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Variations in Amylase and Invertase activities in *Solanum* species (Eggplants) during ripening

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KEY WORDS: Carbohydrate degrading enzymes; Amylase; Invertase; *Solanum spp*.; Eggplants; Ripening

ABSTRACT: *Solanum* species (eggplants) are edible, highly valued constituents of the Nigerian food and indigenous medicines. In this study, the activities of enzymes involved in carbohydrate degradation such as amylase and invertase were evaluated in two *Solanum* species viz *Solanum melongena* (round and oval varieties) and *Solanum aethiopicum* during different ripening stages. The activity of amylase was found to reduce significantly in both varieties of *Solanum melongena* ($p < 0.01$) while there was a non - significant reduction in amylase activity of *Solanum aethiopicum* ($p > 0.01$) from the unripe to the overripe stage. Also, invertase activity in both varieties of *Solanum melongena* was observed in this study to be significantly reduced ($p<0.01$) from the unripe to the overripe stage, with a non - significant reduction in invertase activity occurring in *Solanium aethiopicum* ($p > 0.01$). The variations observed in the activities of these carbohydrate degrading enzymes correlate with the decrease in glucose level that has been reported during ripening in *Solanum* species; thereby confirming the ripe and overripe eggplants as nutritionally good diet for diabetic patients and individuals, who are watching their weight.© JASEM

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Introduction: *Solanum* species, which are also known as eggplants are commonly consumed almost on daily basis by both rural and urban dwellers in various sub-Saharan African countries such as Nigeria. The use of eggplants, forms part of the traditional sub-Saharan African culture (Shalom *et al*., 2011). Wide variations exist within the colour, vegetative and fruit anatomy of the eggplant species, (Osei *et al*., 2010). *Solanum melongena*'s fruits are white in colour and small with two varieties which are round or oval in shape, while that of *Solanum aethiopicum* are largely oval shaped, white in colour with green stripes. The fruits of both *Solanum* species, when ripe or overripe are yellow and red in colour, respectively (Agoreyo and Nwachukwu, 2012).

Starch is the main storage form of carbohydrate in plants. Carbohydrate is transported as sucrose from photosynthetic tissues via the phloem to other tissues of the plants such as the rapidly growing tissues known as the sink tissues or starch storage organs such as fruits, where the sucrose is used to synthesize starch. Sucrose transported to the sink tissues are catabolised to produce energy, while the storage starch that is produced in the starch storage organs is broken down following specific developmental cue such as ripening in fruits (Stanley *et al.*, 2005).

One of the most important enzymes of carbohydrate degradation in plants is amylase, which rapidly degrades starch into soluble substrates such as maltose and glucose for other enzymes to metabolise (Richard, 2002; Rejzek *et al.*, 2011). Maltose and glucose can be converted to glucose-6-phosphate which can be metabolized by the glycolytic pathway. Also, sucrose utilization as a source of energy in plants depends on its cleavage into glucose and fructose by the carbohydrate degrading enzyme, invertase (Roisch *et al.*, 2003; Ruan *et al.*, 2010).

Evaluation of the status of amylase, one of the enzymes responsible for the initiation of starch utilization during the ripening of banana fruits (*Musa sapientum*) showed low activity at the onset of the climacteric and a concomitant enhancement that is parallel to the respiratory climacteric (Surendranathan *et al*., 2004). Changes in amylase activity have also been reported during ripening of mango fruits (*Mangifera indica*). The climacteric rise in mango fruit was reported to be marked by an appreciable increase in the activity of amylase (Lima *et al*., 2001). In *Cucumis melo* which is commonly

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called muskmelon or cantaloupe, a progressive increase in the amylase activity has been observed with the degradation of starch throughout the period of ripening in the fruit. The increase in amylase activity in muskmelon fruit was also found to correlate with the increase in various energy consuming processes such as respiration and ripening (Menon and Ramana, 2012). Enzymes capable of degrading starch have also been detected in the plastids of tomato (*Solanum lycopersicum*). In ripe tomato, the amount of starch was reported to be strongly reduced due to intense degradation. Moreover, high activity of β – amylase has been found during ripening in tomato (Bian *et al.*, 2011). Kanwal *et al*. (2004) have also correlated the amylase activity in fruits with the ripening process and rise in respiration. Furthermore, Lajalo (2001) cited that the starch contents of fruits are degraded in a complex process during ripening period involving amylase.

