC synthase) to 5- methylthioadenosine and 1-amino cyclopropane-1-carboxylic acid (ACC), which is the precursor of ethylene. ACC is finally oxidized to ethylene, CO_2 and HCN by the enzyme 1-amino cyclopropane-1-carboxylic acid oxidase (ACC oxidase). This final step of the ethylene biosynthetic pathway requires ferrous ions (Fe^{2+}) and ascorbic acid as cofactors (Zarembinski and Theologis, 1994; Bleecker and Kende, 2000; Wang *et al*., 2002). The cyanide (HCN) produced along with ethylene in the oxidative breakdown of ACC which is catalysed by the enzyme ACC oxidase (Peiser *et al*., 1984), does not accumulate; it is effectively trapped and detoxified by the activity of the β-cyanoalanine synthase (CAS) ubiquitous in ethylene-producing tissues (Yip and Yang, 1988).

During ripening, partial disassembly of the fruit cell wall is largely responsible for softening and textural changes. The cell wall becomes increasingly hydrated as the pectin rich middle lamella is modified and partially hydrolysed, resulting in an ease with which one cell can be separated from another, which in turn affects the final texture of the ripe fruit as ripening progresses (Crookes and Grierson, 1983; Carey *et al*., 1995; Smith *et al*., 2002). These large changes in pectin structure, has been attributed to the action of polygalacturonase (Cooper *et al*., 1998; Chun and Huber, 2000; Brummell and Harpster, 2001). Although polygalacturonase (PG) is not the major determinant of fruit softening, transgenic fruit with low PG activity are slightly firmer, more resistant to splitting, mechanical damage and pathogen infection (Grierson and Schuch, 1993; Langley *et al*., 1994; Wang *et al*., 2005). Softening accompanying ripening proved to be significantly reduced in transgenic tomato fruit with suppressed β-galactosidase activity, an enzyme that serves to remove pectic galactan side chains (Carey *et al*., 1995; Smith and Gross, 2000; Brummell and Harpster, 2001; Smith *et al*., 2002; Wang *et al*., 2005).

This study was undertaken to determine ACC oxidase activity, the production of ethylene, the activities of polygalacturonase and the glycosidases in plantain (*Musa paradisiacal* L.).

Ethylene production in the plantain was also determined under controlled atmosphere (CA) and regular atmosphere (RA). These experiments were carried out in order to monitor the production of ethylene during the ripening process of the plantain and to show how controlling the production of ethylene by using postharvest technology can enhance the shelf life of the plantain. Determination of the activity of the glycosidases and polygalacturonase is also a measure of how ripe the fruit is; the greater the enzyme activity the riper the fruit.

The information resulting from this study will be of great help to the agricultural sector of Jamaica, where the poor shelf-life of many tropical fruit has resulted in the lost of much needed foreign exchange.

MATERIALS AND METHODS

Plant material: Mature, green and unripe plantain (*Musa paradisiaca* L. cv. French) fruits which were obtained from a local market in Papine, St. Andrew, Jamaica; were allowed to ripen naturally at room temperature (30±2°C) and samples were collected at various ripening stages.

Chemicals: All reagents were of analytical grade and were purchased from Sigma (St. Louis, MO, USA) and BDH Chemicals (Poole, UK). Alumina F-1 (80/100 mesh) was purchased from Supelco (Bellefonte, USA).

Equipment: Beckman (J-21) centrifuge was supplied by Beckman Coulter Fullerton, California USA. A Pye unicam gas chromatograph (series 204) and a Pu 4810 computing integrator were from Philips (Cambridge, England). A Cecil CE 9000 series spectrophotometer was from Cecil Instruments Ltd., Cambridge, England.

ACC oxidase activity: ACC oxidase activity was measured by the method of Williams and Golden (2002) with slight modifications. The pulp and peel of the plantain samples were diced separately and one gram (1 g) each was put into a 13 mL reaction vial containing 2.5 mL of 0.1 M Tris-HCl incubation buffer (pH 7.2), consisting of sodium

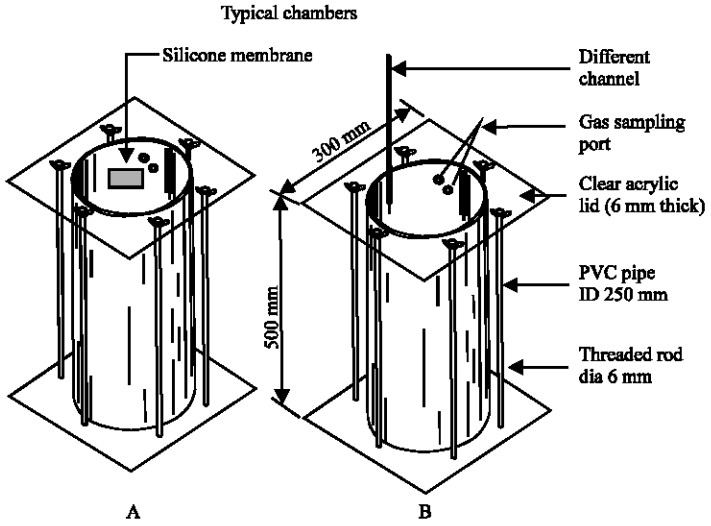

Fig. 1: Chambers used in controlled atmosphere experiments. A: chamber fitted with silicone membrane. B: Chamber fitted with diffusion channel

