CURRENT BOTANY

Curr. Bot. 1(1):22-28, 2010

REGULAR ARTICLE

Effect of ethrel on phenolic changes during ripening of off-season fruits of mango (*Mangifera indica* L. var. Neelum)

T. Venkatesan and C. Tamilmani

Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

KEYWORDS

Fruit ripening, Phenols, Peroxidase, Polyphenoloxidase and Catalase

CORRESPONDENCE

T. Venkatesan, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

CB Volume 1, Year 2010, Pages 22-28

ABSTRACT

The present investigation is aimed at studying the effect of ethrel on the ripening of off–season fruits of *Mangifera indica* L. var. Neelum. The control fruits were kept in the laboratory naturally while the experimental fruits were treated with different concentrations of ethrel (100, 200 and 300ppm). In control fruits, partial ripening led to incomplete metabolic changes, which did not alter the presence of sourness in the fruits. Hence, they were not fit to be eaten. On the other hand, the fruits treated with different concentrations (100, 200 and 300ppm) of ethrel ripened on 13th day, 11th day and 9th day respectively after treatment. The colour changed from green greenish to yellow and the fruits were palatable in nature. The colour changed from green to greenish yellow to yellow. On the other hand, in the control fruits, partial ripening led to incomplete metabolic changes, which did not alter the presence of sourness in the fruits, and hence, they were not fit to be eaten. All the studies were carried out using the peel and the pulp of fruit tissues individually and the following results were obtained during the process of ripening. The phenols decreased during ripening, both in the treated and control fruits. On the other hand, the activity of peroxidase, polyphenoloxidase and catalase increased. Among the different 100, 200 and 300 ppm ethrel treatments, the 200 ppm alone had the optimum effect on the ripening of off–season fruits of *Mangifera indica* L. var. Neelum.

Introduction

Fruit ripening and senescence may be regarded as an oxidative process involving marked alterations in fruit metabolism and the acitivity of a number of enzymatic systems, including those related to the regulation of reactive oxidative species (Masia, 1998). The phenolic compounds are a structurally diverse class of plant secondary metabolites. Generally they possess an aromatic ring bearing one or more hydroxyl substituents (Robards, *et al.,* 1999). As widely distributed non-nutrient biologically active compounds, phenolics are also reported to have multiple effects on tissue maturation processes, defense mechanisms (Kubo and Matsumoto, 1984) and sensory qualities of plant-derived food products, including astringency, bitterness and aroma (Macheix, *et al.,* 1990). Phenols are the by-product of the metabolism of aromatic amino acids (Neish, 1964). Phenolic compounds enjoy a wide distribution in the plant kingdom and they are particularly prominent in fruits where they are important in determining colour and flavour. Normally phenolic content decreases as the fruit matures (Williams, 1959). The level of phenolics in fruits varies widely from species to species, variety to variety, season to season and location to location. The great majority of the phenolic components found in fruits have no characteristic taste when tasted at low concentration in this pure form. The exception to this general rule is the sourness associated with phenolic acids, the astringency of condensed flavours and the bitterness associated with some of the citrus flavonoids (Van Buren, 1970). Aziz *et al.* (1976) observed a general decline in total phenolic content in the pulp of banana fruit during ripening. The loss of phenol was more rapid in the peel. Similar results have been obtained in the studies of Venkaiah and Babu (1977) Lima *et al.*, (2000), Tomas-Barberan *et al.*, (2001); and Tamilmani (2005) and Prakash and Tamilmani (2002). The polyphenol content of mango is high at the early part of the growth, which decreases during the ripe stage and remains fairly steady (Lakshminarayana *et al.*, 1970). The loss of astringency in the course of ripening is associated with the loss of phenolic content (Selvaraj and Kumar, 1989). In sapota, similar observation was reported by Lakshminarayana and Subramanyam (1966). Polyphenolic compounds commonly serve as a protective mechanism in plants, warding off predator and microbiological attack. Many factors affect polyphenolic concentrations, including cultivar differences, growing conditions, maturity, and postharvest handling of fruit (Hakkinen and Torronen, 2000; Lakshminarayana *et al.*, 1970; Selvaraj and Kumar, 1989; Wang and Lin, 2000). Abiotic stresses such as excessive UV, heat and chilling temperatures, wounding and drought that are introduced before or after fruit harvest may affect the biosynthetic pathways of secondary metabolites (Cisneros – Zevallos, 2003), as fruits attempt to protect themselves. Under normal ripening conditions, mango polyphenol content is highest during fruit growth, decreasing with ripening (Lakshminarayana *et al.*, 1970, Selvaraj and Kumar, 1989). Tamilmani (2005) studied phenolic degradation during the ripening of bitter gourd fruit (*Momordica charantia* L.). Riberio et al. (2008) studied phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera india* L.) varieties. Gruz *et al.* (2011) studied phenolic acid content and radical scavenging activity of extracts from medilar (*Mespilus germanica* L.) fruit at different stages of ripening.

