POLYPHENOLOXIDASE AND PERIOXIDASE ACTIVITY DURING OPEN AIR RIPENING STORAGE OF PINEAPPLE (*ANANAS COMOSUS* L.), MANGO (*MANGIFERA INDICA*) AND PAPAYA (*CARICA PAPAYA*) FRUITS GROWN IN DAR ES SALAAM, TANZANIA

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ABSTRACT

*Polyphenoloxidase (PPO) and perioxidase (POD), the enzymes responsible for causing browning and change in texture and flavor of fruits and vegetables, were extracted and measured in harvested mature pineapple (*Ananas comosus L.*), mango (*Mangifera indica*) (Viringe and Dodo varieties) and papaya (*Carica papaya*) fruits during off vine, open air, room temperature ripening storage. The initial (at harvest) average PPO activity values in Δ Optical Density (OD) per minute per cm^3 of enzyme solution were 0.00074, 0.00083 and 0.0010 for early, mid and late season pineapple fruits respectively. The initial average PPO activity values in ΔOD/min/ cm^3 of enzyme solution were 0.00152, 0.00121 and 0.0010 for early, mid and late season 'Viringe' mango fruits, respectively and 0.0054, 0.0041 and 0.0024 for early, mid and late season 'Dodo' mangoes. For papaya fruits, early, mid and late season fruits had initial average PPO activities of 0.00252, 0.00143 and 0.00085 Δ OD/min/cm^3, respectively. The PPO activity decreased continuously during the open air ripening storage of all the fruits while the POD activity increased during ripening storage. Variations in PPO and POD enzyme activity were observed across the season and during the ripening period.*

Key words: Polyphenoloxidase, perioxidase, mango, papaya, pineapple

INTRODUCTION

Quality factors such as colour, flavour and texture in fruits are controlled by enzymes. Any adverse changes whether chemical or physical in the fruit caused by activation of the appropriate enzyme will normally lower quality and/or nutritional value of the fruit (deMan 1990). Polyphenoloxidase (PPO; EC.1.10.3.1) and perioxidase (POD; EC 1.11.1.7) are related to discolouration (browning) and changes in texture and flavour in fruits (Vamos-Vigyazo 1981).

Enzymatic browning occurs in fruits and vegetables after bruising, cutting or during storage. This results from oxidation of phenolic compounds by PPO (Macheix *et al.* 1990). The PPO is a copper-containing enzyme which can undergo reversible oxidation and reduction in the process of hydroxylation and oxidation (Mayer and Harel 1979). In hydroxylation Cu^{+} is oxidized to Cu^{2+} and in oxidation (Mayer 1987) Cu^{2+} is reduced to Cu^{+}. This enzyme catalyses two reactions, the hydroxylation of monophenols to the corresponding orthodihydroxy compounds called cresolase activity and the oxidation of ortho-dihydroxy phenols to orthoquinones called catecholase activity (Macheix *et al.* 1990).

Orthoquinones are highly reactive and readily polymerise after their spontaneous conversion to hydroxyquinones – hence the dark coloured pigments. The reactions require molecular oxygen (Richard-Forget *et al*. 1992, Rouet-mayer *et al*. 1993). With few exceptions this reaction is undesirable due to development of unpleasant colours and flavours and a loss of nutrients (Goupy

et al. 1995). The substrates for the PPO enzymes are phenolic compounds present in plant tissues, mainly flavonoids. Specific examples of PPO substrates are chlorogenic acid, caffeic acid, dicatechol, protocatechinic acid, tyrosine, catechol, dihydroxypheylalanine, pyrogallol and catechins (deMan 1990).

The products of PPO action, particularly the quinones, are very important as antifungal agents, reducing the ability of fungi to penetrate tissues that have suffered minor physical damage (Vamos-Vigyazo 1981). The relative resistance to fungal diseases of different varieties of onions and apples has been correlated with their PPO activities and quinone content (Coulate 1984). PPO activity is pH dependant and varies with the source of the enzyme and with the substrate (Vamos-Vigyazo 1981). Dijkstra and Walker (1991) found the optimum pH of an apricot (*Prunus armeniaca*) PPO to be 6.6. Luh and Phithalcpal (1972) found the optimum activity of PPO extracted from Halford peaches occurred at a pH of 6.2. Enzyme preparations obtained from the same fruits or vegetables at various stages of maturity differ in the optimum pH of activity.

