

ACTIVITY OF THE ENZYMES ASSOCIATED WITH FRUIT RIPENING AND QUALITY DETERIORATION IN BARBADOS CHERRY (*MALPIGHIA GLABRA* L.)

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Barbados cherry, native to Trinidad, is grown commercially as an ornamental plant and also for the nutrient rich fruit. Mature fruits were found to be rich source of ascorbic acid and phenols. Polygalacturonase (PG), pectin methyl esterase (PME) and cellulase- the major cell wall hydrolyzing enzymes associated with fruit ripening were isolated and assayed. The activity of PG and PME expressed a significant increase from unripe to half ripe and declined towards the pre ripe stage of fruit development. An interesting feature noticed here was the gradual increase in activity of PG and PME during post ripe stage. During the early stages of Barbados cherry development, PPO activity and the level of ascorbic acid gradually decreased, whereas in the post-ripening stage PPO activity increased. Similarly, POD activity decreased during development of the fruits but was high in the pre-ripening and post-ripening stages. Polyphenol oxidase (PPO), a quality-deteriorating enzyme of tropical fruits exhibited a gradual increase in activity. The enzyme was found to be extremely active in the later stages of fruit development and ripening. The occurrence of low level of phenols and high level of PPO activity in ripe fruits clearly indicates the physiological correlation of the enzyme with the substrates. A negative correlation was noticed between PPO and ascorbate content during fruit development and maturation. The significantly higher activity of PPO and POD strongly suggest their role in the deterioration of fruit quality *via* browning, leaching and perishability during post harvest storage.

Keywords: Ascorbate; Cellulase; Fruit ripening; Pectin methylesterase; Peroxidase; Phenols; Polygalacturonase; Polyphenol oxidase.

Introduction

Fruit ripening is a genetically programmed event that is characterized by a series of biochemical and physiological processes that alter fruit color, flavor, aroma, and texture¹. Extensive cell wall modifications occur during ripening and are thought to underlie processes such as fruit softening, tissue deterioration, and pathogen susceptibility. Pectins are a major class of cell wall polysaccharides that are degraded during ripening, undergoing both solubilization and depolymerization. Modifications in cell wall polymers during ripening are intricate and considered to involve the co-ordinated and interdependent action of a range of cell wall-modifying enzymes and proteins such as polygalacturonase (PG), pectin methyl esterase (PME), β -galactosidase, α -L-arabinofuranosidase, endo-(1,4) β -D glucanase, expansin, and xyloglucan endotransglycosylase². These

modifications are regulated at least in part by the expression of genes that encode cell wall-modifying enzymes³. Phenoloxidase enzymes are endogenous to fruits and vegetables catalyse the production of quinones from their phenolic constituents. This enzyme catalyses the hydroxylation of monophenols to diphenols, and further oxidation of diphenols to quinones, in the presence of molecular oxygen. The generated unstable highly reactive quinones subsequently react with themselves, amino acids and proteins evolving in to brown, black or red heterogeneous polymers responsible for quality loss in many fruits⁴. The Barbados cherry (*Malpighia glabra* L.) is a subtropical plant indigenous to Trinidad. The three lobed soft, juicy, thin-skinned fruits soften readily and which in turn makes transportation and storage of the fruit difficult. Therefore, to improve the quality, storage and processing characteristics of the fruits, it is necessary

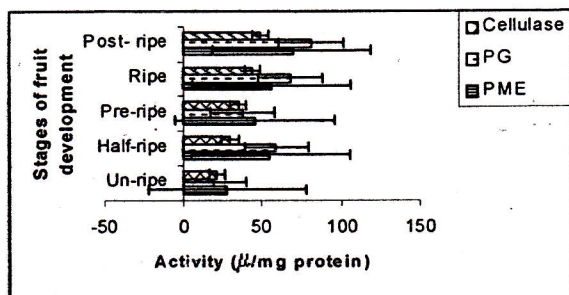


Fig. 1. Activity of pectin methyl esterase (PME), polygalacturonase (PG) and cellulase during fruit development.

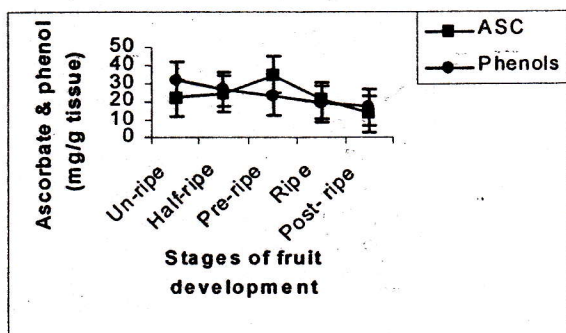


Fig. 3. Total ascorbate and phenol content during fruit development.

to understand the biochemistry of ripening. However, no information is available regarding the enzymic changes occurring during ripening of Barbados cherry. The present study was focused on the role of the major cell wall hydrolyzing enzymes polygalacturonase (PG), pectin methyl esterase (PME) and cellulase, and quality deteriorating enzymes polyphenoloxidase (PPO) and peroxidase (POD) during fruit development and maturation.