The involvement of different kinds of enzymes in the ripening of fruits is amply documented (Mattoo *et al.*, 1968; Subramanyam *et al.*, 1972; Sacher, 1973; Joshi and Shiralkar, 1977; Yoshioka, 1979; Presis and Levi, 1980; Lal *et al.*, 1985;

Steup, 1988; Rothan and Nicolas, 1989; Selvaraj and Rajivkumar, 1989; Prabha and Bhagyalakshmi, 1998; Reddy and Srivastava, 1999; Ketsa *et al.*, 1999). Some of the hydrolytic and oxidative enzymes increase during the ripening of fruits (Leopold and Kriedmen, 1975; Mayer and Harel, 1981); Poorinima singh and Dwiredi 2008; and Jang and Moon, 2011). The enzyme catalase is closely related to peroxidase in structure and function, and both the enzymes are sometimes considered together as hydroperoxidase. There appears to be two functions of catalase in plants: The first is to dispose of excess H_2O_2 produced during oxidative metabolism of all organisms, and the second is to utilize H_2O_2 in the oxidation of alcohols, phenols and other H-donors in a manner similar to peroxidase. Peroxidase catalysed reactions may be envisioned as an integral aspect of the biochemistry of ageing cells, since sub-cellular organelles undergo degeneration with subsequent decompartmentation of enzymes and substrates (Dilley, 1970). Plant cells contain several different peroxidases. They are located in the cytoplasm, in the cell walls (Gordon and Alldridge, 1971), and in the chloroplasts (Martinoia *et al.*, 1982). Chloroplast peroxidase was found only in the membrane fraction and not in the stroma fraction (Kuroda *et al.*, 1990). Thomas *et al.* (1981) and Lal *et al.* (1985) observed an increase in the activity of the enzymes catalase and peroxidase during ripening of fruits. Peroxidase and catalase activities increased during ripening of banana (Haard, 1973; Haard and Tible, 1973 and Desai and Deshpande, 1978). Catalase increases during fruit maturation and ripening (Pal and Selvaraj, 1987). The enzyme has been purified 32-fold from ripe fruit (Chan *et al.*, 1978). The enzyme has a pH optimum of 6.1 and is stable when frozen, through inactivated by acidification to pH 3.5. It has an apparent molecular weight of 160 k.Da; the enzyme is rapidly inactivated by heat, losing 90% activity after three minutes at 60°C. The catalase and peroxidase activities during the ripening of fruits of cucumber (Miller *et al.*, 1988; 1990), papaya (Pal and Selvaraj, 1987 and Silva *et al.*, 1990), tomato (Rothan and Nicolas, 1989) and sapota (Rao and Chundawat, 1989) were studied. The results showed an increased activity of these two enzymes during ripening in sapota and papaya.