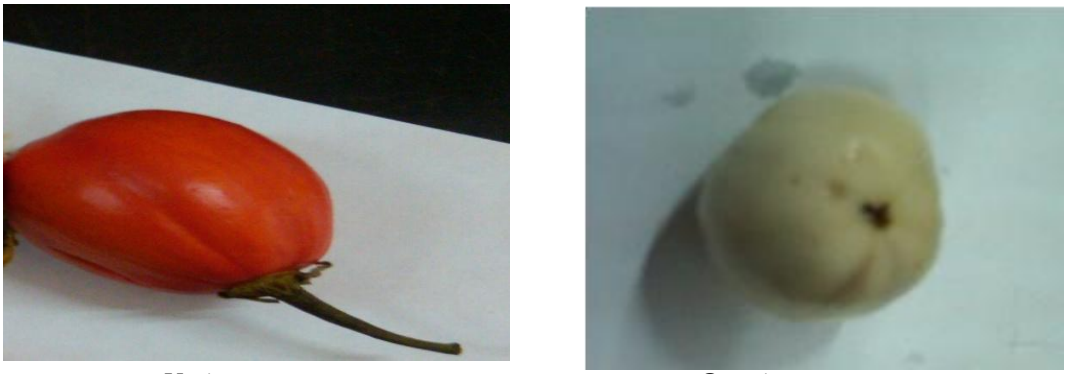
In tomato, sucrose has been reported to be hydrolysed by invertase into fructose and glucose that are required for metabolism during ripening. Enhanced invertase activity also correlated with increased invertase gene expression during tomato ripening (Yu *et al*., 2011). Increased invertase activity has also been reported in mango fruit during ripening (Rahman *et al.*, 2011). The relationship between sugar profiles and sucrose metabolizing enzymes in banana during fruit development and ripening showed that invertase was the main determinant in the regulation of sucrose level of fruit. Bananas with low levels of sucrose were reported to have an increased invertase gene expression (Fils – Lycaon *et al*., 2011). Also, maximum invertase activity was reported to occur in ripe muskmelon fruit during ripening (Menon and Ramana, 2012). Zanor *et al.* (2009) reported that invertase is a key enzyme in plant growth and development.

This present study was undertaken in *Solanum* species in order to evaluate the activities of amylase and invertase involved in the degradation of starch and sucrose, respectively during ripening. Moreover, this study was also carried out to determine if there is a correlation between the activities of amylase and invertase and the level of glucose that has been reported to decrease during ripening in *Solanum* species.

MATERIALS AND METHODS

***Plant Material*:** Fruits of *Solanum melongena* (oval and round varieties) and that of *Solanum aethiopicum* were purchased from a local market in Benin City. Analyses were carried out on the different ripening stages of the fruits (unripe, ripe and overripe). The change in fruit colour from white or white with green stripes (unripe stage) to yellow (ripe stage) and red (overripe stage) was used to determine the ripening stages.

Solanum melongena (oval variety)



Unripe stage Overripe stage

Solanum melongena (round variety)

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Unripe stage Overripe stage

Solanum aethiopicum

Fig. 1: Fruits of *Solanum melongena* (oval and round varieties) and *Solanum aethiopicum* that were used in this study.

Enzyme Extraction For Amylase: Amylase was extracted by the method of Davies and Ross (1987). Ten grammes of each *Solanum* species (eggplant) were homogenized by grinding with a chilled mortar, pestle and acid washed sand in 10ml of 0.1M citrate phosphate buffer, pH 6.0 containing 20 mM calcium chloride which stabilized the enzyme. The homogenate was filtered through two layers of cheese cloth to remove cell debris. The filtrate was centrifuged at 10,000 g for 15 min. The supernatant served as the crude extract for the enzyme assay. The supernatant was used for total amylase assay, it served as a source of alpha and beta amylase.