ascorbate (30 mM), sodium hydrogen carbonate (30 mM) and glycerol (10% v/v); together with 0.1 mL of 20 mM ACC and 0.05 mL of 80 mM $FeSO_4$. The vials were sealed with rubber septa and incubated at room temperature (30±2°C) for 2 h. Ethylene produced was measured by a gas chromatograph from head-space analysis of the 13 mL reaction vials.

Ethylene production: Ethylene produced by intact plantain was also measured at CA and RA. Fruits were weighed and placed in laboratory scale storage chambers, composed of a length of PVC pipe, 30 cm long and 17 cm internal diameter. The extremities of each unit were fitted with a square sheet of acrylic 5 mm thick, held in place by threaded rods. At one end of the chamber, the acrylic lid served as a cover and was removable to facilitate opening and closing of the chamber (Fig. 1). The cover was transparent, allowing for visual sample inspection during storage. A diffusion channel made of copper tubing (11 cm in length and 3 mm in internal diameter) was installed onto the acrylic cover of each chamber in order to control the oxygen (Gariépy *et al*., 1986; Ramachandra 1995; Ratti *et al*., 1997; Stewart *et al*., 2005). Neoprene gaskets were used at both ends of the chamber to ensure air tightness. The control chamber was not fitted with the diffusion channel tubing. The gas concentration in the sealed chambers was established by flushing the containers with 100% N_2 and allowing the atmosphere within the chamber to be modified by the diffusion channel system. The control chamber contained regular atmosphere (RA). In this study the diffusion channel (11 cm × 3 mm i.d) used, gave a final oxygen concentration of 5%, down from 21%; this was determined by a Hewlett Packard gas chromatograph fitted with a thermal conductivity detector. The weight of the plantain placed in the chambers was on an average 0.74 kg and the experiment was carried out at 25°C.

Ethylene was measured in both the diced and intact samples, using a Pye Unicam series 204 Gas Chromatograph (GC) fitted with a flame ionization detector. The flame ionization detector was coupled to a column [column dimensions: 6 mm (o.d.) ×4 mm (i.d.) ×1.5 m] containing alumina packing (F-1, 80/100 mesh). The results were recorded by a Philips pu 4810 integrator. A gas syringe was used to remove 1 mL of gas from the head-space of the reaction vial and injected into the injector of GC. The GC parameters were: injector temperature 120°C; detector temperature 200°C; oven temperature 75°C; sensitivity 4×10; carrier gas N_2: flow rate through the column was 30 mL min^{-1}. The amount of ethylene produced was quantified using the method of Lizada and Yang (1979), in which ACC was quantitatively converted to ethylene and a standard curve for ethylene generated.

Extraction of polygalacturonase: Polygalacturonase was extracted from the pulp and peel of mature plantain at the unripe, ripe and overripe stages, according to the method of Okolie and Obasi (1992) with slight modifications. Ten gram each, of the plantain pulp and peel were sliced separately and homogenized in 30 mL of chilled 0.4 M sodium chloride solution. The cold homogenate was

filtered through four layers of cheesecloth and centrifuged in a Beckman centrifuge (Model J-21) at 10,000 g for 5 min. The supernatant was use as the crude extract for the assay of polygalacturonase activity.

Assay of polygalacturonase: Polygalacturonase was also assayed according to the method of Okorie and Obasi (1992). A 0.5 mL sample of the crude extract was incubated in a test tube at room temperature (30±2 °C) with 0.5 mL of 1 g L^{-1} polygalacturonic acid in 0.2 M sodium phosphate buffer (pH 6.8). After 30 min, the tube was transferred into boiling water for 5 min to stop the reaction. The reducing sugar liberated was estimated by the method of Nelson and Somogyi (1952). The equivalent galacturonate released was read off a calibration curve and polygalacturonase activity expressed as micromoles galacturonate equivalents released per minute per gram fresh weight (Fw).

Extraction of glycosidases: Glycosidases were extracted from the pulp and peel of mature plantain at the unripe, ripe and overripe stages using a modified method of Golden *et al.* (1993). Twenty gram each, of the plantain pulp and peel were sliced separately and homogenized with 20 mL of 0.05 M Na-Citrate buffer (pH 4.5). The homogenate was filtered through two layers of cheesecloth and centrifuged in a Beckman centrifuge (Model J-21) at 10,000 g for 10 min. The supernatant was then used as the crude extract for the assay of glycosidases activities.

