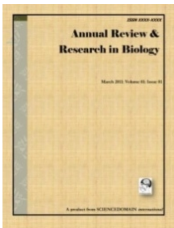

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Changes in Amylase Activity, Soluble Sugars and Proteins of Unripe Banana and Plantain during Ripening

Isaac Olusanjo Adewale[1*], Adenike Adefila[1] and Tosin Babatunde Adewale[1]

[1]*Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.*

Authors' contributions

This work was carried out in collaboration between all authors. Author IOA designed the study and wrote the manuscript. Author AA supervised part of the work. Author TBA carried out the experiment. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

The profiles of amylase activity, soluble sugars and proteins in unripe plantain (*Musa paradisiaca*) and for a comparison, unripe banana (*Musa sapientum*), from harvest to ripening were investigated to provide basic information about their nutritional status at the different stages of the ripening process. Unripe plantain contained about a quarter of the soluble sugars found in ripe plantain. Conversion of the insoluble sugar (starch) to soluble sugar by amylase during ripening was an almost linear process. And as such, activity of amylase in plantain was highest at the ripe stage (2400 ± 120 Units/mg protein) and lowest immediately after harvest when it was still green (700±100 Units/mg protein). The soluble proteins increased from first day when it was harvested, at maximum around the third day and began to decline gradually. In contrast, amylase activity was highest in unripe banana (3900 ± 310 Units/mg protein) and decreased rapidly to a very low value (100 ±15 Units/mg protein) when it was fully ripened. The soluble sugar level in unripe banana was 3.8 ±0.5 mg/ml and around 6.0 ±1.0 mg/ml of extract when fully ripened. The soluble protein was 3 ± 1.0 mg/ml of extract when unripe and increased nine folds to a value of 27± 3.0 mg/ml of extract when fully ripened. The overall results suggest that the highest amount of starch, and the lowest amount of soluble sugars were present on the day when the mature plantain was just harvested.

**Corresponding author: Email: olusanjo2002@yahoo.co.uk, iadewale@oauife.edu.ng;*

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1. INTRODUCTION

Plantain is a green boat shaped fruit when freshly harvested and gradually turns yellow as the fruit ripens. To a large extent, the shade of colour depends essentially on the stage of ripening. The same goes with its close relation-banana which is smaller in size and has a thinner skin. Plantain is usually cooked before eaten whereas banana is not, rather it can be eaten once ripened or used in dessert [1,2]. Plantain can be eaten, boiled, roasted, grilled or fried. Unripe plantain and banana are traditionally processed into flour especially in the South Western Nigeria probably for reasons of preservation. Banana is also similarly treated. Plantain is reputed to have high dietary fibres, very low in fat and cholesterol, very rich in potassium, magnesium and phosphate. It contains complex carbohydrate that is slowly hydrolysed into soluble sugars when consumed. It also has more starch than banana [3] Hence plantain in unripe form, cooked or processed into flour is good for people who need to carefully control their plasma sugar concentration and for people taking diuretics, because of its high potassium content. It also has many other health benefits [4,5]. On the other hand, banana fruits are also as rich as plantain in micronutrients and other nutritionally important molecules and contain 22.8% carbohydrates, which is lower than that of plantain [6]. The starch in banana is however not slow-digesting. Thus, it is quickly converted to glucose and released for energy generation, when consumed.

There is the renewed interest in the consumption of plantain especially among the diabetics because of the awareness that its starch is slowly broken down [3]. Flours derived from banana or plantain are not visually distinguishable, and as such, there is always the tendency to erroneously accept that products derived from plantain or banana, are nutritionally identical and can substitute for one another. Furthermore, there is scanty information on the relationship between amylase activity and its reducing sugar products and soluble proteins in these fruits, especially from the perspectives of consumers with some peculiar health challenges.

We have therefore decided to provide basic information on the flux of these molecules at different stages after harvest to ripening.