Enzyme Assay For Amylase: Total amylase activity was determined by the method of Davies and Ross (1987) with some modifications. 3 ml of assay mixture containing 5mg/ml of starch, 0.1 M sodium fluoride, 0.2M sodium acetate buffer (pH 4.8) were added to a test tube. The reaction was started by the addition of 1 ml of the crude enzyme extract to the test tube. The tube was incubated at room temperature (30^0C) for 15 min, after which the reaction was stopped by the addition of 2ml of 2M sodium hydroxide to the test tube. The reaction was carried out in triplicate and the control did not contain any crude enzyme. The reducing sugar liberated was measured by the method of Nelson (1944), as modified by Somogyi (1952). 1 ml of Nelson - Somogyi R4 reagent was added to 0.5ml of the reaction mixture and boiled for 20 minutes. It was then cooled, after which 1 ml of R3 solution was added. It was shaken vigorously to remove carbon dioxide and allowed to stand for 10 min. 10ml of water were added and the absorbance was read at 600nm. The release of reducing sugars in each reaction mixture was quantified using a glucose calibration curve. Amylase activity was expressed as µmole glucose equivalent released $min^{-1}g^{-1}$ fresh weight.

*Enzyme Extraction For Invertase***:** Invertase was extracted using the method of Passam and Barret (1977). Ten grammes of each *Solanum* species (eggplant) were homogenized by grinding with a chilled mortar, pestle and acid washed sand in 10ml of 10 mM sodium phosphate buffer pH 7.0 containing 1% PVP (polyvinyl pyrrolidone) to mop up the polyphenols and 0.5 mM DTT (dithiothreitol) which protects the sulphurhydryl group. The homogenate was filtered through two layers of cheese cloth, to remove cell debris. The filtrate was centrifuged at 15000 g for 15 min. The supernatant served as the crude extract for the enzyme assay. The supernatant was used for total invertase assay.

*Enzyme Assay For Invertase***:** Total Invertase activity was determined by the method of Passam and Barret (1977) with some modifications. 2 ml of a standard sucrose solution (10%) in 50 mM sodium acetate buffer (pH 5.0) were added to a test tube. The reaction was started by the addition of 1 ml of the crude enzyme extract to the test tube. The test tube was incubated for 3 hours, after which the reaction was stopped by the addition of 1ml of 2 M sodium hydroxide to the test tube. The reaction was carried out in triplicate and the control did not contain any crude enzyme. The release of reducing sugars in each reaction mixture was then measured by the method of Nelson (1944), as modified by Somogyi (1952). The

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release of reducing sugars in each reaction mixture was quantified using a glucose calibration curve. Invertase activity was expressed as µmole glucose equivalent released $min^{-1}g^{-1}$ fresh weight.

*Statistical Analysis***:** Analysis of variance (ANOVA) was evaluated by the statistical and presentational system software (SPSS). Tukey- Kramer multiple comparison test was employed to determine the statistical differences among the means.

RESULTS

The fruits of *Solanum melongena* (oval and round varieties) and *Solanum aethiopicum* are shown in Fig. 1. Significant decrease ($p < 0.01$) was observed in amylase activity in *Solanum melongena* (round variety) during ripening. The amylase activity decreased by 7.1% from the unripe to the ripe stage, while it decreased by 65.2% from the unripe to the overripe stage (Fig. 2a). The invertase activity of *Solanum melongena* (round variety) decreased significantly ($p < 0.01$) during ripening. Although, there was an initial increase of 7.8% from the unripe to the ripe stage, which later decreased by 43.6% from the ripe to the overripe stage (Fig. 2b).