Assay of glycosidases: The glycosidases assayed for were; β-D- galactosidase, β-D-glucosidase and N-acetyl-β-D-glucosaminidase. The method of Golden *et al.* (1993) with slight modifications was also used for this assay. The assay mixture contained 1 mL of extraction buffer (pH 4.5), 0.4 mL of 4 mM of the appropriate substrate (p-nitrophenyl-β-D-galactopyranoside; p-nitrophenyl-β-D-glucopyranoside; p-nitrophenyl-N-acetyl-β-D-glucosaminide and *o*-nitrophenyl-β-D-galactopyranoside) and 0.3 mL of the crude extract. Incubation was carried out at 37°C for 10 min. After 10 min of incubation, 1 mL of 0.3 M Na_2CO_3 was added and the resulting absorbance was determined at 405 nm with Cecil CE 9000 series spectrophotometer (Model No. CE 9050). One unit of enzyme activity is the amount of enzyme converting 1 μmole of PNPG per min to nitrophenol.

Inhibition studies: Inhibitors such as $Na_2S_2O_4$ (sodium dithionite: 1 mM) and $Na_2S_2O_5$ (sodium metabisulphite: 1 mM) [0.2 mL each] were used to study the inhibition of ethylene production, while 1 mM $CuSO_4$, 2 mM $FeSO_4$, 2 mM galactose, 1 mM $Na_2S_2O_5$, 1 mM $CoCl_2$, 1 mM Phenyl-Hg-acetate and 1 mM $HgCl_2$ (0.4 mL each) were used to study the inhibition of β-D-galactosidase activity in the plantain pulp.

Statistical analysis: For statistical analysis the student's t-test was performed.

RESULTS

ACC oxidase activity: The activity of ACC oxidase, the enzyme that catalyses the final step in the biosynthesis of ethylene, was higher in the peel of the plantain samples than in the pulp in all the ripening stages. There was a significant ($p<0.01$), sharp increase in ACC oxidase activity at the onset of ripening (more green than yellow stage) and a significant ($p<0.01$), rapid decline in the ripe plantain fruit in both the pulp and peel. While ACC oxidase activity was undetectable in the pulp and peel of the overripe fruit at the yellow with large brown patches stage (Table 1).

Sodium dithionite and Sodium metabisulphite inhibited ACC oxidase activity in the sprung green stage of the unripe plantain pulp; however, they were weak inhibitors (Table 2).

Ethylene production: The pattern of change in the rate of endogenous ethylene production in the intact plantain samples stored at regular atmosphere (RA) and controlled

Table 1: ACC oxidase activity in the pulp and peel of ripening plantain

Ripening stages	ACC oxidase activity (Ethylene: ng h^{-1} g^{-1} Fw)	ACC oxidase activity in peel (Ethylene: ng h^{-1} g^{-1} Fw)
Hard green*	ND	0.36± 0.01
Sprung green*	0.45±0.01	0.85±0.03
More green than yellow**	8.63±0.07	21.99±0.04
More yellow than green^{+}	1.51±0.08	13.48±0.04
Yellow with green-tip^{+}	0.52±0.06	3.64±0.07
Fully yellow^{+}	0.45±0.02	2.49±0.02
Yellow with few brown patches (flecking)$^{++}$	0.17±0.02	1.20±0.01
Yellow with large brown patches^{++}	ND	ND

Each value represents the mean±SE of duplicates, ND: Not Detected, Fw, Fresh weight, * Unripe stages, ** Onset of ripening, $^{+}$ripe stages, $^{++}$Overripe stag

Table 2: The effect of inhibitors on ACC oxidase activity in plantain pulp; in the sprung green stage (unripe)

Inhibitor	Concentration (mM)	ACC oxidase activity (Ethylene: ng h^{-1} g^{-1} Fw)	Inhibition (%)
None	----	0.44±0.12	----
Sodium dithionite	1.0	0.30±0.05	31.8
Sodium metabisulphite	1.0	0.33±0.04	25.0

Each value represents the mean±SE of triplicates, Fw: Fresh weight

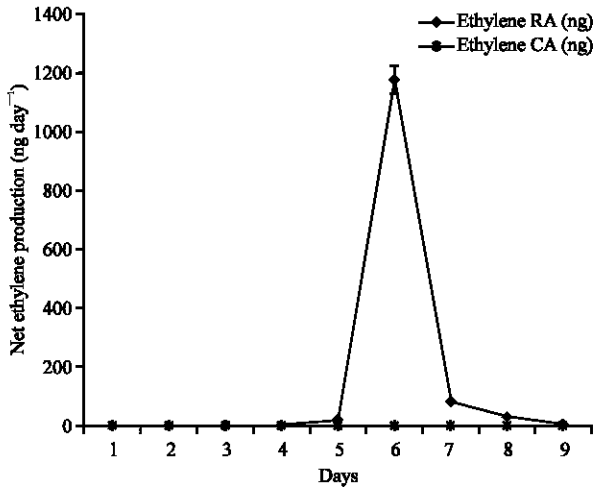

Fig. 2: Endogenous ethylene production in intact plantain at RA and CA (diffusion channel)