An increase in fruit peroxidase activity at the time of ripening has been documented in blueberries (Miesle *et al.*, 1991), papaya (Silva *et al.*, 1990), peaches (Quesada *et al.*, 1992) tomatoes and pears (Frenkel, 1972) and grapes (Calderon *et al.*, 1993). Increases in peroxidase activities in fruits and vegetables also have been associated with decreased quality (Burnette, 1977). Peroxidase are also present in ripening bananas. These enzymes catalyse, in the presence of H_2O_2, the oxidation of substrates such as phenols, aromatic secondary and tertiary amines, leucodyes, ascorbic acid and certain heterocyclic compounds such as indoles (Haard and Tobin, 1971). Most forms of peroxidase have been reported in bananas and they appear to vary between clones (Jarret and Litz 1986). The exact role of these enzymes in ripening fruit is unclear. Toraskar and Modi (1984) observed differing isoenzyme patterns between chill-injured and healthy banana fruit. An increase in cell wall-associated peroxidase activity in bananas has been reported to occur with the initiation of the climacteric rise in respiration (Haard, 1973). Peroxidase activity is present in fruit thoughtout development (Tan and Weinheimer, 1976) and reaches a minimum at the start of the climacteric, and then increases during ripening (Pal and Selvaraj, 1987; Da Silva *et al.*, 1990). The amounts of the different isoenzymes (n=6) vary during fruit development (Tan and Weinheimer, 1976), with at least four peroxidase isoenzymes being present in quarter–ripe fruit (Sawato, 1969). These can be separated into soluble and ionically–bound forms. (Da Silva *et al.*, 1990) having molecular weights of 41 kDa and 54 kDa, respectively. Poorinima singh and Dwivedi (2008) report the effect of 1-Methylcyclopropene (1-MCP) and ethrel on antioxidant levels in mango fruit during ripening. Use of 1-MCP is applied commercially to delay ripening while ethrel is used to accelerate ripening of climacteric fruits.1-MCP treatment led to decreased levels of H_2O_2 and lipid peroxidation, concomitant with increased activities and isozymes of catalase and superoxide dismutase as compared to respective controls. On the other hand ethrel treatment led to an increase in H_2O_2 and lipid peroxidation, concomitant with a decrease in the activities and isozymes of catalase and superoxide dismutase. Guaiacol peroxidase could be detected in the control or in treated fruits, activity of ascorbate, peroxidase was found to drastically increase in the presence of ethrel while 1-MCP treatment led to only a marginal increase in ascorbate, oxidase.

The activity of the enzyme polyphenoloxidase during ripening of fruit is reviewed by Mayer and Harel (1981). The polyphenoloxidase comprises a large group of enzymes, all of which are characterized by their ability to utilize molecular oxygen during the oxidation of phenolic substrates (Mayer and Harel, 1981). The polyphenol content of mango is high at the early part of the growth, which decreases during ripe stage and remains fairly steady (Lakshminarayana *et al.*, 1970). The loss of astringency in the course of ripening is associated with a loss of phenolic content (Selvaraj and Kumar, 1989). Increased polyphenoloxidase activity during ripening of 'malgoa' and 'Harumanis' mangoes paralleled the decrease in the ascorbic acid content of the fruit (Lazan *et al.*, 1986). Polyphenoloxidase activity increased marginally from harvest maturity to early ripe stage followed by decline during ripening in the varieties, Banganapalli, Dasheri, Fazli, and Langra, and declined steadily from harvest maturity to ripe stage in Alphonso, Suvarnarekha, and Totapari (Selvaraj and Rajiv Kumar, 1989). Galeazzi *et al.* (1981) observed an increase in the activity of polyphenoloxidase during ripening of banana. Polyphenoloxidase, the enzyme responsible for oxidation of polyphenols, increases in activity during ripening (Mowlah and Itoo, 1982), and this may be relevant to the reduction in the astringency of ripe guavas. Polyphenoloxidase (PPO) and peroxidase (POD) are the enzymes involved in the browning process of apple. Browning occurs almost instantly when the cell structure is destroyed, and the enzyme and substrate are mixed. PPO catalyses the hydroxylation of monophenols (Monophenolase) and Oxidation of O-disphenols to O-quinones (diphenolase), which subsequently polymerise to yield undesirable brown pigments in the presence of oxygen (Espin, *et al.,* 1998). Jang and Moon (2011) studied the inhibition of polyphenoloxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. Polyphenoloxidases comprise a large group of enzymes which can utilize molecular oxygen for the oxidation of phenolic substances. They are divided into two main groups: the catechol oxidases and the laccases which have been classified under the general name monophenol monooxygenase. The activity of PPO in grape berries is considerably affected by several factors, such as cultivar, developmental stage, and environmental conditions (Mayer and Harel, 1979; Wisseman and Lee, 1980; Sapis *et al.*, 1983 a,b). Polyphenoloxidase showed an increase in the course of ripening in 'Malgoa' and 'Harumanis' (Lazan *et al.*, 1986), and the increase occurred concomitantly with the decline in ascorbic acid levels. In the freshly harvested fruit, the acitivity measured was $<0.1\ A_{420\ nm}\ min^{-1}.g^{-1}$ fresh weight, using catechol as substrate. In contrast, the polyphenoloxidase activity, measured by using pyrogalol as substrate, generally declined in the course of ripening in several Indian varieties including the 'Alphonso' (Selvaraj and Kumar, 1989). Therefore the aim of the study was to find out the oxidative key enzyme in the phenolic dynature during ripening mango fruit.

