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Biochemical Investigation on the activities of Acid and Alkaline Phosphatases in two varieties of *Carica papaya* L. (Pawpaw) during ripening

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KEY *WORDS*: Ripening; Phosphatases; *Carica papaya*; Agric pawpaw; Local pawpaw

ABSTRACT: The activities of acid phosphatase and alkaline phosphatase were investigated in two varieties of ripening *Carica papaya* fruit; Oblong-shaped variety which is also known as 'Agric pawpaw' and Pear-shaped variety which is also known as 'Local pawpaw'. Acid phosphatase activity decreased significantly ($p < 0.01$) throughout the ripening stages from 0.0186±0.0006 to 0.0037 ± 0.0002µmol/min/g fresh weight in the Oblong-shaped variety: 'Agric pawpaw' and from 0.0134 ± 0.0008 to 0.0068 ± 0.0004µmol/min/g fresh weight in the Pear-shaped variety: 'Local pawpaw'. Conversely, alkaline phosphatase activity was found to increase significantly ($p < 0.01$) throughout the ripening stages from 0.0044 ± 0.0006 to 0.0135 ± 0.0006µmol/min/g fresh weight in the Oblong-shaped variety: 'Agric pawpaw' and from 0.0074 ± 0.0012 to 0.033 ± 0.0023µmol/min/g fresh weight in the Pear-shaped variety: 'Local pawpaw'. These results suggest that acid phosphatase is the main non-specific phosphatase that is responsible for the production and supply of inorganic phosphate in the unripe stage of *Carica papaya* fruits, while alkaline phosphatase is responsible for the production and supply of inorganic phosphate in the overripe stage. Acid phosphatase and alkaline phosphatase could be obtained from the unripe and overripe stages of *Carica papaya* fruits respectively for research purposes, for commercial exploitation and industrial gains.

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Introduction: Carica papaya, which is also known as papaya or pawpaw belongs to the family Caricaceae. It is cultivated in most subtropical and tropical countries. *C. papaya* is an economically important fruit tree, whose fruit is consumed worldwide in the fresh state or used as processed products. (Jaime *et al*., 2007). Ripe fresh *C. papaya* fruits are commonly eaten for breakfast and dessert. They are also used in making fruit salads, fresh drinks, ice cream, jelly, jam, marmalade, canned fruit balls or cubes in syrup, crystallized fruit, candies, and paste. Unripe, green fruits are usually pickled or used as vegetables (Geetha and Thirumaran, 2010). The unripe fruit is also a source of papain, a proteolytic enzyme that hydrolyses protein and as such it is used in the preparation of food as meat tenderizer and is of industrial importance. Papain is also used in medications for the treatment of hard tissues on the skin (Hewitt *et al*., 2000; Chovatia *et al*., 2010; Seenivasan *et al*., 2010; Da Silva *et al*., 2010). Although the fruit of *C. papaya* is edible, the fruit and other parts of the plant are also being used throughout Africa for their medicinal properties (Afolabi *et al*., 2011). The fruit has been reported to have antihelminthic activity (Okeniyi *et al*., 2007).

The fruit of *C. papaya* has different shapes; it may be ovoid-oblong, spherical, cylindrical, and pear-shaped or may have grooves (Zhou *et al*., 2004; Chen *et al*., 2007). *C. papaya* fruit is a climacteric fruit and it exhibits a characteristic rise in ethylene production during ripening; which is accompanied by fruit softening, change in colour, the development of a strong aroma and production of reducing sugar from polysaccharides. Genes that are associated with the development of fruit aroma during ripening have been identified in *C. papaya*. (Devitt *et al*., 2006).

Acid phosphatase (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1) are non - specific phosphatases that are grouped as acid or alkaline, based on their optimum pH below or above pH 7. They are enzymes that catalyse the hydrolysis of phosphate esters to produce inorganic phosphate and their main function in plants is to supply the inorganic phosphate that is required for the

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maintenance of cellular metabolism (Tabaldi *et al*., 2007; Mishra *et al*., 2008). Alkaline phosphatase is also employed in molecular biology for the construction of recombinant plasmids because of their ability to cleave 5′ terminal phosphate from DNA fragments (Vemuri *et al*., 2010).