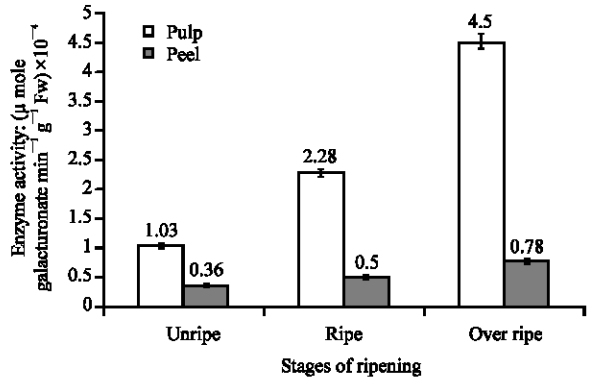

Fig. 3: Polygalacturonase activity in plantain pulp and peel

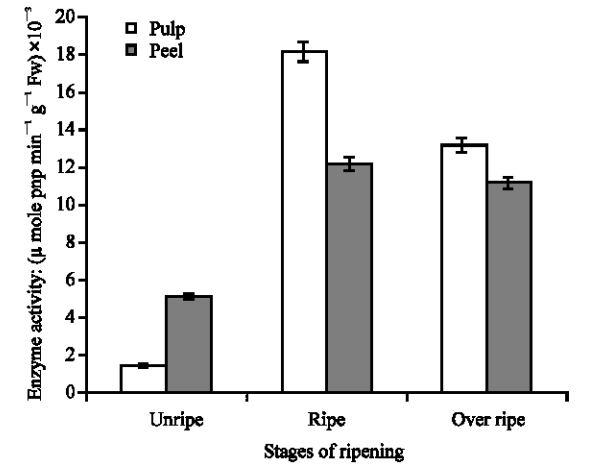

Fig. 4: β-D-Galactosidase activity in plantain pulp and peel

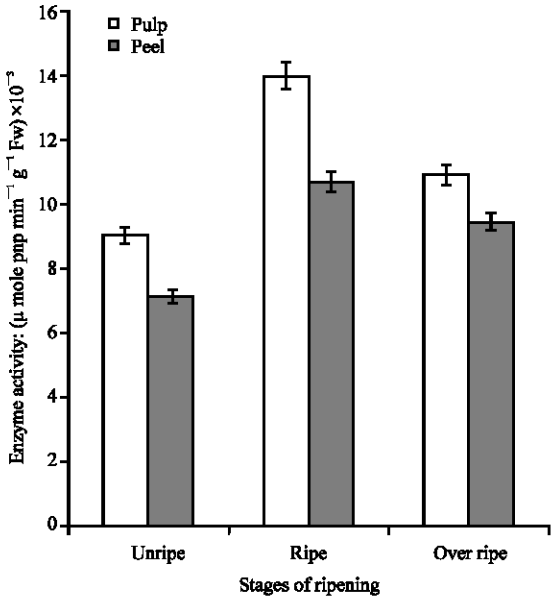

Fig. 5: β-D-Glucosidase activity in plantain pulp and peel

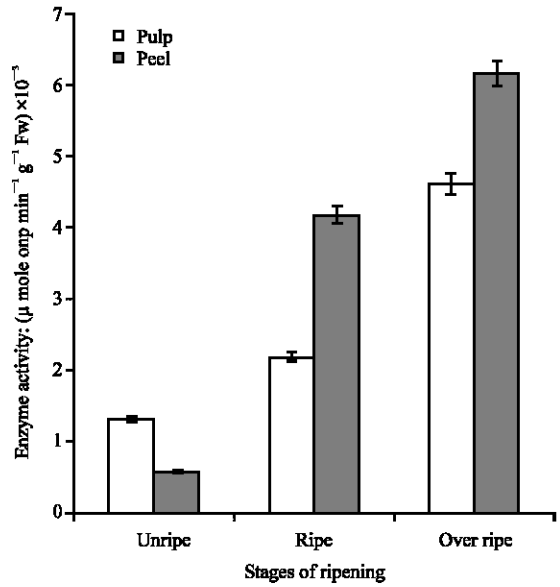

Fig. 6: β-D-Galactosidase activity in plantain pulp and peel: 0NPG as substrate

atmosphere (CA) is shown in Fig. 2. At CA no ethylene was detected over the nine (9) day period, however at RA ethylene was detected at day 6 (1174 ng) but declined sharply at day 7 (83 ng). At day 9 no ethylene was detected at RA.

