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Acid phosphatase and alkaline phosphatase activities in ripening fruit of *Musa Paradisiaca* L.

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Abstract

The activities of non – specific acid phosphatase and alkaline phosphatase were examined during ripening in the fruit of plantain (*Musa paradisiaca* L. cv. False horn). This study was carried out in order to determine the activities of these phosphohydrolytic enzymes in supplying inorganic phosphate that is required for the maintenance of various cellular metabolism such as the conversion of starch to sugar and the associated rise in respiration that occur during ripening in plantain. Acid phosphatase activity in plantain was found to increase significantly ($P < 0.001$) in all the ripening stages by 1.7 to 8.2 fold (from 2.05 ± 0.43 to 16.77 ± 0.08 µmol/min/g fresh wt.). On the other hand, alkaline phosphatase activity decreased significantly ($P < 0.001$) from the hard green stage (unripe) to the yellow with green tips stage (ripe) by 1.2 to 9.1 fold (from 2.34 ± 0.05 to 0.26 ± 0.03 µmol/min/g fresh wt.) and subsequently increased, from the fully ripe stage to the yellow with large black patches stage (overripe) by 1.9 to 2.5 fold (from 0.49 ± 0.09 to 0.64 ± 0.01 µmol/min/g fresh wt.). The results suggest that acid phosphatase is the main non – specific phosphatase that is responsible for the production and supply of inorganic phosphate during ripening in plantain fruits.

Key words: acid phosphatase, alkaline phosphatase, *Musa paradisiaca*, plantain, ripening

Introduction

Acid and alkaline phosphatases have been traditionally classified as being acid and alkaline, due to their optimum $_{P}H$ activity, above $_{P}H$ 7.0 or below $_{P}H$ 7.0 (Barret – Lannard et al., 1982; Sharma et al., 2004). Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) are enzymes that catalyze the removal of inorganic phosphate (orthophosphate) from organic phosphate esters, in acidic and alkaline media respectively (Panara et al., 1990; Vincent et al., 1992; Asmar et al., 1995). These phosphatases are ubiquitous in plants, animals and microorganisms (Lee, 1988; Duff et al., 1994; Jeong et al., 2003). Acid and alkaline phosphatases in plants play a major role in the supply and metabolism of inorganic phosphate for the maintenance of cellular metabolism (Tabaldi et al., 2007; Mishra and Dubey, 2008). Acid phosphatase has been reported to function in the maintenance of significant inorganic phosphate mobility during banana fruit ripening (Turner and Plaxton, 2001). Alkaline phosphatase has also been reported to be involved in the breakdown and mobilization of starch and sucrose, for the biosynthesis of essential oil in lemongrass (*Cymbopogon flexuosus* Steud) Wats (Ganjewala et al., 2010). Mishra and Dubey (2008) also reported the inhibitory effect of Arsenite (As_2O_3) on the activities of acid and alkaline phosphatases in rice (Oryza sativa L.) seedlings, which resulted in a decline in the level of the phosphate pool. LePS2 gene encodes acid phosphatase in tomato (*Lycopersicon esculentum*) and it was induced in tomato plant in the absence of inorganic phosphatase (Baldwin et al., 2001). Ripening in plantain (*Musa paradisiaca* L.) is associated with various biochemical processes, one of which is the conversion of starch to sugars (Marriot et al., 1981). The activities of the key enzymes responsible for the conversion of starch to sugars in plantain, during ripening have also been reported to increase. One of the key enzymes that are involved in the starch sugar transformation in plantain is phosphorylase. Phosphorylase catalyses the phosphorolytic cleavage of starch, which results in the production of sugar phosphatase that are required for the enhanced respiratory and synthetic processes that occur during ripening in plantain (Iyare and Ekwukoma, 1992). The phosphorolysis of starch by phosphorylase occurs in the presence of inorganic phosphate which can be obtained from the hydrolysis of esters of orthophosphate, by acid and alkaline phosphatases (Vincent et al., 1992). Inorganic phosphate plays a vital functional role in energy transfer and metabolic regulation and is also an important structural constituent of many biomolecules. Consequently, inorganic phosphate metabolism is of critical importance in plant developmental processes (Julie et al., 2000; Bozzo et al., 2002). This study was therefore carried out to determine the activities of acid and alkaline phosphatases in producing and supplying inorganic phosphate for the various biochemical processes that require it during ripening in plantain. The information obtained in this study will be relevant in the purification and characterization of these phosphohydrolytic enzymes in plantain fruits.

