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Changes in the Activities of Some Carbohydrate Degrading Enzymes During Ripening of *Musa paradisiaca* and *Musa sapientum*

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Abstract: The activities of starch phosphorylase, total amylase, α -amylase, β -amylase and invertase were monitored in the pulp of post harvest plantain and banana from unripe to the very ripe state. The activities of all the enzymes assayed for increased significantly ($p < 0.01$) as assessed by student t-test. For plantain and banana, the enzymes; starch phosphorylase, β -amylase and invertase had their highest activity prior to the climacteric peak, while total amylase and α -amylase activities were highest at the climacteric. This indicates that starch phosphorylases and β -amylase are important in the initial starch to sugar conversion, while total and α -amylases attained their highest activity at the climacteric peak when a large part of starch had already disappeared. The absolute values obtained showed higher enzyme activities for plantain than for banana, this could be a reflection of higher starch content of plantain.

Key words: Ripening, amylase, invertase, phosphorylase

INTRODUCTION

In the equatorial belt of Africa, plantain and cooking bananas are the main staple foods^[1]. Starch constitutes a major proportion of the nutrient in unripe plantain and banana and most of these are converted to soluble sugars during ripening with approximately 5% lost as CO₂ in respiration^[2]. The predominant sugar in ripe plantain and banana is sucrose but hexoses appear after sucrose and ultimately exceed sucrose concentration^[2]. The disappearance of starch reserves during plantain ripening is very fast, it drops from 27% to less than 1%, sucrose concomitantly increase twelve fold^[3].

Starch to sucrose transformation during ripening of plantain and banana involves several enzymes and more than one pathway. Amylases participates in starch hydrolysis but are probably not linked to sucrose synthesis^[4]. Amylase belongs to a large family of Ca²⁺-protein, which share several structural features^[5]. Invertase catalyses the reaction that converts sucrose into fructose and glucose, these sugars are generally predominant in rapidly expanding juvenile tissues^[6], these tissues have very high invertase activities. Mature sucrose storing tissues have low invertase activity and sucrose phosphate synthase is presumed to be the major sucrose synthesizing enzyme^[7]. Hubbard *et al.*^[2] reported that neutral invertase activity remained constant during

banana ripening, while acid invertase activity increased steadily through out the sampling period. Starch degeneration in banana appears to be mediated primarily by starch phosphorylase^[3]. Early studies with banana showed a substantial increase in phosphorylase activity associated with the starch to sugar conversion^[8]. Improved enzyme extraction procedures^[9] have shown that phosphorylase activity of unripe bananas is sufficient to account for the rapid loss of starch during ripening^[3] but there is a slow reduction in phosphorylase activity during the climacteric^[4].

The aim of this study was to monitor the changes in some enzyme (total amylase, α -amylase, β -amylase, invertase and starch phosphorylase) activities during the ripening of banana and plantain.

MATERIALS AND METHODS

Plant materials: Two bunches of mature green plantain (*Musa paradisiaca*) and 2 bunches of mature green banana (*M. sapientum*) were purchased from local markets. The plantain and banana were identified at the Department of Botany, University of Benin, as French Variety and Dwarf Cavendish Eastern Variety, respectively. The bunches were stored in a well aerated room. This study was conducted in October, 1998.

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Enzyme extractions: Crude enzyme extracts were prepared from the pulp of the plantain and banana. The first samples were taken on the day of purchase (green state) and samples were collected every day as they changed from green to greenish yellow to yellowish green to yellow and finally to yellow with brown flecks, indicating the very ripe state and the onset of spoilage.

All extractions were carried out on ice using ice-cold reagents.

Starch phosphorylase: Phosphorylase was extracted according to the method of Gerbrandy and Doorgeest^[10].

Amylase: Amylase was extracted by the method of Davies and Ross^[11].

Invertase: Invertase was extracted using the method of Passam and Barret^[12].

Enzyme assay

Starch phosphorylase: Starch phosphorylase activity was determined using the Gerbrandy and Doorgeest^[10] method.

Total amylase: Total amylase was determined by the method of Davies and Ross^[11].

α -amylase: α -Amylase activity was assayed using the method of Davies and Ross^[11] as described for total amylase except that the substrate starch was replaced with limit dextrin at a final concentration in the assay medium of 2.5 mg cm⁻³.

β -amylase: β -Amylase activity was calculated as the difference between total amylase and α -amylase.

Invertase: Invertase was determined by the method of Passam and Barret^[12].

Statistics: Values are expressed as mean \pm SEM. Student t-test was used to analyse samples. A $p < 0.01$ was considered statistically significant.

RESULTS

Post harvest observation of plantain and banana in storage: The plantain and banana bunches were stored in a well aerated room after the removal of the first fingers for the Day 1 analysis. At this stage, the fingers were green and firm. The description greenish-yellow indicates the onset of ripening, while yellow with brown flecks indicates the onset of spoilage.

Enzyme activity changes in plantain: Table 1 shows the post harvest changes in the activities of starch phosphorylase, total amylase, α -amylase, β -amylase and invertase in plantain. All enzyme activities increase to peak and then decrease slightly. Significant changes ($p < 0.01$) were observed in the enzyme activities at the end of the sampling period.

Enzyme activity changes in banana: Table 2 shows the post harvest changes in the activities of starch phosphorylase, total amylase, α -amylase, β -amylase and invertase in banana. All enzyme activities increase to a peak and then started to decrease. Significant increases ($p < 0.01$) were observed in the enzyme activities at the end of the sampling period.

DISCUSSION

Marriot *et al.*^[13] reported that the pulp of unripe plantain contains 27% carbohydrate while that of banana contains 20-25% carbohydrate and most of these carbohydrates are present as starch. During ripening, the starch content of plantain and banana is converted to sugar which is predominantly sucrose initially and hexoses as ripening proceeds into the over ripe state.