Material and Methods

The detached fruits of *Mangifera indica* L. var. Neelum were selected for the present study. The off-season (September to February) green mature unripe fruits were harvested from Auroville near Pondicherry union territory, India and stored in cortons in the Department of Botany at room temperature 28±2°C with the relative humidity of 85 per cent. They were treated with different concentrations of ethrel (100, 200 and 300 ppm). All the experiments were conducted with seven replicates. The peel and pulp of the fruit material were used to study the ripening process.

Total phenols were extracted and estimated, following the method of Chandramohan, et al. (1973), and quantitative

estimation was done based on Bray and Thorpe (1954) method. Three grams of fruit material was homogenized in 10 ml of 80 per cent ethanol. The homogenate was filtered through a cheese cloth. The residue was extracted with 80 per cent ethanol and the filtrate was made upto 15 ml (1 g of material in 5 ml of ethanol). To 1 ml of the ethanolic extract, 1 ml of folin-ciocalteau reagent and 2 ml of 20 per cent sodium carbonate were added. The mixture was boiled in a boiling water bath for 1 minute. Then it was cooled immediately in running water and the volume was raised to 25 ml. The colour intensity was read at 725 nm in a Spectronic-20. The phenolic content was expressed in catechol equivalent.

Fruit material weighing about 100 mg was homogenized with 10 ml of 1.0 N phosphate buffer (pH 7.0) in a prechilled mortar and pestle. The homogenate was centrifuged at 24,000 rpm at 4° C in a refrigerated centrifuge for 30 minutes, and the aliquot was used as the source of the enzyme. Peroxidase activity was assayed by the method of Kumar and Khan (1982). Assay mixture of polyphenoloxidase contained 2 ml of 0.1 M phosphate buffer (pH 7.0) 1 ml of 0.01 M pyrogallol and 1 ml of well-diluted enzyme extract. This was incubated for 5 minutes at 25°C after which the reaction was stopped by adding 1 ml of 2.5 N H_2SO_4 to the assay mixture. The amount of purpurogalin formed was determined by taking the absorbance at 420 nm. The enzyme activity was expressed in absorbance units. Polyphenoloxidase activity was assayed by the method of Kumar and Khan (1982). Assay mixture of polyphenoloxidase contained 1 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml of 0.01 M pyrogallol and 1 ml of well-diluted enzyme extract. This was incubated for 5 minutes at 25°C after which the reaction was stopped by adding 1 ml of 2.5 N H_2SO_4 to the assay mixture. The amount of purpurogalin formed was determined by taking the absorbance at 420 nm. The enzyme activity was expressed in absorbance units. Catalase activity was assayed by the method of Vir and Grewal (1975). 2.5 ml of 0.1 M phosphate buffer (pH 6.4) was taken in a cuvette, and 0.1 ml of 2 per cent H_2O_2 and 0.2 ml of tissue extract was added. The reaction was read using a spectrophotometer at 230 nm for 75 seconds with an intervel of 15 seconds, after the addition of peroxidase. The cuvette containing tissue extract and buffer alone was used to adjust the absorbance to zero. The control was maintained using boiled tissue extract.