Inorganic phosphate is an important structural constituent of many biomolecules and phosphorylated intermediate of energy metabolism and it is of critical importance to plant's physiological and biochemical processes (Vance *et al*., 2003; Vance *et al*., 2010). Acid phosphatase has been reported to play crucial role in the physiological and biochemical processes that occur in ripening fruits of Musa paradisiaca L. (Agoreyo, 2010). This study was therefore carried out to investigate the activities of acid and alkaline phosphatases in two varieties of *C. papaya* during ripening and to also determine if the fruits could be processed for their enzymes; so that acid and alkaline phosphatases in these fruits could be used for research purposes, for commercial exploitation and industrial gains.

MATERIALS AND METHODS

Plant Material: Two varieties of *C. papaya* fruits that are oblong and pear shaped and are also known as 'Agric pawpaw' and 'Local pawpaw' respectively, were purchased in their unripe green state from a local Market in Benin City. These fruits were allowed to ripen normally, while samples were collected from them in the unripe, ripe and overripe stages for the analyses of acid and alkaline phosphatases.

Oblong- shaped variety: 'Agric pawpaw' Pear- shaped variety: 'Local pawpaw'

Figure 1: The two varieties of *Carica papaya* fruits that were used in this study

Enzyme extraction for non-specific acid phosphatase (EC 3.1.3.2): 5 g of the fruit of each *C. papaya* variety were ground in a chilled mortar, with acid washed sand and 20 ml of chilled 50 mM Tris - HCL buffer (pH 7.6) containing 1 mM EDTA. The homogenate was filtered through double layers of cheesecloth and centrifuged at 20,000 g for 20 minutes. The supernatant was used as the crude extract for the enzyme assay (Murray, 1980).

Enzyme Assay For Non-Specific Acid Phosphatase (EC 3.1.3.2): Acid phosphatase activity was assayed by adding 100 µl (0.1 ml) of the enzyme extract to 1ml of 3 mM α-Naphthylphosphate in 60mM Sodium Citrate (pH 5.3). The reaction mixture was incubated at 37° C for 5 minutes, after which absorbance was read at 405nm every minute for another 5 min in order to determine the ΔA/minute (change in absorbance per minute). The assay was performed in triplicate and acid phosphatase activity expressed as µmol α-Naphthol released $min^{-1}g^{-1}$ fresh weight.

Enzyme Extraction For Non-Specific Alkaline Phosphatase (EC 3.1.3.1): 5 g of the fruit of each *C. papaya* variety were ground in a chilled mortar, with acid-washed sand and 20 ml of chilled 0.05 M sodium carbonate buffer (pH 10). The homogenate was filtered through double layers of cheesecloth and centrifuged at 20,000 g for 20 minutes. The supernatant was used as the crude extract for the enzyme assay (Agoreyo, 2010).

Enzyme Assay For Non-Specific Alkaline Phosphatase (EC 3.1.3.1): Alkaline phosphatase activity was assayed by adding 0.5 ml of the enzyme extract to 0.5 ml of 3.6 mM Sodium thymolphthalein

monophophate in 0.2 M 2-Amino-2-methyl-1-propanol buffer (pH 10.2) containing 1 mM magnesium chloride. The reaction mixture was then incubated at 37° C for 10 minutes. The reaction was terminated by the addition of 2.5 ml of 0.1 M sodium hydroxide containing 0.1 M sodium carbonate. The absorbance was read at 590 nm and the amount of sodium thymolphthalein released was estimated. The assay was performed in triplicate and the alkaline phosphatase activity expressed as µmol sodium thymolphthalein 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

Figure 1 shows the two varieties of *C. papaya* that were used in this study. The acid phosphatase activity of *C. papaya* fruit (Oblong-shaped variety: 'Agric pawpaw') showed significant decrease ($p < 0.01$) during ripening. The activity decreased by 38.98 % (1.6 fold) from the unripe to the ripe stage, while it decreased by 80.47 % (5.1 fold) from the unripe to the overripe stage (Fig. 2a). On the other hand, the alkaline phosphatase activity increased, but not significantly ($p > 0.01$) from the unripe stage to the ripe stage by 43.97 % (1.8 fold) during ripening. The alkaline phosphatase activity however increased significantly ($p < 0.01$) from the unripe to the overripe stage by 67.68 % (3.1 fold) from the unripe to the overripe (Fig. 2b).