2. MATERIALS AND METHODS

2.1 Materials

Bunches of banana and plantain fruits, straight from harvest, were usually purchased from local markets within Ile-Ife environ, Osun State, South-western Nigeria.

2.2 Chemicals/ Reagents

All reagents were of analytical grade and were purchased from Sigma Chemical Company, St Louis, MO, USA or BDH Chemicals Limited, Poole, England.

2.3 Methods

2.3.1 Sample preparation/ Extraction of soluble sugars and proteins

A bunch of the banana or plantain fruits was divided into 7 groups and kept in the dark at room temperature of about 25ºC. Each group contained 1 or 2 fingers of plantain or 3-5 fingers of banana. On the first day, homogenate of the pulp was prepared and on the 3rd, 5th and 7th day, homogenates of the other groups were similarly prepared.

2.3.2 Pulp homogenate preparation

Fingers of plantain or banana fruit were peeled and cut into pieces. About 30 g of this was weighed into 70 ml of 10 mM sodium phosphate buffer pH 6 containing 1 mM EDTA and homogenized for 1 min. The resulting homogenate was centrifuged for 30 min at 4000 rpm to obtain a clear supernatant.

2.3.3 Amylase and total soluble sugar measurement

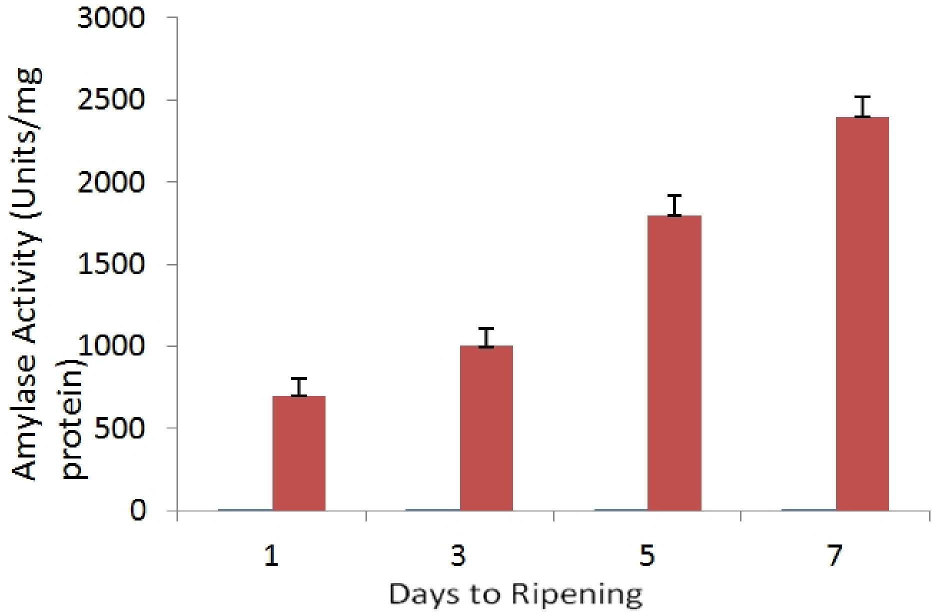
The amount of reducing sugars released from potato starch by the crude amylase in the extract was quantified using the dinitrosalicylic acid (DNSA) method [7]. Briefly 0.2 and 0.5 ml aliquots of boiled and unboiled extracts were incubated with 1% soluble starch in phosphate buffer pH 6. The volumes were made equal with water and incubated at room temperature for 5 min. Colour was developed by boiling for 5 min after the addition of DNSA solution and absorbance was read at 470 nm. Reducing sugar released was calculated taking into cognizance the amount of reducing sugar that was originally present in the extract, which was subtracted from the total sugar estimated from the calibration curve. In a similar manner, the amount of reducing end in the extract was determined. The amount of sugar present was similarly read-off from the standard curve. A unit of amylase activity was defined as the amount of enzyme that catalysed the liberation of reducing ends from potato starch equivalent to 1µg of D-glucose per minute.