The degree of browning depends upon such factors as amount and type of phenolic compounds, enzyme, pH of the medium, availability of oxygen, temperature and time of exposure to the air after peeling (Luh and Phithalcpal 1972). Vámos-Vigyázó *et al.* (1976) indicated that enzymatic activity was the main factor involved in browning, whereas others (Prabha and Patwardhan 1985 and 1986) indicated that it was the phenolic content of the fruit. Lourenço *et al*. (1992) related the browning tendency of sweet potato cultivars to phenolic content, especially to the levels of chlorogenic acid and isochlorogenic acid which compose 80 % or more of the total phenolic content present in the root. According to Harel *et al*. (1970) both factors contributed to browning and Macheix *et al*. (1991) pointed out that these factors depended on the physiological stage of the fruits. Coseteng and Lee (1987) found that among seven varieties of apple cultivars, the degree of browning was correlated with PPO activity in four varieties and with phenolic content for three others, whereas Klein (1987) found no correlation for 22 New Zealand varieties.

Differences in the affinity of the enzyme for the substrate were reported to be another factor that determined the degree of browning (Luh and Phithalcpal 1972, Kahn 1997). Normal fruit develop internal browning as a consequence of over maturity and senescence. Fruits that have stems show more browning than those with no stems (Westwood 1978).

There are contradicting results in the PPO activity reports for various fruits during ripening; however, most fruits show a decrease in activity during ripening. During early stages of peaches fruit ripening the PPO activity was found to be high, which then decreased (Luh and Phithalepal 1972). The decrease was accompanied by a decrease in *o*-dihydroxyphenols. Grapes (Machiex *et al*. 1991), avocardo (Vanini *et al*. 2010) and mangoes (Joshi and Shiralkar 1977) had a decrease in PPO activity during ripening. In pears, the PPO activity first decreased and then steadily increased with the rate of browning of the fruit (Ranadive and Haard 1972).

MATERIALS AND METHODS

Sample collection

Fruit samples were collected from Dar es salaam, Tanzania. The pineapple, mango and papaya fruits were picked at the mature green stage in batches of five for the PPO & POD activity determination. The determinations were done at intervals of two days from the day of harvest. This was repeated during the early, mid and late

seasons of the different fruits. Once picked the fruits were transported to the Chemistry Department, University of Dar es Salaam, for further investigation. Only fruits that did not show any visual signs of bruises, cuts, blackening, etc. were used for the study.

Enzyme extraction

Minced fresh fruit (10 g) were homogenized with 20 cm^3 0.05 M potassium phosphate buffer at pH 6.0 in a blender at 2 ˚C. The mixture was then filtered through glass wool and centrifuged at 6000 r.p.m at 4 ˚C for 20 minutes. The extract was kept at 1 ˚C in a rubber-stoppered test tube containing a few drops of toluene (Sanchez-Ferrer 1989).

Polyphenoloxidase (PPO) determination

The enzymatic activity was determined by measuring the increase in optical density using a CIBA - CORNING Colorimeter model 252. A 0.01 M catechol solution (2.5 cm^3) and 22 cm^3 of a 0.05 M phosphate buffer (pH 6.0) were mixed and kept at 30.0 ± 0.5 ˚C for 10 minutes in a water bath. The enzyme extract (0.5 cm^3) was added to the above mixture with further mixing achieved by swirling. The optical density was then measured at 430 nm. A reference cuvette containing the solvent was placed in the sample and solvent compartments and the colorimeter was zeroed until a zero absorbance was displayed. The sample cuvette was then used for measurements on the samples. At intervals of 2 minutes absorbency readings were made on the reacting mixture. Duplicate determinations were carried out and the average result recorded. PPO activity was obtained by determining the initial slope of the curve obtained by plotting optical density at 430 nm against time in minutes. The results are expressed as the increase in ΔOD/min/cm^3 of enzyme solution (Luh and Phithalcpal 1972).