Materials and methods

Plant material

Unripe, green plantain bunch (*Musa paradisiaca* L. cv. False horn) was purchased from a local market in Benin City, Nigeria

Table 1. Physical changes of the plantain fruits during ripening

Stages of ripening*	Weight of plantain fruit (g)	Weight of pulp (g)	Weight of peel (g)	Pulp to peel ratio
Hard green	281.0	158.0	123.0	1.29: 1
Sprung green	271.5	161.0	110.5	1.46: 1
More green than yellow	236.5	136.5	100.0	1.36: 1
More yellow than green	218.0	139.5	78.5	1.78: 1
Yellow with green tips	204.0	130.0	74.0	1.76: 1
Fully yellow	226.0	149.5	76.5	1.95: 1
Yellow with few black patches	224.0	145.5	76.5	1.90: 1
Yellow with large black patches	202.0	129.5	72.5	1:79: 1

*Changes in the colour of the peel as indices of the stages of ripening

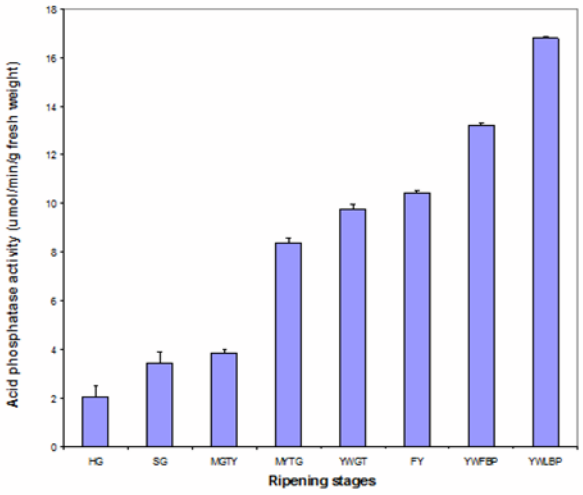

Fig 1. Acid phosphatase activity of plantain (*Musa paradisiaca* L. cv. False horn) at various ripening stages; HG – Hard green, SG – Sprung green, MGTY – More green than yellow, MYTG – More yellow than green, WGT – Yellow with green tips, FY – Fully yellow, YWFBP - Yellow with few black patches, YWLBP – Yellow with large black patches

The plantain samples were allowed to ripen normally at room temperature (30 ± 2 ^{o} C). Samples were collected at various ripening stages; viz, hard green, sprung green, more green than yellow, more yellow than green, yellow with green tips, fully yellow, yellow with few black patches, yellow with large black patches. The plantain samples were weighed and used for enzyme extraction and assay.

Enzyme extraction for non-specific and acid phosphatase (EC 3.1.3.2)

The enzyme extraction was done according to the method of Murray (1980). Five grammes (5g) of the plantain sample were ground in a chilled mortar, with acid – washed sand and 20 ml of chilled 50 mM Tris – HCl buffer ($_{p}$H 7.6) containing 1 mM EDTA. The homogenate was filtered through double layers of cheesecloth and centrifuged at 20,000 g for 20 min. The supernatant was used as the crude extract for the enzyme assay.

Enzyme assay for non-specific acid phosphatase (EC 3.1.3.2)

Acid phosphatase activity was assayed by adding 0.2 ml of enzyme extract to 1 ml of 5.5 mM *p* – Nitrophenol phosphate (*p* – NPP) in 55 mM citrate buffer ($_{p}$H 4.8). The reaction mixture was incubated at 37^{o} C and the reaction was terminated by the addition of 10 ml of 200 mM NaOH. Absorbance was read at 405 nm and the amount of *p* – nitrophenol released was estimated. The assay was performed in triplicate and acid phosphatase activity expressed as µmol *p* – nitrophenol released min^{-1} g^{-1} fresh wt.

Enzyme extraction for non-specific alkaline phosphatase (EC 3.1.3.1)

Five grammes (5g) of plantain sample were ground in a chilled mortar with acid - washed sand and 20 ml of chilled 0.05 M sodium carbonate buffer ($_{p}$H 10).The homogenate was filtered through double layers of cheesecloth and centrifuged at 20, 000 g for 20 min. the supernatant was used as the crude extract for the enzyme assay.

Enzyme assay for non-specific alkaline phosphatase (EC 3.1.3.1)

Alkaline phosphatase activity was assayed by adding 0.05 ml of enzyme extract to 0.5 ml of 3.6 mM sodium thymolphthalein monophosphate in 0.2 M 2–Amino–2–methyl–1– propanol buffer ($_{p}$H 10.2) containing 1 mM magnesium chloride. The reaction mixture was incubated for 10 min at 37^{O} C and the reaction was terminated by the addition of 2.5 ml of 0.1 M sodium hydroxide containing 0.1 M sodium carbonate. Absorbance was read at 590 nm and the amount of sodium thymolphthalein released was estimated. The assay was performed in triplicate and alkaline phosphatase activity expressed as µmol
sodium thymolphthalein released min^{-1} g^{-1} fresh wt.