2.3.4 Soluble protein determination

The protein concentrations of the extracts were measured following the method of Bradford [8]. Bovine serum albumin was used as the standard protein. The protein content was expressed as mg/ml of extract.

3. RESULTS AND DISCUSSION

Our observations showed that mature plantains become ripened on the 7th day after harvest in agreement with similar studies earlier carried out by others [9]. However, it takes an average of five days for banana to ripen in this study. Figs. 1a and b are a plot of the summary of the amylase profiles in plantain and banana extracts from the day of harvest to the ripening stage.



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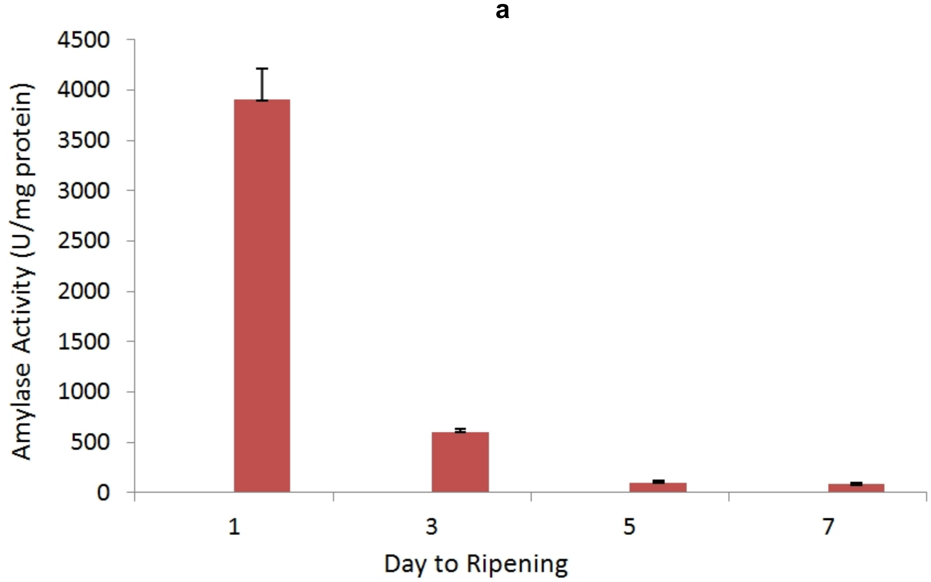

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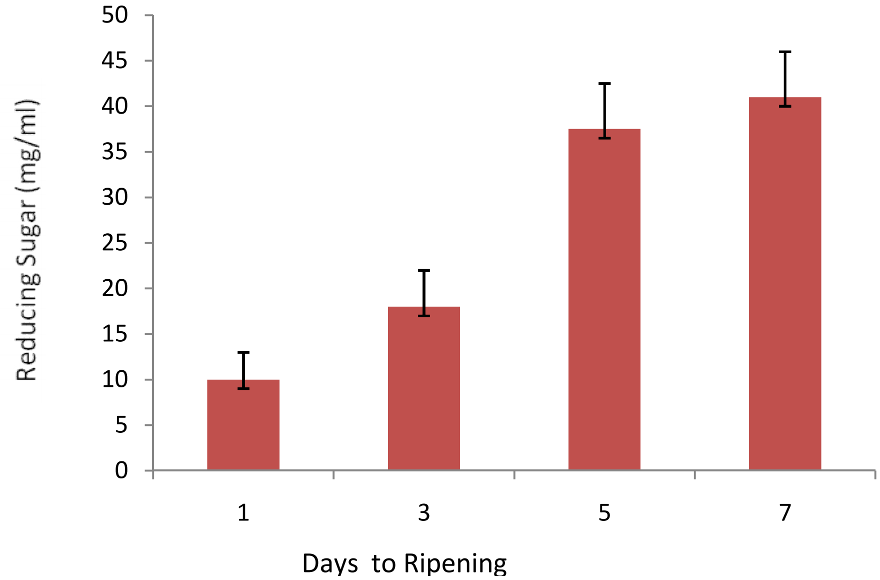
Fig. 1. Profile of amylase from harvest to ripening stage