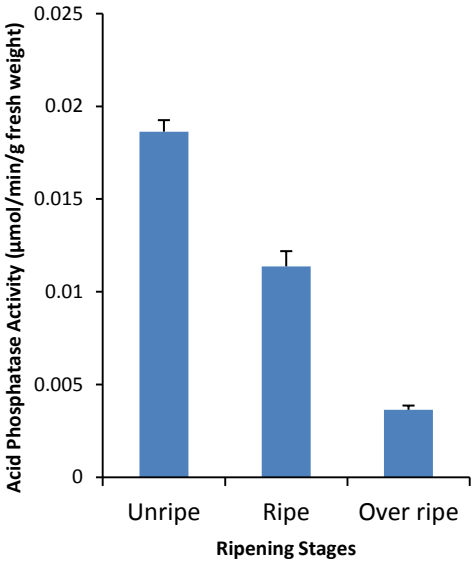

Fig 2a: Acid phosphatase activity of *Carica papaya* fruit (Oblong- shaped variety: 'Agric pawpaw') at various ripening stages

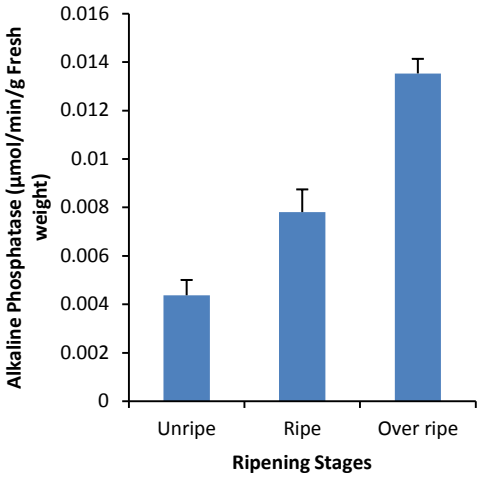

Fig Fig 2b: Alkaline phosphatase activity of *Carica papaya* fruit (Oblong- shaped variety: 'Agric pawpaw') at various ripening stages

Acid phosphatase activity of *Carica papaya* fruit (Pear-shaped variety: 'Local pawpaw') was also found to decrease significantly ($p < 0.01$) during ripening. The decrease in activity was 28.83 % (1.4 fold) from the unripe to the ripe stage, while the activity decreased by 49.16 % (2.0 fold) from the unripe to the overripe stage (Fig. 3a). However, alkaline phosphatase activity increased but not significantly ($p > 0.01$) from the unripe to ripe stage by 42.05 % (1.7 fold). The alkaline phosphatase activity increased significantly by 61.26 % (2.6 fold) from the unripe to the overripe stage (Fig. 3b).

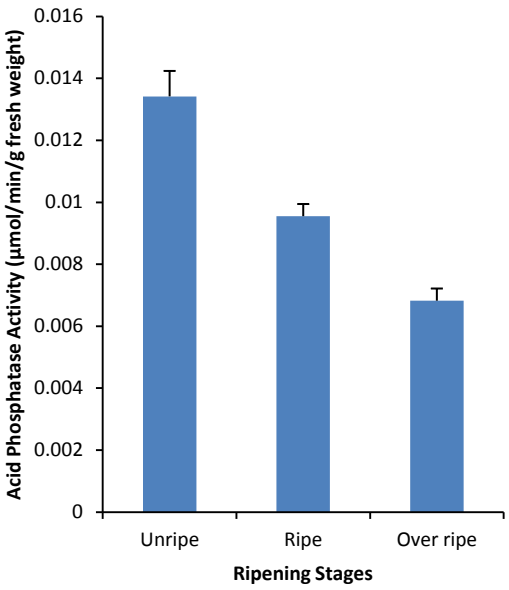

Fig 3a: Acid phosphatase activity of *Carica papaya* fruit *(*Pear-shaped variety: 'Local pawpaw') at various ripening stages