Result and discussion

Acid phosphatase activity increased continuously in the plantain fruit, throughout the period of ripening (Fig. 1). Activities of some enzymes such as amylase, invertase and phosphorylase have also been found to increase during ripening (Iyare and Ekwukoma, 1992). However, the activity of alkaline phosphatase decreased from the hard green stage (unripe) to the yellow with green tip stage and subsequently increased gradually, until the plantain was overripe (Fig. 2). Moreover, the activity of acid phosphatase was higher than that of alkaline phosphatase in all the ripening stages except for the hard green stage (Figs. 1 – 2). During fruit ripening, peel colour has been reported to be an important characteristic which often serves as one of the major criteria used to determine whether a fruit is ripe or unripe (Williams, 2002). In this study, the changes in the colour of the peel were used as indices of ripening (Table 1). It has also been reported that during ripening, the $_{p}$H of plantain decreases due to increase acidity, until it reaches a value of 4.0 and then increases gradually thereafter (Thomas et al., 1983; Collin and Dalnic, 1991). Since, acid and alkaline phosphatases' activities are affected by $_{p}$H (Barret – Lannard et al., 1982), the increase in acid phosphatase activity observed during ripening in this study, suggests that the low $_{p}$H of plantain

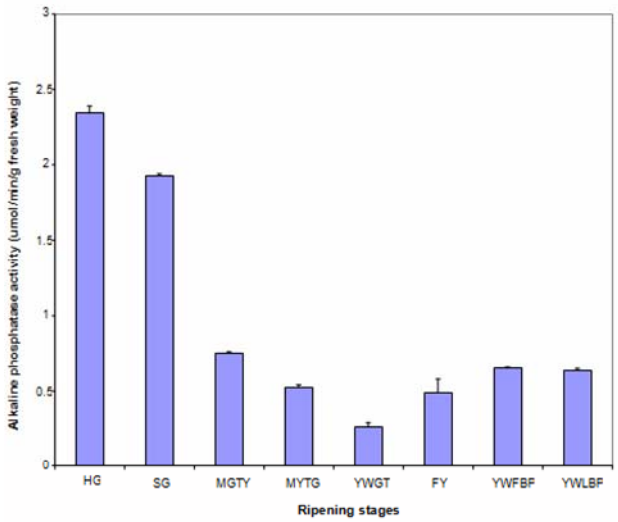

Fig 2. Alkaline phosphatase activity of plantain (*Musa paradisiaca* L. cv. False horn) at various ripening stages; HG – Hard green, SG – Sprung green, MGTY – More green than yellow, MYTG – More yellow than green, YWGT – Yellow with green tips, FY – Fully yellow, YWFBP – Yellow with few black patches, YWLBP – Yellow with large black patches.

during ripening was optimal for the activity of this enzyme. On the other hand, the low $_{P}$H of plantain during ripening was not optimal for the activity of alkaline phosphatase as observed by its decrease in activity in this study. Decreased $_{P}$H, due to increased acidity as ripening progressed in plantain influenced the activity of these two enzymes; by increasing acid phosphatase activity and decreasing alkaline phosphatase activity (Figs. 1–2). Plant acid phosphatases have been reported to be induced under developmental conditions such as flowering, fruit ripening and seed germination (Turner and Plaxton, 2001; Bozzo et al., 2002). Phosphatases are usually considered as playing a role in the production and transport of inorganic phosphate, which is necessary for a large number of metabolic reactions (Osagie, 1992). For instance, during the ripening of plantain, starch is converted to sugar phosphatase by phosphorylase and this conversion requires inorganic phosphate produced by phosphatase. From this study, the results suggest that the inorganic phosphate required for starch phosphorolysis during ripening in plantain is produced mainly by acid phosphatase (Fig. 1). The increase in the activity of acid phosphatase in ripening plantain fruit in this study, correlate with that of Kamenan and Diopoh (1982), in which they reported the presence of acid phosphatase in yam tubers (Dioscorea spp.) and also suggested that the presence of the acid phosphatase in these yam tubers seemed related to that of phosphorylase, thus suggesting a possible role of phosphatase in the production of phosphate necessary for starch phosphorolysis in yams. The sweet taste of ripe plantain, apart from the activity of amylase which provide sugar (Iyare and Ekwukoma, 1992), may also be due to the accumulation of glucose formed mainly, by the action of acid phosphatase on accumulated glucose – 1 - phosphate (G-1-P) and glucose – 6 – phosphate (G-6-P) obtained from the cleavage of starch during ripening. Murray (1980) reported that acid phosphatase from the seed coats of developing pea seeds, although showed invariably highest activity against the model substrate *p* -NPP but also showed a broad acceptance of physiological substrates such as glucose – 6 – phosphate (G-6-P) , 3 – phosphoglycerate (3 – PG) and ADP.

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