Perioxidase (POD) determination

2.0 cm^3 of enzymatic extract was mixed with 20 cm^3 distilled water in a 50 cm^3 beaker. 1.0 cm^3 of 0.5% H_2O_2 solution was added to the solution followed by 1.0 cm^3 of 0.5% guaiacol solution. The mixture was well mixed and 4 cm^3 was used as the reacting mixture. Absorbency at 460 nm was recorded at intervals of 2 minutes, duplicate determinations carried out and the average reported. POD activity was determined from the initial slope of the curve obtained by plotting optical density against time in minutes. The results are expressed as the increase in ΔOD/min/mL of enzyme solution (Woods and Aurand 1977).

RESULTS

Proximate composition of fruits

The proximate composition (moisture, total acidity, sugars, ash, crude fibre, crude fat and ascorbic acid content) of the pineapple, mango and papaya fruits has been reported elsewhere (Othman 2011, Mbogo and Othman 2009, Othman 2009).

PPO activity in pineapple

The PPO activities in the pineapple fruit decreased sharply during the first 2-4 days of ripening. Early and mid-season fruits had a similar trend in the decrease in activities to the 4^{th} day then the activities remained constant to the 8^{th} day. The PPO activities in the late-season fruits decreased to the 2^{nd} day of storage ripening, remained constant to the 6^{th} day and then dropped to 0.00065 ΔOD/min/cm^3 at day 8 (Figure 1). Late season fruits had the highest initial PPO activity at the time of harvest.

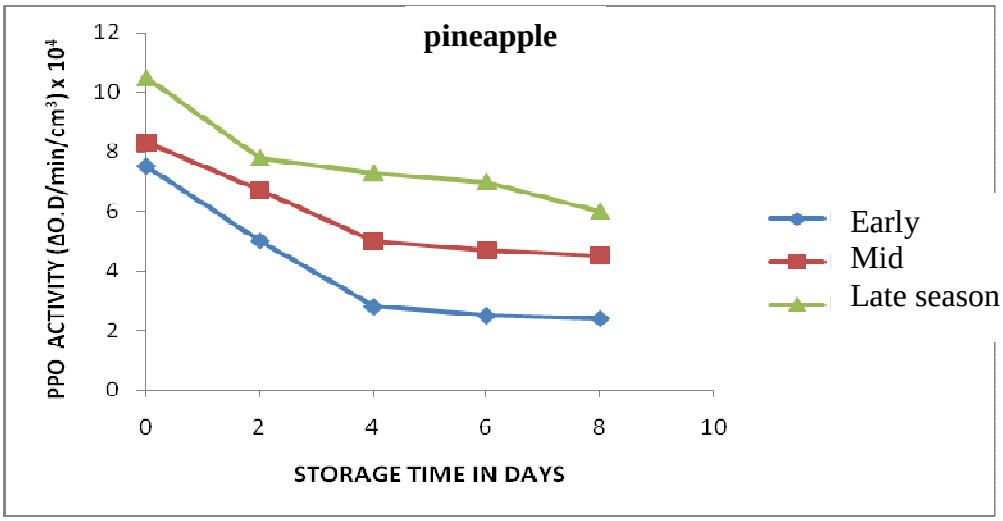

Figure 1: PPO activity in pineapples grown in Dar es Salaam

PPO activity in Papaya

The PPO activity in the papaya fruits decreased continuously throughout the 8 days of ripening storage. Early and mid and late-season fruits had a similar trend in the decrease in activity with post harvest ripening (Figure 2). Late season fruits had the highest initial PPO activity at the time of harvest. There was a large decrease in PPO activity during the first two days of ripening-storage. Early-season papaya fruits had the highest PPO activity throughout the eight day storage time while late-season fruits had lowest PPO activity.