Polygalacturonase activity: Polygalacturonase activity was higher in the plantain pulp than in the peel. The activity was lowest in the unripe fruit (1.03×10^{-4} μmole

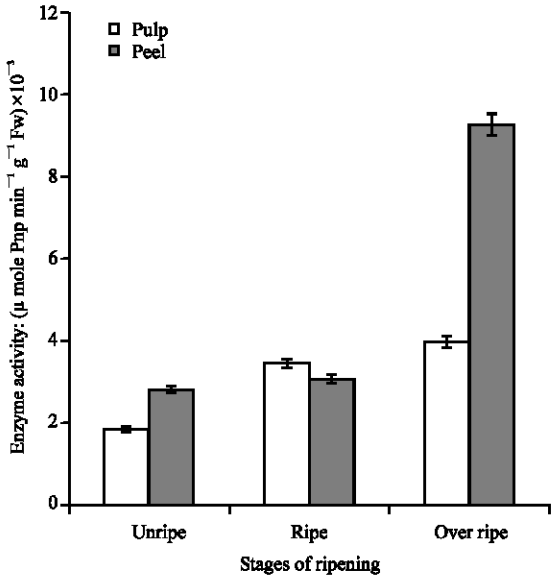

Fig. 7: N-acetyl-β-D-Glucosaminidase activity in plantain pulp and peel

Table 3: Effect of inhibitors on the activity of β-D-galactosidase in the ripe stage of plantain pulp

Inhibitor	Concentration (mM)	ACC oxidase activity (Ethylene: ng h^{-1} g^{-1} Fw)	Inhibition (%)
None	-	17.8±0.0	-
$CuSO_4$	1	16.2±0.5	9.0
$FeSO_4$	2	8.9±0.1	50.0
Galactose	2	6.0±0.1	66.3
$CoCl_2$	1	6.3±0.1	64.6
$Na_2S_2O_5$	1	6.0±0.12	66.3
Phenyl-Hg-acetate	1	3.3±0.3	81.5
$HgCl_2$	1	2.6± 0.11	85.4

Each value represents the mean±SE of triplicates, Fw: Fresh weight

galacturonate min^{-1} g^{-1} Fw) and highest in the overripe fruit (4.5×10^{-4} μmole galacturonate min^{-1} g^{-1} Fw) in both the pulp and peel (Fig. 3).

Glycosidases activities: β-D-Galactosidase was highest in the ripe plantain fruit (18.2 x 10^{-3} μmole PNP min^{-1} g^{-1} Fw-pulp) and lowest in the unripe fruit (1.48×10^{-3} μmole PNP min^{-1} g^{-1} Fw-pulp) (Fig. 4). The same is true for β-D-glucosidase (Fig. 5). However, β-D-galactosidase activity was highest in the over-ripe plantain (6.16×10^{-3} μmole ONP min^{-1} g^{-1} Fw-peel), when o-nitrophenyl-β-D-galactopyranoside (ONPG) was used as substrate. β-D-Galactosidase showed a higher substrate specificity for p-nitrophenyl-β-D-galactopyranoside (PNPG) than for (ONPG). N-acetyl-β-D-glucosaminidase activity was also highest in the over-ripe plantain (9.26×10^{-3} μmole PNP min^{-1} g^{-1} Fw-peel) samples (Fig. 6 and 7).

Various inhibitors were used to inhibit β-D-galactosidase activity in the ripe plantain pulp and their effects on its activity are shown in Table 3.

DISCUSSION

ACC oxidase activity in naturally ripened plantain fruit (*Musa paradisiacal* L. cv. French) in this study was higher in the peel than in the pulp in all the ripening stages (Table 1). The difference between the peel and pulp with respect to ethylene biosynthesis in banana has also been investigated by Ke and Tsai (1988). They found that 1-amino cycloproprane-1-carboxylic acid (ACC) content and ACC oxidase activity were different in the peel and pulp during ripening; moreover, ethylene production was mainly from the pulp. Agoreyo and Golden (unpublished observation) also observed that ACC oxidase activity in another cultivar of plantain (*Musa paradisiaca* L. cv. Horn) was higher in the pulp than in the peel. These results suggest that ACC oxidase activity varies in the pulp and peel of the same cultivar. Also, ACC oxidase activity is either higher in the pulp or peel, depending on the type of cultivar.