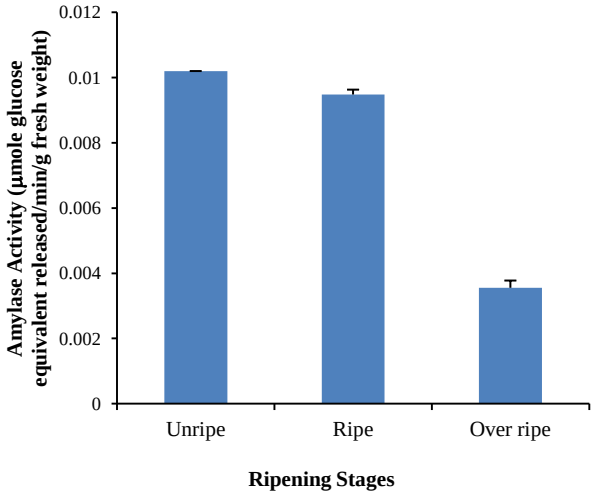

Fig. 2a: Amylase activity of *Solanum melongena* (round variety) at various ripening stages

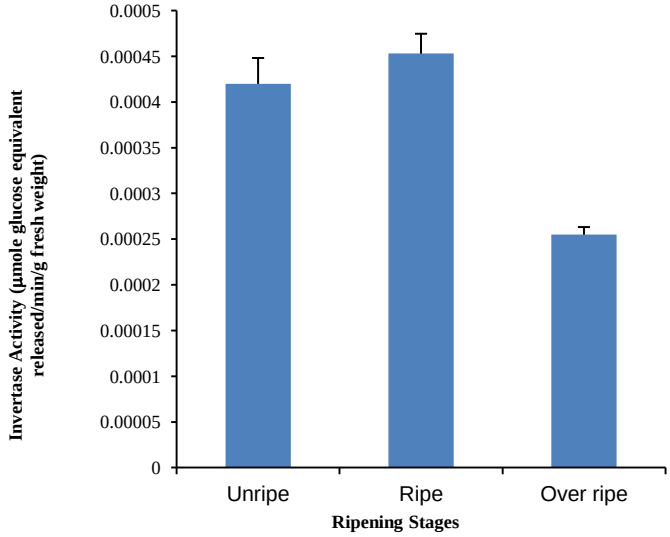

Fig 2b: Invertase activity of *Solanum melongena* (round variety) at various ripening stages

Amylase activity of *Solanum melongena* (oval variety) also decreased significantly ($p < 0.01$) during ripening. The amylase activity decreased by 50% from the unripe to the ripe stage, while, the activity decreased by 57.9% from the unripe to the overripe stage (Fig. 3a). Invertase activity in *Solanum melongena* (oval variety) decreased significantly ($p < 0.01$) by 36.7% from the unripe to the ripe stage and by 40% from the unripe to the overripe stage (Fig. 3b).

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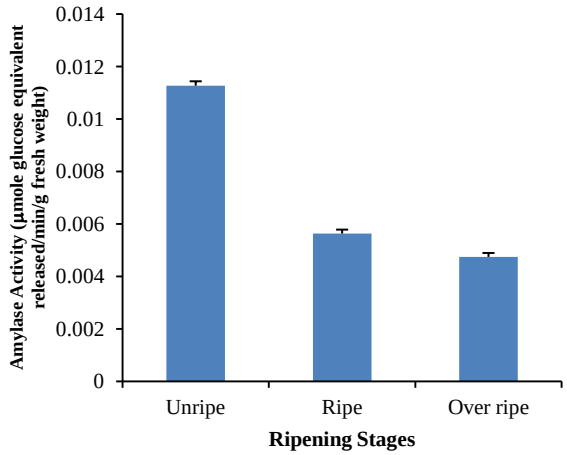

Fig 3a: Amylase activity of *Solanum melongena* (oval variety) at various ripening stages