The specific activity of amylase in plantain (a) and banana (b) in the 30% homogenate of the pulp extracts from harvest to ripening stage was calculated as enzyme units/ mg protein. The amylase activity was measured by incubating the extracts with 1% potato starch, together with the appropriate controls. The reducing ends released were quantified using DNSA method. The values are an average ± SEM of four independent determinations.

For plantain fruits, the specific activity of alpha amylase was at the lowest on the first day of harvest and rose up to the highest level on the 7th day. The action of amylase on starch results in the depolymerization of the starch with concomitant release of reducing ends, as such, the soluble sugar also increased in a similar manner and was highest on the 7th day of harvest (Fig. 2a).



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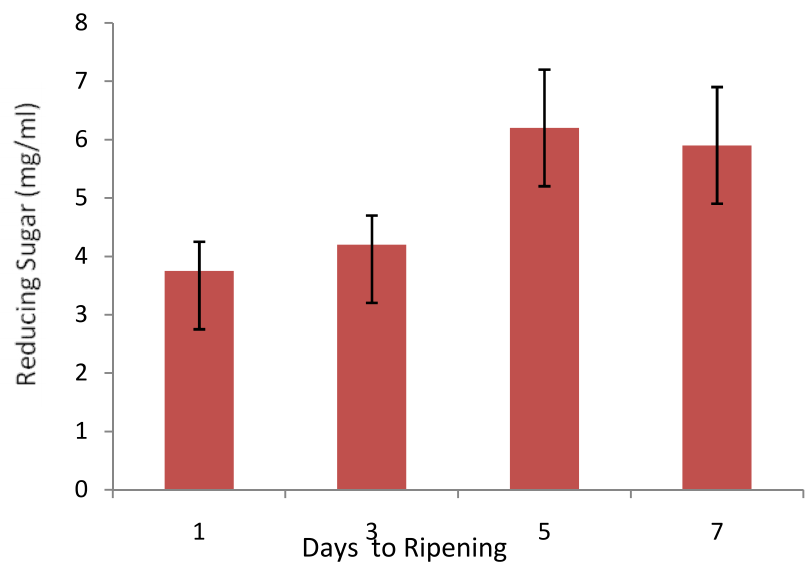

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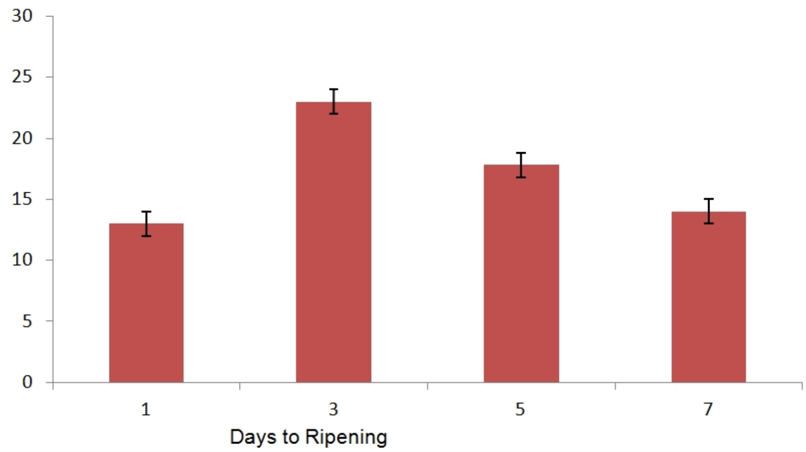
Fig. 2. Reducing sugar profile in plantain and banana pulp extracts
The reducing sugar concentration in the 30% homogenate of plantain pulp (a) and banana (b) from harvest (Day 1) to ripening (Day 7) were determined using DNSA method. Other conditions are as stated in the text. The values plotted are an average ± SEM of four independent determinations