Results and Discussion

The table–1 shows the changes of phenolic content, which occur during the ripening of *Mangifera indica* fruits. The total phenolic content decreased during the course of ripening, both in the peel and pulp of treated and control fruits. The percentage of loss was more in the 200ppm ethrel treated fruits than in the 100, 300ppm and control. Phenolic compounds enjoy a wide distribution in the plant kingdom, and they are particularly prominent in the fruits where they are important in determining colour and flavour. Normally phenolic content decreases as the fruit matures (Williams, 1959). The level of phenolics in fruits varies widely from species to species, variety to variety, season to season and location to location. The great majority of the phenolic components found in fruits have no characteristic taste when tasted at low concentration in this pure form. The exception to this general rule is the sourness associated with phenolic acids, the astringency of condensed flavours and the bitterness associated with some of the citrus flavonoids (Van Buren, 1970). Aziz et al. (1976) observed a general decline in the total phenolic content in the pulp of banana fruits during ripening. The loss of phenol is more rapid in the peel. The polyphenol content of mango is high at the early part of the growth, which decreases during the ripe stage and remains fairly steady (Lakshminarayana et al., 1970). The loss of astringency in the course of ripening is associated with the loss of phenolic content (Selvaraj and Kumar, 1989). In Sapota, similar observation was reported by Lakshminarayana and Subramanyam (1966). Under normal ripening conditions, mango polyphenol content is highest during fruit growth, decreasing with ripening (Lakshminarayana *et al.*, 1970, Selvaraj and Kumar, 1989). Polyphenolic compounds commonly serve as a protective mechanism in plants, warding off predator and microbiological attack. Many factors affect polyphenolic concentrations, including cultivar differences, growing conditions, maturity, and post-harvest handling of fruits (Hakkinen and Torronen, 2000; Lakshminarayana *et.al.*, 1970; Selvaraj and Kumar, 1989; Wang and Lin, 2000). Abiotic stresses such as excessive UV, heat and chilling temperatures, wounding and drought that are introduced before or after fruit harvest may affect the biosynthetic (Cisneros – Zevaloos, 2003), as fruits attempt to protect themselves. Under normal ripening conditions, mango polyphenol content is highest during fruit growth, decreasing with ripening (Lakshminarayana et.al., 1970, Selvaraj and Kumar, 1989). These observations indicate a correlation between fruit ripening and loss of secondary metabolic substrates. Similarly Tamilmani (2005) studied phenolic degradation during the ripening of bitter gourd fruit (*Momordica charantia* L.). Riberio et al. (2008) studied the phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera india* L.) varieties. Gruz *et al.* (2011) studied the phenolic acid content and radical scavenging activity of extracts from medilar (*Mespilus germanica* L.) fruits at different stages of ripening. The decreased phenolic content during ripening was associated with the increased activity of oxidative enzymes such as peroxidase, polyphenoloxidase, and catalase. The activities of the enzymes Perixidase, Polyphenoloxidase and Catalase gradually increased during the course of ripening, both in the treated and control fruits. The Peroxidase, Polyphenoloxidase and Catalase activities were higher in the peel than in the pulp throughout the ripening period. Among the three enzymes, the Polyphenoloxidase activity was more than that of Peroxidase and Catalase, both in the control and treated fruits. Among the different treatments of ethrel during the ripening, the fruits treated with 200ppm ethrel had more activities of Peroxidase, Polyphenoloxidase and Catalase than that of the 100, 300ppm and control. (Tables–2, 3 & 4). Similar increases in the activity of the enzyme catalase and peroxidase have also been reported by Rothan et al. (1989) and Silva et al. (1990). The increases in peroxidase activities in fruits and vegetables also have been associated with decreased quality (Burnette, 1977). Poorinimasingh and Dwivedi (2008) report the effect of 1-Methylcyclopropene (1-MCP) and ethrel on antioxidant levels in mango fruit during ripening. Use of 1-MCP is applied commercially to delay ripening while ethrel is used to accelerate the ripening of climacteric fruits.1-MCP treatment led to decreased levels of H_2O_2 and lipid peroxidation, concomitant with increased activities and isozymes of catalase and superoxide dismutase as compared to respective controls. On the other hand ethrel treatment led to an increase in H_2O_2 and lipid peroxidation, concomitant with a decrease in the activities and isozymes of catalase and superoxide dismutase. Guanaco peroxidase could to be detected in the control or in treated fruits, activity of ascorbate, Peroxidase was found to drastically increase in the presence of ethrel, while 1-MCP treatment led to only a marginal increase in ascorbate oxidase. In this study, as the enzymes catalase, peroxidase and polyphenoloxidase increased during ripening, the amount of total phenols decreased. This gives an indication that the decreased phenolic content may be due to the oxidation of phenols by the enzymes, catalase, peroxidase, and polyphenoloxidase. Of these three enzymes, polyphenoloxidase activities were the highest while catalase activities were the lowest. This shows that the polyphenoloxidase is the key enzyme involved in the oxidation of phenols. Polyphenoloxidase, the enzyme responsible for oxidation of polyphenols, increases the activity during ripening (Mowlah and Itoo, 1982), and this may be relevant to the reduction in the astringency of ripe guavas

Table 1: Effect of ethrel on the phenolic changes during the ripening of off-season fruits of *Mangifera indica* L. var. Neelum (Values are Mean ± SE of 7 samples expressed in mg/g fr. wt.)