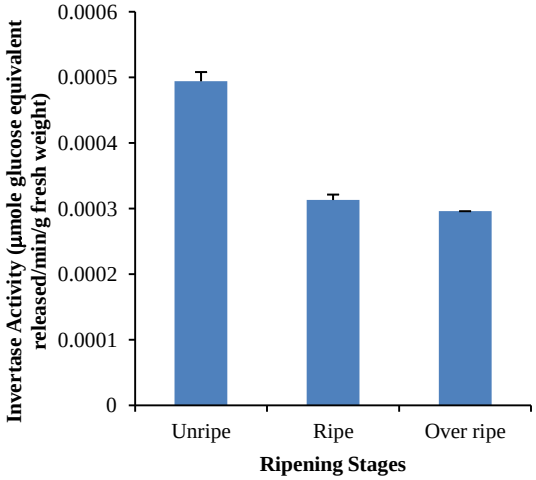

Fig 3b: Invertase activity of *Solanummelongena* (oval variety) at various ripening stages

Non - significant decrease ($p>0.01$) was observed in the amylase activity of *Solanum aethiopicum* during ripening. The decrease in activity was 2.5% from the unripe to the ripe stage and 15.4% from the unripe to the overripe stage (Fig. 4a). The invertase activity of *Solanum aethiopicum* was also found to decrease non - significantly ($p>0.01$) during ripening; however, there was 21.9% increase in activity from the unripe to the ripe stage, which later decreased by 26.1% from the ripe to the overripe stage (Fig 4b).

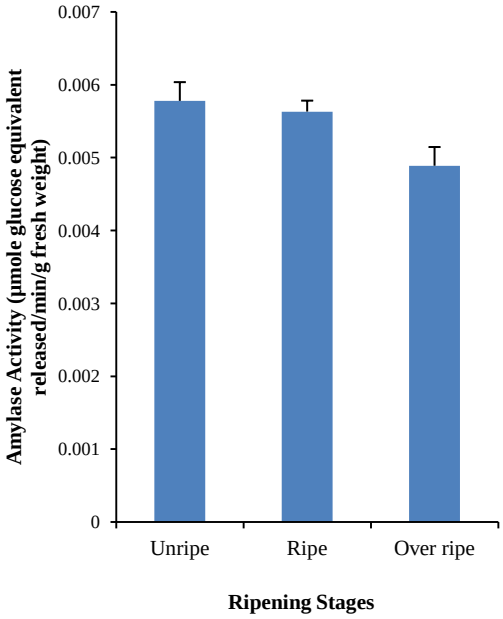

Fig 4a: Amylase activity of *Solanum aethiopicum* at various ripening stages

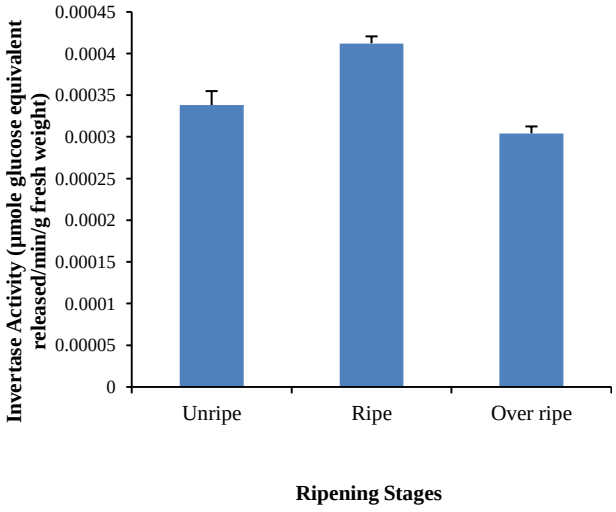

Fig 4b: Invertase activity of *Solanum aethiopicum* at various ripening stages

DISCUSSION

Fruits constitute a nutritionally indispensable component of human diet. They play a vital role in human nutrition by supplying dietary fibre, minerals, vitamins and antioxidants such as carotene that are essential for health (Prasanna *et al*., 2007).

Fruit ripening is a highly coordinated phenomenon involving a series of physiological and biochemical changes such as colour change, increased respiration and hydrolysis of polysaccharides, especially starch (Agoreyo *et al*., 2012). Ripening in most fruits is associated with increased sugar production and sweetness, which is as a result of the hydrolysis of starch and sucrose by the enzymes amylase and invertase (Osamu, 2000).