Materials and Methods

Isolation and assay of Polygalacturonase (PG): Frozen tissue (1 g) was homogenized in 1 M sodium acetate buffer containing 0.2% sodium dithionite and 1% PVP with PMSF⁵. The enzyme assay was carried out by measuring the reducing groups released from polygalacturonic acid⁶. One unit of the enzyme catalyzed the liberation of one nmole of galacturonic acid in one second under the conditions of the enzyme assay. Specific activity was expressed as $\mu\text{mg}^{-1}\text{ protein}$.

Pectin methyl esterase (PME): Isolation of the enzyme was carried out following the method of Lazan *et al.*⁷. 5g fruit tissue was homogenized in cold 0.1M sodium citrate

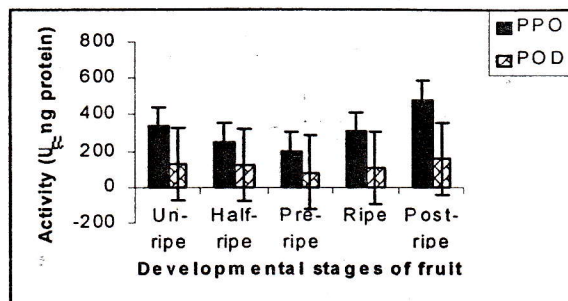


Fig. 2. Activity of polyphenol oxidase (PPO) and peroxidase (POD) during fruit development.

(pH 4.5) containing 1 M sodium chloride, 10 mM β -mercaptoethanol and 1% (w/v) soluble polyvinyl pyrrolidone (PVP-40). The homogenate was centrifuged at 15000 rpm for 30 min at 4°C and the supernatant was used as enzyme source. PME activity was assayed using 1% (w/v) pectin containing 2.5 mM CaCl_2 and 0.5 ml enzyme extract. 25 ml of 1% pectin solution containing 2.5 mM CaCl_2 was adjusted to pH 7.5 and placed in a constant temperature (30°C). The enzyme solution was then added, the pH was immediately readjusted to 7.5; and the time was noted. 0.01 M NaOH was added at the rate required keeping the mixture at pH 7.5 for 15 min. Heat killed enzyme extract was used in the assay mixture as blank⁸. 1 unit of enzyme activity was defined as the milliequivalent of ester hydrolyzed/min/g of enzyme.

Cellulase: Fruit tissue was ground in a homogenizing medium consisting of 0.02 M sodium phosphate buffer (pH 7.0) containing 0.02 M EDTA, 1% Triton X100, 0.02 M cysteine HCl and 1mM PMSF. The homogenate was filtered and centrifuged at 10,000 rpm for 20 min. The activity was determined by measuring the reducing groups released from carboxymethyl cellulose. The reaction mixture containing 0.25 ml of crude enzyme, 0.25 ml of 100 mM sodium acetate buffer (pH 5.0), 0.5 ml 1% carboxymethyl cellulose solution was inoculated at 37°C for 30 min⁵.

Isolation and assay of Peroxidase (POD): POD was isolated and assayed following the method of Goliber⁹ and Ingham¹⁰. One unit of POD is the amount of enzyme required to oxidize 1 μM of guaiacol by H_2O_2 at test condition.

Isolation and assay of Polyphenol oxidase (PPO): PPO was extracted and assayed according to the method of Oktay *et al.*¹¹. The activity of PPO was determined spectrophotometrically by recording the increase in absorbance at 420 nm for 10 min. One unit of the enzyme activity is defined as an increase in absorbance of the

mixture at 420 nm of 0.1/min and /mL of enzyme solution. *Estimation of total phenols*: Total phenol content of fruit tissues was estimated by the method of Mayr *et al*¹². The total phenols/g tissue was calculated from the standard graph.

Estimation of ascorbate: Ascorbate was extracted and quantified as per the methodology of Ranganna¹³.

Results and Discussion

The fruits take a period of 30 days for complete maturation. The activity of PG and PME was assayed from different stages of fruit development. No considerable level of activity was expressed by PME and PG in the initial stages while a profound increase occurred at the second stage and decreased gradually (Fig. 1). The decrease in the activity of PG and PME may be correlated with the high ascorbate content. In the later stages of fruit maturation PG and PME activity increased substantially suggesting their role in softening of wall tissues. Cellulase activity increases *at par* with the fruit development and maturation (Fig. 1).