During ripening, as observed in this study, ACC oxidase activity also increased sharply at the onset of ripening and then declined rapidly in the ripe fruit, been undetectable in the overripe fruit (yellow with large brown patches) in both the pulp and peel (Table 1). Similarly a sharp rise and fall in the rate of endogenous ethylene production was also observed in intact plantain during ripening (Fig. 2). This pattern of change in the rate of endogenous ethylene production correlates with what has been observed in naturally ripened banana (Liu *et al*., 1999). At the onset of ripening, a burst in ethylene production and ACC oxidase activity occurs in many climacteric fruits such as the banana (Lelièvre *et al*., 1997; Liu *et al*., 1999) the same was observed for plantain in this study. In most climacteric fruits, ethylene production during ripening has been widely recognized to have a climacteric pattern in parallel with the respiration rate; however, ethylene production in banana and avocado declines in the early climacteric phase, resulting in a small peak. This correlates with what was observed in the plantain in this study (Fig. 2). Liu *et al.* (1999) suggest that this rapid decline could be due to the decreased contents of ascorbate and iron, inhibitors of enzyme activity or other unknown factors. However, the results of this study (Table 1) in which these cofactors (ascorbate and iron) were added to the reaction vials, showed a sharp rise and fall in ACC oxidase activity, similar to that exhibited by the plantain in the production of endogenous

ethylene (Fig. 2). Moreover, ascorbic acid has been found to increase in plantain during ripening (Agoreyo *et al*., 2003). It seems that inhibitors of enzyme activity or other unknown factors may be responsible for this sharp rise and fall in ethylene production in banana, avocado and plantain fruits.

Intact plantain stored in controlled atmosphere (CA) using the diffusion channel, showed a reduction in the rate of endogenous ethylene production and did not ripen (as shown in this study: Fig. 2). While those stored in the regular atmosphere (RA) showed the normal rise in ethylene production that characterize climacteric fruit during ripening. The rise in endogenous ethylene production in plantain started on day 5 and declined on day 7 (Fig. 2). This showed that there is a difference in the rate of change of ethylene production in the plantain. Moreover, the result also showed that CA with diffusion channel can help to delay ripening and extend the shelf-life of the fruit by reducing endogenous ethylene production. Stewart *et al*. (2005) have also demonstrated that CA using the diffusion channel lowered the percentage oxygen that is available to the fruit, delayed ripening and extended the shelf- life of bananas.

Sodium dithionite and sodium metabisulphite, reducing reagents that consume oxygen, inhibited ACC oxidase activity in the sprung green stage by 31.8 and 25%, respectively (Table 3). Both sodium dithionite and sodium metabisulphite abolished ACC oxidase activity in pawpaw (Dunkley and Golden, 1998) and also inhibited the enzyme by 82.9 and 89.5%, respectively in breadfruit (Williams and Golden, 2002). The inhibition shown by sodium dithionite and sodium metabisulphite resulted from their consumption of oxygen, required by ACC oxidase for its activity.

Polygalacturonase activity increased with ripening in both the pulp and peel of plantain. The increase in the pulp was higher than that of the peel. The polygalacturonase activity in the pulp increased from the unripe to the ripe and over-ripe stages by 123 and 337%, respectively (Fig. 3). While that of the peel increased from the unripe to the ripe and overripe stages by 39 and 117%, respectively (Fig. 3). The polygalacturonase activity was highest in the overripe stage in both the pulp and peel of the plantain (Fig. 3). Experiments with transgenic tomato fruit in which polygalacturonase mRNA and protein levels were suppressed have demonstrated that the softened texture of a ripe fruit does not result directly from polygalacturonase-mediated modifications to the pectin network (Brummell and Harpster, 2001). Down-regulation of polygalacturonase does, however, delay fruit senescence and enhance resistance to postharvest pathogens (Langley *et al*., 1994). Polygalacturonase is therefore known to be associated with fruit ripening as well as softening of over-ripe fruit (Tucker *et al*., 1980; Brummell and Harpster, 2001).

The glycosidases activities of the pulp and peel of plantain, showed the same general trend except for N-acetyl-β-D-glucosaminidase (Fig. 4-7). Activities tended to peak at the ripe stage, having increased from lower values in the unripe fruit and then declined in the over-ripe stage. β-D-Galactosidase showed the greatest increase, more than nine - fold in the plantain pulp and more than two-fold in the peel. β-D- Galactosidase also showed higher substrate specificity for p-nitrophenyl-β-D- galactopyranoside (PNPG) than for o-nitrophenyl-β-D-galactopyranoside (ONPG). For N-acetyl-β-D-glucosaminidase, the activity increased continuously with ripening; the highest activity was observed in the overripe stage (Fig. 7). It has long been known that increased activity of many glycosidases accompanies fruit ripening and these results correlate with the findings of Golden *et al*. (1993), on coffee berries (*Coffea arabica*). The β-D- galactosidase from coffee berries increased during ripening, displayed activity against p-nitrophenyl-β-D-galactopyranoside (PNPG), lactose, arabinogalactan and galactan. It was suggested that β-D-galactosidase plays a role in cell wall degradation such as occurs during ripening. Carey *et al*. (1995) reported that this class of β-D-galactosidase that acts on PNPG did not display activity against galactan and did not markedly change during ripening in tomato. However, Balasubramaniam *et al*. (2005) showed that this class of β-D-galactosidase from ripe carambola fruit (*Averrhoa carambola* L. cv. B10) displayed activity against PNPG, galactan and arabinogalactan. They suggested that the ability of β-D-galactosidases to hydrolyze synthetic PNPG substrate implies that they may have an exo-acting activity. Softening accompanying ripening proved to be significantly reduced in transgenic tomato fruit with suppressed β-D-galactosidase activity, that specifically act on galactan (Carey *et al*., 1995; Smith and Gross, 2000; Brummell and Harpster, 2001; Smith *et al*., 2002; Wang *et al*., 2005).