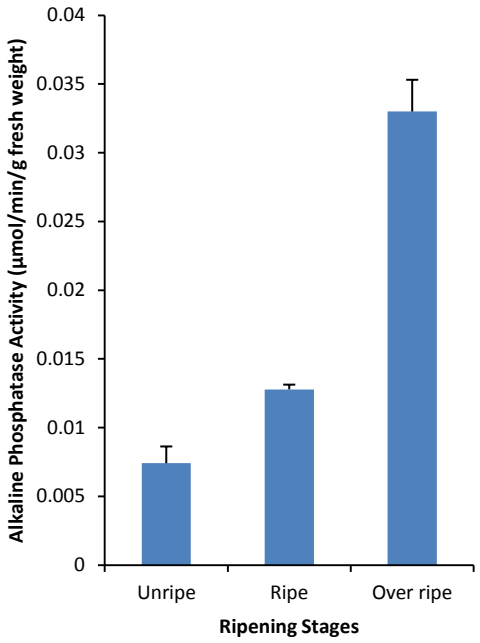

Fig Fig 3b: Alkaline phosphatase activity of *Carica papaya* fruitfruit (Pear-shaped variety: 'Local pawpaw') at various ripening stages

DISCUSSION

The activity of acid phosphatase was found to decrease significantly ($p < 0.01$) from the unripe to the overripe stage in the two varieties of *C. papaya* that were used in this study. The acid phosphatase activity was highest in the unripe stage of both varieties (0.0186 ± 0.0006 µmol/min/g fresh weight for Oblong-shaped variety: 'Agric pawpaw' and 0.0134 ± 0.0008 µmol/min/g fresh weight for Pear-shaped variety: 'Local pawpaw') (Figs 2a & 3a). The alkaline phosphatase activity however, increased significantly ($p < 0.01$) from the unripe to the overripe stage in two varieties of *C. papaya*. The activity of alkaline phosphatase was highest in the overripe stage in both varieties ((0.0135 ± 0.0006 µmol/min/g fresh weight for Oblong-shaped variety: 'Agric pawpaw' and 0.0330 ± 0.0023 µmol/min/g fresh weight for Pear-shaped variety: 'Local pawpaw') (Figs 2b & 3b). The findings of Luthfunnesa *et al.* (2006), in which they observed an increase in the pH of *C. papaya* fruit during ripening corroborates with the increase in activity that was observed in alkaline phosphatase and the decrease in activity of acid phoshatase in this study. Agoreyo (2010) also reported that since the activities of acid and alkaline phosphatases are affected by pH; then the non specific phosphatase that is most functional at a particular stage of the developmental process of a plant, is determined by the plant's physiological pH at that period. Increase in acid phosphatase activity and decrease in alkaline phosphatase activity however have been reported in plantain fruits (*Musa paradisiaca* L.) during ripening , which is due to the increase in acidity of the fruit that results in a low pH. This low pH favours the optimal activity of acid phosphatase in plantain, during ripening (Agoreyo, 2010). Acid phosphatase has also been reported to function in the maintenance of inorganic phosphate mobility during banana fruit ripening (Turner & Plaxton, 2001)); while alkaline phosphatase has been reported to be involved in the breakdown and mobilization of starch and sucrose for the biosynthesis of essential oil in lemongrass (Ganjewala *et al*., 2010).

Comparison of the activities of acid and alkaline phosphatases in the two varieties of *C. papaya* fruits that were used in this study, showed that acid phosphatase was higher in the Oblong-shaped variety: 'Agric pawpaw' than that of Pear-shaped variety: 'Local pawpaw' in all the ripening stages except in the overripe stage (Figs 2a & 3a). Whereas, the activity of alkaline phosphatase was higher in the Pear-shaped variety: 'Local pawpaw' than that of Oblong-shaped variety: 'Agric pawpaw' in all the ripening stages in this study

(Figs 2b & 3b). The fast perishability of *C. Papaya* fruits and lack of storage facilities in some countries where they are grown, result in a lot of wastage. This wastage could be prevented by obtaining acid phoshatase from the unripe Oblong-shaped variety: 'Agric pawpaw' and alkaline phosphatise from the overripe Pear-shaped variety: 'Local pawpaw' for research purposes, for commercial exploitation and industrial gains. Ripening in fruits is associated with respiration, in which sugar phosphates are used. In climacteric fruits such as C. Papaya the rate of respiration is high and the production of the sugar phosphates that are used for respiration requires inorganic phosphate that is produced by acid and alkaline phosphatases from phosphate esters. (Taiz and Zeiger, 2002).

These results suggest that acid phosphatase is the main non-specific phosphatase that is responsible for the production and supply of inorganic phosphate in the unripe stage of *Carica papaya* fruits, while alkaline phosphatase is responsible for the production and supply of inorganic phosphate in the overripe stage.

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