The profile of amylase in banana was however in contrast to that of plantain. Specific activity was highest on the first day (3800 Units/mg protein; Fig. 1b) and then decreased rapidly to a very low value (100 Units/mg protein) towards ripening. The reducing sugar was between 3.8 mg/ml of extract on the first day of harvest to 5.8 mg/ml towards the ripening period (Fig. 2b), suggesting no correlation between amylase activity and the reducing sugar level in banana unlike plantain. The reason for this is not yet clear. Perhaps, some other starch degrading enzyme may be responsible for starch depolymerization in banana unlike plantain. Another possible explanation is that starch is turned into non-reducing sugar directly since most soluble sugar in banana occur in non-reducing form such as sucrose [10] The total soluble protein profiles are shown in Fig. 3a and b.



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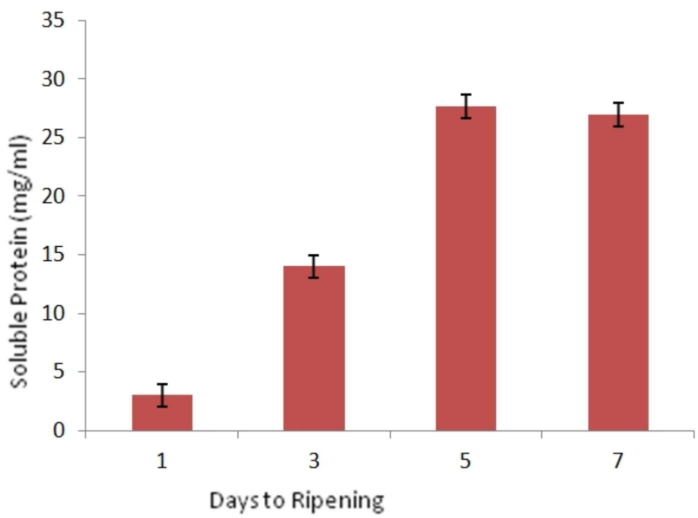

b

Fig. 3. Profile of soluble protein concentration in plantain and banana extracts
The concentration of soluble proteins in plantain (a) and banana extracts (b) from day 1 (harvest day) to the ripening stage (Day 7) were determined using Bradford method. Bovine serum albumin was used as the standard protein. The values plotted are an average ± SEM of four independent determinations.

The concentrations of soluble protein at harvest and during ripening in both the plantain and banana were similar. In plantain, the concentrations increased from the point of harvest on the first day to approximately twice the value on the third day before a gradual reduction toward ripening (Fig. 3a). In the case of banana, lowest concentration was found on the day of harvest and steadily rose to 9 times of this value on the 5th day up to the point of ripening.

Overall, the results permit us to conclude that in the case of banana, amylase only plays a significant role at the unripe stage, probably depolymerizing it to the extent that other carbohydrate hydrolyzing enzymes can take over. Since sucrose phosphate synthase and starch phosphorylase among others have been implicated in this process [3,11], we suggest that these enzymes may be involved at this stage together with invertase. This will account for relatively unvarying concentration of reducing sugars during the stages of ripening. Since there was a positive correlation between amylase activity and reducing sugar concentration in the case of plantain, we believe amylase plays a predominant role in the formation of soluble sugars and in particular reducing sugars in plantain.

Again, the soluble proteins were at the highest concentration in banana towards ripening period whereas, in plantain, protein concentrations were highest on the 3rd day and gradually declined towards ripening period almost in a linear manner. Since plantain has the lowest soluble sugar just at harvest, on the first day, in addition to its higher slowly-digesting starch concentration [3], it is better consumed or processed at this stage for those who need to control carefully their blood sugar level. The soluble sugar distribution in banana and plantain also confirm that in processed form, banana flour cannot substitute for that of plantain.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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