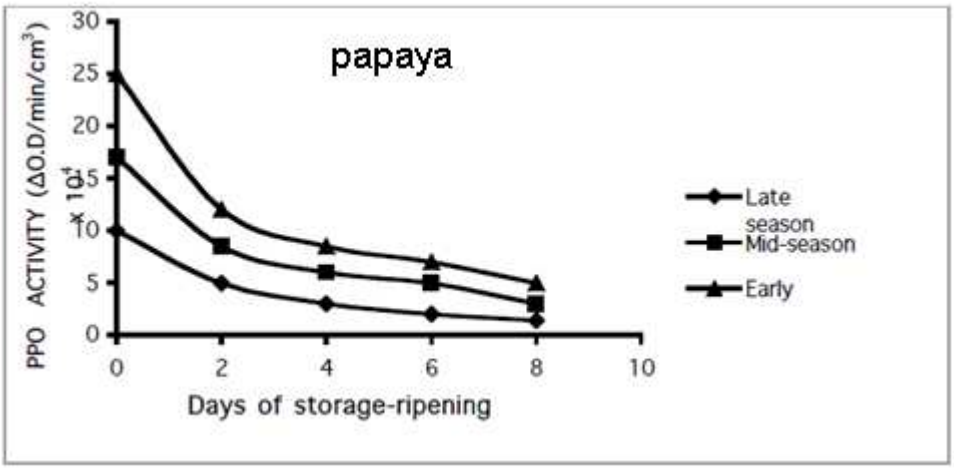

Figure 2: PPO activity in papaya fruits grown in Dar es Salaam (Othman 2009)

PPO activity in mango

The PPO activity in the mango fruits, Viringe and Dodo varieties, decreased continuously throughout the 8 days of ripening storage. Early and mid and late-season fruits had a similar trend in the decrease in activity with post harvest ripening (Figures 3(a) and 3(b)). Early season fruits had always the highest initial PPO activity at the time of harvest. Dodo-mango had higher initial PPO activity than Viringe-mango.

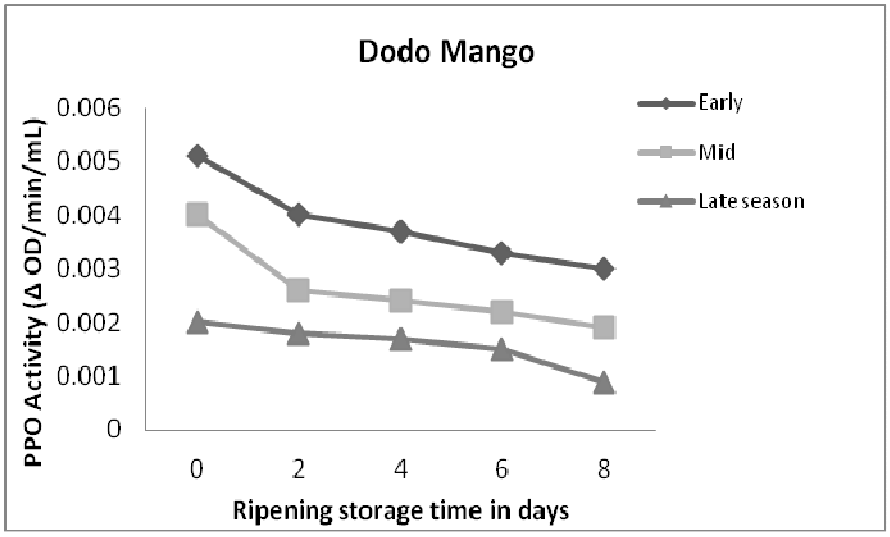

Figure 3(a): PPO activity in Dodo mango fruits from Dar es Salaam during open air storage ripening.