Table 3 shows the effect of various inhibitors on the activity of β-D-galactosidase of ripe plantain pulp. Mercuric chloride inhibited the enzyme by 85.4%, exhibiting the highest inhibition. While $CuSO_4$ gave the lowest inhibition (9%). Galactose inhibited the enzyme by 66.3%, suggesting that it may have a negative feed back inhibition on the enzyme.

CONCLUSION

Postharvest technology revealed that the shelf-life of the plantain can be enhanced by using the diffusion channel system to suppress the production of ethylene. This was achieved by lowering the percentage of oxygen that was available to the plantain. The activity of all the enzymes that were assayed increased steadily from the unripe up to the ripe stage and then declined rapidly at the over-ripe stage. These findings are in keeping with climacteric fruits, the plantain being no exception.

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REFERENCES

Abeles, F.B., P.W. Morgan and M.E. Saltveit, 1992. Ethylene in Plant Biology. 2nd Edn., Academic Press, New York, USA.

Agoreyo, B.O., P.A.P. Aweh and F.O. Agoreyo, 2003. Effects of different storage conditions on the ascorbic acid content of plantain (*M. paradisiaca*). Biosci. Res. Commu., 15: 239-244.

Balasubramaniam, S., H.C. Lee, H. Lazan, R. Othman and Z.M. Ali, 2005. Purification and properties of a β-galactosidase from carambola fruit with significant activity towards cell wall polysaccharides. Phytochem., 66: 153-163.

Bleecker, A.B. and H. Kende, 2000. Ethylene: A gaseous signal molecule in plants. Annu. Rev. Cell. Dev. Biol., 16: 1-18.

Brummell, D.A. and M.H. Harpster, 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant. Mol. Biol., 47: 311-340.

Carey, A.T., K. Holt, S. Picard and R. Wilde *et al*., 1995. Tomato exo-(1-4)-β-D-galactanase; isolation, changes during ripening in normal and mutant tomato fruit and characterization of a related cDNA clone. Plant Physiol., 108: 1099-1107.

Chandler, S., 1995. The Nutritional Values of Bananas. In: Bananas and Plantains. Gowen, S. (Ed.), Chapman and Hall, London, UK., pp: 468-480.

Chang, C. and A.B. Bleecker, 2004. Ethylene Biology: More than a gas. Plant. Physiol., 136: 2895-2899.

Chun, J.P. and D.J. Huber, 2000. Reduced levels of β-subunit protein influence in tomato fruit firmness, cell wall ultrastructure and PG2-mediated pectin hydrolysis in excised pericarp tissue. J. Plant Physiol., 157: 153-160.

Cooper, W., M. Bouzayen, A. Hamilton, C. Barry, S. Rossall and D. Grierson, 1998. Use of transgenic plants to study the role of ethylene and polygalacturonase during infection of tomato fruit by *C. gloeosporioides*. Plant Pathol., 47: 308-316.

Crookes, P.R. and D. Grierson, 1983. Ultrastructure of tomato fruit ripening and the role of polygalacturonase isozymes in cell wall degradation. Plant Physiol., 72: 1088-1093.

Dunkley, H.M. and K.D. Golden, 1998. ACC oxidase from Carica papaya: Isolation and characterization. Physiol. Plant, 103: 225-232.

Dupille, E., C. Rombaldi, J.M. Lelievere, J.C. Cleyet-Marel, J.C. Pech and A. Latche, 1993. Purification, properties and partial amino-acid sequence of 1-amino cyclopropane-1-carboxylic acid oxidase from apple fruits. Planta, 190: 65-70.

Gariépy, Y., G.S.V. Raghavan and R. Theriault, 1986. Controlled atmosphere storage of celery with the silicone membrane system. Int. J. Refrig., 9: 234-240.

Golden, K.D., M.A. John and E.A. Kean, 1993. β-Galactosidase from Coffea arabica and its role in fruit ripening. Phytochemistry, 34: 355-360.

Grierson, D. and W. Schuch, 1993. Control of Ripening. Philosophical Transactions of the Royal Society of London, Series B., 342: 241-250.

John, P. and J. Marchal, 1995. Ripening and Biochemistry of the Fruit. In: Bananas and Plantains. Gowen, S. (Ed.), Chapman and Hall, London, UK., pp: 434-467.

Ke, L.S. and P.L. Tsai, 1988. Changes in the ACC content and EFE activity in the peel and pulp of banana fruits during ripening in relation to ethylene production. J. Agric. Ass. China, 143: 48-60.