In this study, the activity of starch phosphorylase in plantain increased to its peak before the ripe state, indicating that phosphorylase is involved in the initial conversion of starch to sugar (Table 1). This result agrees with that of Areas and Lajolo^[3] they reported that starch degradation in banana appears to be mediated primarily by phosphorylase. Singh and Sanwell^[8], also reported a substantial increase in phosphorylase activity during the conversion of starch to sugar in banana ripening. Banana starch phosphorylase activity increased to its peak at the ripe state, with a sharp fall recorded the next day (Day 7) indicating that most of the phosphorylase activities occurs before the climacteric peak (Table 2).

The total amylase and α -amylase of both plantain and banana followed the same trend. They increase steadily, reaching their peak at the ripe state and then declined slightly. The β -amylase activity increased to their peak before the ripe state and then began a steady decline. Garcia and Lajolo^[14] reported that α - and β -amylases were noted in all fruit ripening, however that their activities increased significantly only at the climacteric peak when a large part of starch had already disappeared, only β -amylase activity increases before the onset of the respiratory peak and parallel to starch decreases. This observation corroborates with the results obtained in this study.

Table 1: Enzyme activity ($\mu\text{mol}/\text{min}/\text{g}$ fresh weight) changes in plantain

Day	Texture	Colour	Starch phosphorylase	Total amylase	α -amylase	β -amylase	Invertase
1	Firm	Green	0.066 \pm 0.00329	0.038 \pm 0.00069	0.026 \pm 0.00092	0.006 \pm 0.00098	0.001 \pm 0.00029
2	Firm	Green	0.082 \pm 0.00167**	0.040 \pm 0.00092**	0.029 \pm 0.00046**	0.007 \pm 0.00069**	0.012 \pm 0.00046*
3	Firm	Greenish yellow	0.146 \pm 0.01086*	0.043 \pm 0.00110**	0.034 \pm 0.00139*	0.009 \pm 0.00069**	0.016 \pm 0.00052*
4	Firm	Lighter greenish yellow	0.142 \pm 0.00329*	0.053 \pm 0.00000*	0.35 \pm 0.00046*	0.018 \pm 0.00046*	0.016 \pm 0.00052*
5	Soft	Yellowish green	0.113 \pm 0.00075*	0.088 \pm 0.00110*	0.040 \pm 0.00069*	0.048 \pm 0.00046*	0.026 \pm 0.00029*
6	Soft	Yellow	0.107 \pm 0.00075*	0.150 \pm 0.00462*	0.103 \pm 0.00052*	0.048 \pm 0.00491*	0.015 \pm 0.00029*
7	Very soft	Yellow with brown flecks	0.101 \pm 0.00000*	0.145 \pm 0.00289*	0.101 \pm 0.00052*	0.043 \pm 0.00283*	0.012 \pm 0.00029*

Table 2: Enzyme activity ($\mu\text{mol}/\text{min}/\text{g}$ fresh weight) changes in banana

Day	Texture	Colour	Starch phosphorylase	Total amylase	α -amylase	β -amylase	Invertase
1	Firm	Green	0.060 \pm 0.00167	0.036 \pm 0.00029	0.032 \pm 0.00098	0.004 \pm 0.00092	0.005 \pm 0.00046
2	Firm	Green	0.062 \pm 0.00098**	0.041 \pm 0.00266**	0.032 \pm 0.00046**	0.009 \pm 0.00300**	0.015 \pm 0.00052*
3	Firm	Greenish yellow	0.072 \pm 0.00006*	0.079 \pm 0.00190**	0.035 \pm 0.00052**	0.044 \pm 0.00196*	0.015 \pm 0.00029*
4	Firm	Lighter greenish yellow	0.084 \pm 0.00115*	0.128 \pm 0.00052*	0.05 \pm 0.00110*	0.075 \pm 0.00092*	0.013 \pm 0.00000*
5	Soft	Yellowish green	0.097 \pm 0.00167*	0.134 \pm 0.00260*	0.092 \pm 0.00312*	0.042 \pm 0.00410*	0.012 \pm 0.00000*
6	Soft	Yellow	0.115 \pm 0.00237*	0.140 \pm 0.00139*	0.117 \pm 0.00121*	0.023 \pm 0.00248*	0.011 \pm 0.00052*
7	Very soft	Yellow with brown flecks	0.087 \pm 0.00121*	0.135 \pm 0.00110*	0.111 \pm 0.00196*	0.024 \pm 0.00202*	0.010 \pm 0.00029

Results are mean \pm SEM

*Significant ($p < 0.01$) increase compared to day 1 sample

**No significant ($p < 0.01$) increase compared to day 1 sample

Taken alone, the invertase activity of both plantain and banana increased steadily to its peak just before the ripe state and then reduced slightly. The increase in invertase activity is associated with a decline in the sucrose pool and a concomitant increase in hexose sugar concentration as reported by Hubbard *et al.*^[2]. Taken with the other enzymes, invertase had the lowest activity and was relatively constant through out the sampling period. Hubbard *et al.*^[2] reported that neutral invertase activity remained constant during banana ripening while acid invertase activity increased steadily through out the sampling period.

For all enzymes studied, the absolute value showed higher activities for plantain than for banana, this could be indicative of the higher starch content of plantain.

The result from this study indicate that phosphorylase and β -amylase are important in the initial conversion of starch to sugar during the ripening of plantain and banana, while α -amylase activity is highest at the climacteric peak. Also the increase in invertase activity corresponds to the disappearance of the sucrose pool.

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