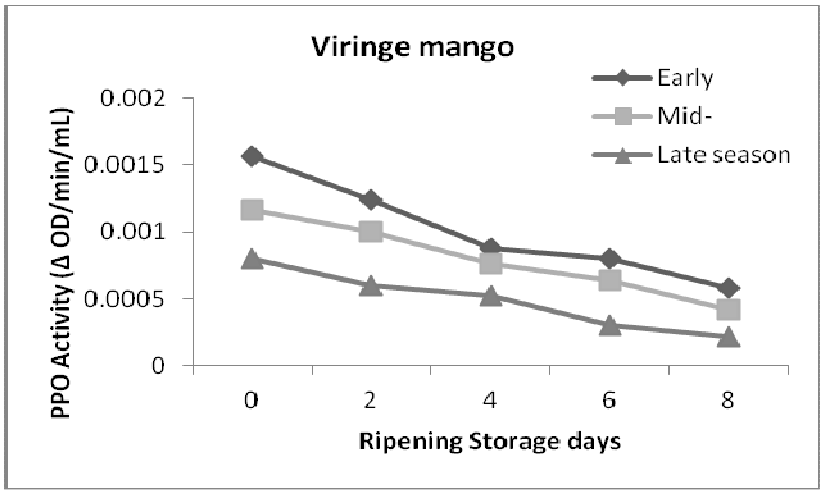

Figure 3(b): PPO activity in Viringe mango fruits from Dar es Salaam during open air storage ripening.

Perioxidase (POD) activity in fruits

POD activity in pineapples

The POD activity in pineapple fruits increases continuously only slightly throughout the eight days of open-air storage ripening. Mid-season fruits exhibited the lowest POD at harvest and during ripening. Early season fruits had the highest POD activity (Figure 4).

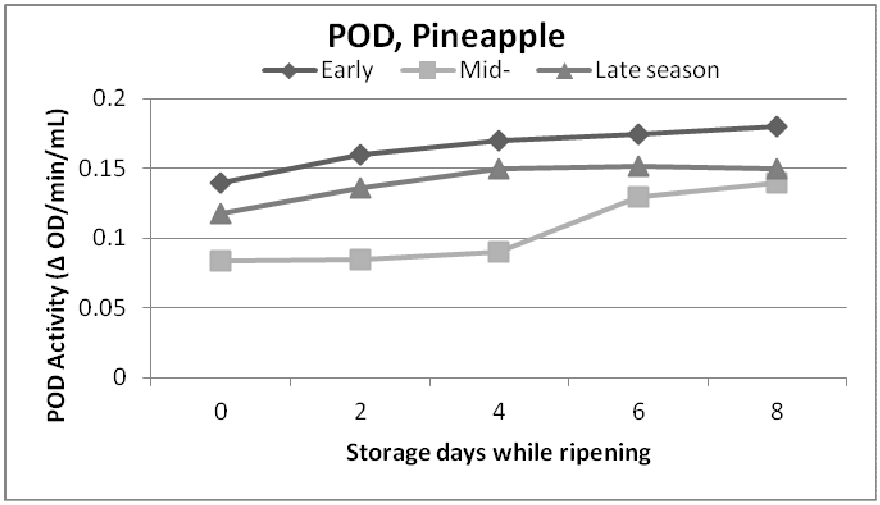

Figure 4: POD activity in pineapple fruits grown in Dar es Salaam

POD activity in 'Dodo' mango and 'Viringe' mango

The results of POD activity measurement of mango fruits are reported in figures 5(a) and (b). Dodo- mango had higher POD values than Viringe-mango. Early season fruits had highest POD activity in Dodo mangoes while late season fruits had highest POD activity in Viringe mangoes. Though there was continual increase in POD values for both mango varieties, Dodo-mango had higher increase during open air storage ripening.

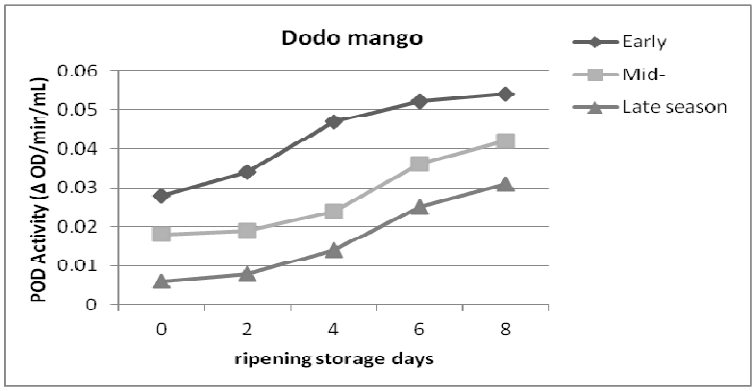

Figure 5(a): POD activity in Dodo mango fruits from Dar es Salaam during open air storage ripening.