Polyphenol oxidase activity gradually decreased during the development of the fruits followed then by an increase during the ripening period, whereas the activity of PPO in the unripe stage of development was higher than in the pre ripe and ripe stages (Fig. 2). The lowest activity was found at the third stage of development. Similar results were also obtained in other studies¹⁴. Peroxidase activity gradually decreased during the development periods, while during the periods of fruit ripening it increased (Fig. 2). Phenolic compounds are thought to be sequestered in cell vacuoles and include flavonols, cinnamic acid derivatives, simple phenols and catechin. Most of these phenolics are intermediates and derivatives of the phenylpropanoid pathways¹⁵. So, in the present study, the increase in PPO and POD activity in the beginning of fruit development may be involved in these processes. POD and PPO degrade fruit anthocyanin indirectly by reacting with D-catechin to form quinones that polymerise with anthocyanin pigments. The increase in both enzyme activities during ripening found in our study may be correlated with anthocyanin metabolism. Ascorbate content varied from 13 to 34.8 mg/g fresh weights (Fig. 3). The highest ascorbic acid content was determined at the pre-ripe stage of the developmental period and the lowest at the post-ripe stage of the fruits. Kadioglu and Yavru¹⁴ obtained similar results with cherry laurel. Similarly a negative correlation was observed between ascorbate content and PPO activity. This interaction may be due to the inhibitory effect of ascorbate on PPO activity, because ascorbate is a natural inhibitor of PPO¹⁶. The results

presented in this work showed that there were important changes in the activities of PPO and POD as well as in the contents of phenols and ascorbate. Ripe Cherry fruits bruise easily and are perishable. This highly perishable nature of the fruits may be correlated with the high profile of activity of the quality deteriorating enzymes PPO and POD at the post ripening stage of fully ripe fruits. Interestingly, a decrease in total phenol content was observed at different stages of fruit maturation, which may be correlated with the activity of PPO and POD in the fruits. In addition, understanding of the biochemical changes and some enzyme activities in cherry, the chemistry of their transformations in the fruit and their functions in physiology, food science, nutrition and health should stimulate interest in maximizing beneficial sensory, nutritional effects of polyphenols in the diet. Such efforts should lead to better foods.

References

1. Brady CJ, Meldrum S K, McGlasson W B and Ali Z M 1983, Differential accumulation of the molecular forms of polygalacturonase in tomato mutants. *J. Food Biochem.* 7 7-14.
2. Brummell DA and Harpster M H 2001, Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. *Plant Molecular Biology* 47 311-340.
3. Fischer RL and Bennett AB 1991, Role of cell wall hydrolases in fruit ripening. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42 675-703
4. Garcia-Carmona F, Valero E and Cabares J 1988, Effect of L-proline on mushroom tyrosinase. *Phytochemistry* 27 1961-1964.
5. Saiprasad G V S, Mythili J B, Nagesh M, Joshi R, Ravisanker K V and Lalitha Anand 2004, A simplified procedure of enzyme extraction for the assay of polygalacturonase and cellulase in some fruits. *J. Food Sci. and Tech.* 41 297- 301.
6. Miller G L 1959, Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Annal. Chem.* 31 426-428.
7. Lazan H, Ali Z M, Liang K S and Yee KL 1989, Polygalacturonase activity and variation in ripening of papaya fruit with tissue death and heat treatment. *Physiol. Plant.* 77 93-98.
8. Lazan H, Selamat MK and Ali ZM 1995, β -galactosidase, Polygalacturonase and pectin esterase in differential softening and cell wall modification during papaya fruit ripening. *Physiol. Plant.* 95 106-112.
9. Ingham LM, Parker M L and Waldron KW 1998, Peroxidase: Changes in soluble and bound forms

- during maturation and ripening of apples. *Physiol. Plant.* **102** 93-100.
10. Gölber T E 1989, Gravitational stress and lignification in aerial vs. submerged shoots of *Hippuris vulgaris*. *Physiol. Plant.* **75** 355-361.
 11. Oktay M, Kufreviöglu I, Kocacaliskan I and Sakiroglu 1995, Polyphenol oxidase from Amasya apple. *J. Food Sci.* **60** 494-496.
 12. Mayr V, Treutter D, Santos-Buelga C, Bauer H and Feucht W 1995, Developmental changes in the phenol concentration of golden delicious apple fruits and leaves. *J. Agric. Food Chem.* **38** 1151-1155.
 13. Ranganna S 1997, *In* : Manual analysis of fruit and vegetable product, 9th edition, Tata Mc Graw Hill, New Delhi.
 14. Kadioglu A and Yavru I 1998, Changes in the chemical content and polyphenol oxidase activity during development and ripening of cherry laurel. *Phyton* (Horn, Austria) **37** 241-251.
 15. Cheng G W and Breen P J 1991, Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *J. Am. Soc. Hort. Sci.* **117** 946-950.
 16. Weaver G and Charley H 1974, Enzymatic browning of ripening bananas. *J. Food Sci.* **39** 1200-1202.