In this study, the activities of amylase and invertase were evaluated in various *Solanum* species during ripening and variations in the activities of these enzymes were observed. In *Solanum melongena* (round variety), amylase activity decreased from 0.01020 ± 0.00001 to 0.00355 ± 0.00022 µmole glucose equivalent released min^{-1} g^{-1} fresh weight, from the unripe to the overripe stage (Figs 2a). Invertase activity in the same variety decreased from 0.00042 ± 0.00003 to 0.00026 ± 0.00001 µmole glucose equivalent released min^{-1} g^{-1} fresh weight, from the unripe to the overripe stage (Figs 2b). Activity of amylase in *Solanum melongena* (oval variety), decreased from 0.01127 ± 0.00017 to 0.00474 ± 0.00015 µmole glucose equivalent released min^{-1} g^{-1} fresh weight, from the unripe to the overripe stage (Figs 3a); whereas invertase activity in the same variety decreased from 0.00049 ± 0.00001 to 0.00030 ± 0.00000 µmole glucose equivalent released min^{-1} g^{-1} fresh weight, from the unripe to the overripe stage (Figs 3b).The amylase activity in *Solanum aethiopicum* decreased from 0.00578 ± 0.00026 to 0.00489 ± 0.00026 µmole glucose equivalent released min^{-1} g^{-1} fresh weight, from the unripe to the overripe stage (Figs 4a), invertase activity also decreased from 0.00034 ± 0.00002 to 0.00030 ± 0.00001 µmole glucose equivalent released min^{-1} g^{-1} fresh weight in the unripe to the overripe stage (Figs 4b). Decrease was observed in the activities of both amylase and invertase in all the *Solanun* species from the unripe to the overripe stage in this study (Figs. 1a to 3b). The pattern of variations in the activities of these enzymes does not correlate with the concomitant enhancement of amylase and invertase activities that have been reported in banana, mango, tomato and muskmelon fruit during ripening (Surendranathan *et al*., 2004; Lima *et al*., 2001; Yu *et al*., 2011; Menon and Ramana, 2012). On the other hand the decrease in the activities of these enzymes correlates with the decrease in the glucose level that has been reported in various *Solanum* species during ripening (Agoreyo and Oghene, 2011). Also, Lima *et al*., (2001) had reported that during ripening in mango fruit, storage starch was hydrolysed to sugar; resulting in an increase in both amylase activity and reducing sugar content. As the mango fruit became overripe traces of starch were detected, which led to a substantial reduction in both amylase activity and reducing sugar content.

Invertase activity decreased throughout the ripening period in *Solanum melongena* (oval variety) but increased non-significantly only in the ripe stage of *Solanum melongena* (round variety) and Solanum *aethiopicum.* Since the glucose level of these three *Solanum* species have been reported to decrease in their ripe stage (Agoreyo and Oghene, 2011), then the glucose produced by the non – significant increase in invertase, apart from being insignificant; could also have been employed for respiration and for the synthesis of carotene that gives ripening *Solanum species* that characteristic yellow and red colours (Beyer *et al*., 2002; Abu and Ukwuannah, 2005). *Solanum melongena* (oval variety) that showed decreased invertase activity throughout the period of ripening had also been shown to have the lowest percentage of glucose in the ripe stage compared to *Solanum melongena* (round variety) and Solanum *aethiopicum* that showed non-significant increase in the ripe stage (Agoreyo and Oghene, 2011).

The result of this study suggests that compositional changes in fruits such as the conversion of starch or sucrose to sugars that results in an increase in sugars especially glucose during ripening, is not very significant in *Solanum* species (eggplants). *Solanum* species, especially in their ripe and overripe stages are therefore useful to individuals that are diabetic and those that are watching their weight. Moreover, *Solanum* species are mostly consumed in their unripe stage and are commonly discarded when they are ripe, especially when overripe; resulting in their being wasted. Therefore, their consumption when ripe or overripe will lead to their effective utilization and also prevent them from being wasted.

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