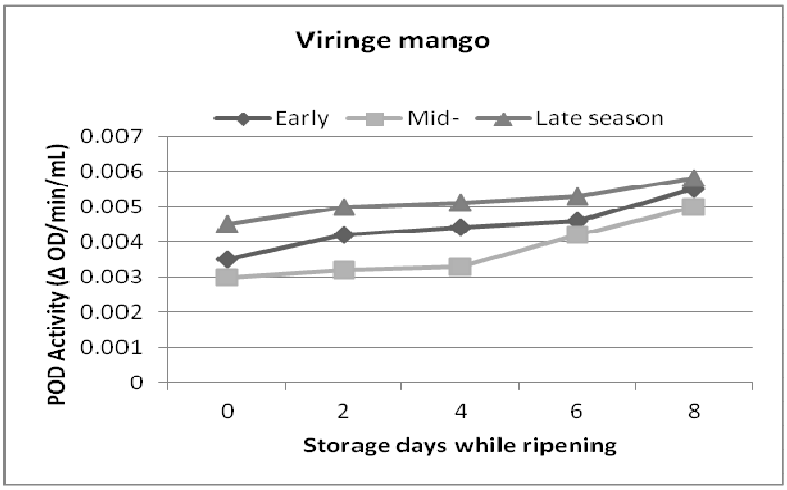

Figure 5(b): POD activity in Viringe mango fruits from Dar es Salaam during open air storage ripening.

POD activity in papaya

The results of measurements of POD activity in papaya fruits are reported in figure 6. At harvest, the POD activity in papaya was the same for all fruits of the three seasons. POD activity only tended to increase slightly during open-air ripening of mid and late season fruits. Throughout the eight days of storage ripening late-season fruits had higher POD activity while late-season fruits had the lowest POD activity. From the day of harvest to day 4, POD activity of papaya were constant for fruits of all three seasons but there was slight increases of POD from day 4 to 6 for both late and mid-season fruits. Early season fruits maintained their original POD activity levels (Figure 6).

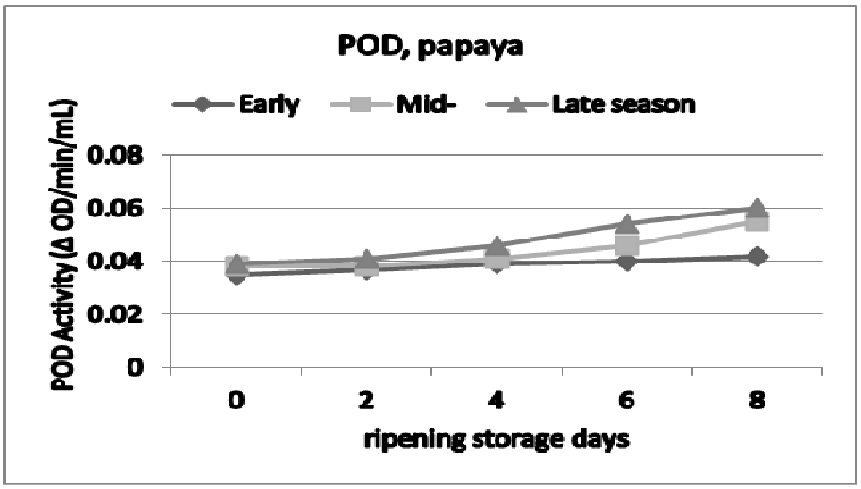

Figure 6: POD activity in papaya fruits grown in Dar es Salaam during ripening

DISCUSSION

PPO and POD activities has been reported to be responsible for general deterioration of fruit quality especially the enzymatic browning of fruits (Vamos-Vigyazo 1981, Arnnok *et al*. 2010).

The experimental observations from this study show the the PPO activity of the fruits showed a decrease during post-harvest ripening. This observed trend is similar to that reported by De Oliviera, (1994) for the ripening period in the fruits of soursops. Sanchez-Ferrer *et al*. (1989) reported a decrease in the PPO activity during the ripening of 'Monastrell' grapes. The PPO activity was strongly cultivar dependent (Vamos-Vigyazo *et al*. 1976) and was found to decrease in peaches (Harel *et al*. 1970), in apricot (Vanini *et al*. 2010) and in mangoes (Joshi and Shiralkar 1977). The PPO activity in pears initially decreased then increased as ripening continued (Ranadive and Haard 1972).