Langley, K.R., A. Martin, R. Stenning, A.J. Murray, G.E. Hobson, W.W. Schuch and C.R. Bird, 1994. Mechanical and Optical assessment of the ripening of tomato fruit with reduced polygalacturonase activity. J. Sci. Food. Agric., 66: 547-554.

Lelièvre, J.M., A. Latché, B. Jones, M. Bouzayen and J.C. Pech, 1997. Ethylene and fruit ripening. Physiol. Plant, 101: 727-739.

Liang, X., N.F. Shen and A. Theologis, 1996. Li^+-regulated 1-amino cyclopropane-1-carboxylate synthase gene expression in *A. thaliana*. Plant J., 10: 1027-1036.

Liu, X., S. Shiomi, A. Nakatsuka, Y. Kubo, R. Nakamura and A. Inaba, 1999. Characterization of ethylene biosynthesis associated with ripening in banana fruit. Plant Physiol., 121: 1257-1265.

Lizada, M.C. and S.F. Yang, 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal. Biochem., 100: 140-145.

Nelson, N. and M. Somogyi, 1952. Notes on sugar determination. J. Biol. Chem., 195: 19-23.

Okorie, P.N. and B.N. Obasi, 1992. Implication of cell wall degrading enzymes in the heat-induced softening of the African pear (*Dacryodes edulis*) J. Sci. Food Agric., 59: 59-63.

Peiser, G.D., T.T. Wang, N.E. Hoffman, S.F. Yang, H.W. Liu and C.T. Walsh, 1984. Formation of cyanide from carbon 1 of 1-amino cyclopropane-1-carboxylic acid during its conversion to ethylene. Proc. Natl. Acad. Sci. USA., 81: 3059-3063.

Price, N.S., 1995. The Origin and Development of Banana and Plantain Cultivation. In: Bananas and Plantains. Gowen, S. (Ed.), Chapman and Hall, London, UK., pp: 1-13.

Ramachandra, M., 1995. Diffusion channels for broccoli storage. M.Sc. Thesis, McGill University, Montreal, Canada.

Ratti, C., H.R. Rabie and G.S.V. Raghavan, 1997. Diffusion channel design for MA storage of vegetables. NABEC-94, Ontario, pp: 94340.

Smith, D.L. and K.C. Gross, 2000. A family of at least seven beta-galactosidase genes is expressed during tomato fruit development. Plant Physiol., 123: 1173-1183.

Smith, D.L., J.A. Abbott and K.C. Gross, 2002. Down-regulation of tomato β-gala-ctosidase results in decreased fruit softening. Plant Physiol., 129: 1755-1762.

Stewart, O.J., G.S.V. Raghavan, K.D. Golden and Y. Gariépy, 2005. MA storage of Cavendish bananas using silicone membrane and diffusion channel systems. Postharv. Biol. Technol., 35: 309-317.

Taiz, L. and E. Zeiger, 2002. Plant Physiol. 3rd Edn., Sinauer Associates, Inc., Publishers, Massachusetts, USA., pp: 223-258.

Thompson, A.K., 1995. Banana Processing. In: Bananas and Plantains. Gowen, S. (Ed.), Chapman and Hall, London, UK., pp: 481-492.

Thompson, A.K. and O.J. Burden, 1995. Harvesting and Fruit Care. In: Bananas and Plantains. Gowen, S. (Eds.), Chapman and Hall, London, UK., pp: 403-433.

Tucker, G.A., N.G. Robertson and D. Grierson, 1980. Changes in polygalacturonase isoenzymes during the ripening of normal and mutant tomato fruit. Eur. J. Biochem., 115: 87-90.

Turner, D.W., 2001. Postharvest Physiology and Storage of Tropical and Subtropical Fruits. In: Bananas and Plantains. Mitra, S.K. (Eds.), CAB International, Oxon, pp: 47-83.

Wang, K.L.C., H. Li and J.R. Ecker, 2002. Ehylene biosynthesis and signaling networks. Plant Cell., 14 (Supple): S131-S151.

Wang, T.W., C.G. Zhang, W. Wu and L.M. Nowack *et al*., 2005. Antisense suppression of deoxyhypusine synthase in tomato delays fruit softening and alters growth and development. Plant Physiol., 138: 1372-1382.

Williams, O.P. and K.D. Golden, 2002. Purification and characterization of ACC oxidase from *A. altilis*. Plant Physiol. Biochem., 40: 273-279.

Yang, S.F. and N.E. Hoffman, 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol., 35: 155-189.

Yip, W. and S.F. Yang, 1988. Cyanide metabolism in relation to ethylene production in plant tissues. Plant Physiol., 88: 473-476.

Zarembinski, T.I. and A. Theologis, 1994. Ethylene biosynthesis and action: A case of conservation. Plant Mol. Biol., 26: 1579-1597.