Mayer and Harel (1979) reported that the PPO activity may be affected by many factors including the developmental period of the fruit *i.e.* whether the fruit is fully matured, ripe or over ripe. In these stages there could be differences in the activities of the enzyme. Lozano *et al*. (1994) found that the PPO activity was greater in young fruits than in fully ripe apples. Other influencing factors may include the pH of the medium, temperature, oxygen availability, amount and type of phenolic compounds present and concentration of ascorbic acid.

Susceptibility to browning, which illustrate the interaction between the PPO enzymes and phenolic compounds in the fruits also decreased with ripening. The results of De Oliviera (1994) for PPO activity in soursops showed that the browning rate was higher in developing fruits and decreased with ripening agrees well with our results. Prabha and Patwardhan (1985) obtained similar observations from studies on apples.

The PPO activity's optimum pH, in most cases between 4.0 and 7.0, has been observed to vary with the source of the enzyme, the substrate and whether it is particulate or soluble nature (Vamos-Vigyazo and Nadudvari-Markus 1982). The type of buffer and the purity of the enzyme affect the optimum pH value as well. Isoenzymes may have a distinct different pH

optima. Not only the optimum pH affects the activity but the relationship between activity and pH was found to differ according to genera, cultivars and substrate (Nicholas *et al*. 1994). In this study the fruit pHs of the solutions were observed to be 3.7, 5.5, and 4.2 for pineapples, papaya and mangoes respectively.

The optimum temperature of activity of the enzyme depends essentially on the same factors as the optimum pH. The enzyme activity of peach was found to increase from a temperature of 3 ˚C to 37 ˚C then decline up to 45 ˚C (Vamos-Vigyazo and Nadudvari-Markus 1982). In banana the PPO enzyme reached its maximum activity at 37˚C (Weaver and Charley 1974). In apples, the Jonathan and Starking varieties, the maximum PPO activity were obtained at 30 ˚C and 25 ˚C respectively (Lazano *et al*. 1994). De Oliviera *et al*. (1994) has reported on changes in the concentration of PPO, POD and other phenolic compounds in soursop fruits during maturation and ripening. Fruits such as banana (Weaver and Charley 1974), mango (Joshi and Shiralkar 1977), some cultivar of grapes (Sanchez-Ferrer 1989), apple (Coseteng and Lee 1987), peaches (Luh and Phithalepal 1972) and medlar fruits (Aydin and Kadioglu 2001) showed reductions in PPO and phenolic compounds with maturation. This observation supports the trend obtained in this study for the PPO activities of the Dar es Salaam mango, papaya and pineapple fruits.

The PPO activity is strongly affected by ascorbic acid content such that this may prevent enzymatic browning caused by the PPO enzymes (Macheix *et al*. 1990). Ascorbic acid reduces quinones on one hand and is a copper chelating agent on the other. It might also act as a pro-oxidant (in the presence of compounds of lower oxidation-reduction potential). The rate of oxygen uptake in the oxidation process, by the PPO of L-tyrosine, DOPA and protocatechuic acid was found to increase by the presence of ascorbic acid (Macheix *et al*. 1990). The quinone reducing capacity of ascorbic acid depends largely on its concentration in solution, that is why sometimes its inhibitory action is not noticed.

CONCLUSION

The action of the enzymes polyphenoloxidase (PPO) and perioxidase (POD) is considered responsible for fruit quality deterioration. PPO and POD causes discoloration (browning) and changes in flavor and bitterness in fruits and vegetables. The browning of fruits such as apples, pears, peaches, apricots and vegetables and potatoes is due to polyphenoloxidase which oxidizes natural phenolic compounds to quinones followed by polymerization leading to browning. A good quality of the pineapple, mango and papaya fruit is the one that is ripe, hence for the best, delicious and edible fruits, they should be eaten or processed while ripe regardless of the period in the season because the activities of PPO were found to decrease during ripening of the fruits and were lowest when the fruits were fully ripened.

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