Days	Peel				Pulp			
	Control	100 ppm	200 ppm	300 ppm	Control	100 ppm	200 ppm	300 ppm
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
1	0.73 ± 0.058	0.72 ± 0.058	0.74 ± 0.059	0.74 ± 0.059	0.13 ± 0.010	0.13 ± 0.010	0.14 ± 0.011	0.13 ± 0.010
3	0.66 ± 0.046	0.68 ± 0.048	0.70 ± 0.049	0.67 ± 0.047	0.11 ± 0.008	0.12 ± 0.008	0.13 ± 0.009	0.12 ± 0.008
5	0.59 ± 0.035	0.64 ± 0.038	0.64 ± 0.038	0.61 ± 0.037	0.10 ± 0.006	0.11 ± 0.007	0.12 ± 0.007	0.11 ± 0.006
7	0.54 ± 0.027	0.60 ± 0.030	0.61 ± 0.030	0.55 ± 0.028	0.09 ± 0.005	0.10 ± 0.005	0.11 ± 0.005	0.10 ± 0.005
9	0.49 ± 0.039	0.56 ± 0.045	0.57 ± 0.046	0.52 ± 0.042	0.08 ± 0.006	0.09 ± 0.007	0.10 ± 0.008	0.09 ± 0.007
11	0.42 ± 0.029	0.52 ± 0.036	0.53 ± 0.037	0.49 ± 0.034	0.07 ± 0.005	0.08 ± 0.006	0.09 ± 0.006	0.08 ± 0.006
13	0.35 ± 0.021	0.48 ± 0.028	0.50 ± 0.030	0.44 ± 0.026	0.06 ± 0.004	0.07 ± 0.004	0.08 ± 0.005	0.07 ± 0.004
15	0.30 ± 0.015	0.42 ± 0.022	0.46 ± 0.023	0.40 ± 0.020	0.05 ± 0.003	0.06 ± 0.003	0.08 ± 0.004	0.06 ± 0.003

SE – Standard Error ppm – Parts per million

Table 2: Effect of ethrel on the peroxidase changes during the ripening of off-season fruits of *Mangifera indica* L. var. Neelum (Values are Mean ± SE of 7 samples expressed in units/mg protein/min)

Days	Peel				Pulp			
	Control	100 ppm	200 ppm	300 ppm	Control	100 ppm	200 ppm	300 ppm
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
1	0.048 ± 0.0038	0.045 ± 0.0036	0.041 ± 0.0033	0.049 ± 0.0039	0.018 ± 0.0014	0.017 ± 0.0013	0.018 ± 0.0014	0.016 ± 0.0012
3	0.058 ± 0.0040	0.051 ± 0.0035	0.046 ± 0.0032	0.065 ± 0.0045	0.026 ± 0.0018	0.023 ± 0.0016	0.024 ± 0.0017	0.021 ± 0.0014
5	0.064 ±0.0038	0.057 ± 0.0034	0.051 ± 0.0030	0.071 ± 0.0042	0.036 ± 0.0022	0.028 ± 0.0017	0.034 ± 0.0020	0.024 ± 0.0014
7	0.072 ± 0.0036	0.065 ± 0.0032	0.058 ± 0.0029	0.079 ± 0.0039	0.045 ± 0.0023	0.035 ± 0.0018	0.038 ± 0.0019	0.029 ± 0.0015
9	0.078 ±0.0062	0.071 ± 0.0056	0.065 ± 0.0052	0.085 ± 0.0068	0.055 ± 0.0044	0.041 ± 0.0032	0.048 ± 0.0038	0.034 ± 0.0027
11	0.085 ± 0.0059	0.078 ± 0.0054	0.072 ± 0.0050	0.092 ± 0.0064	0.066 ± 0.0046	0.051 ± 0.0035	0.058 ± 0.0040	0.045 ± 0.0031
13	0.094 ± 0.0056	0.088 ± 0.0052	0.078 ± 0.0047	0.103 ± 0.0061	0.079 ± 0.0047	0.063 ± 0.0038	0.069 ± 0.0041	0.056 ± 0.0033
15	0.105 ± .0052	0.096 ± 0.0048	0.085 ± 0.0042	0.118 ± 0.0059	0.098 ± 0.0049	0.077 ± 0.0039	0.085 ± 0.0042	0.067 ± 0.0034

SE – Standard Error ppm – Parts per million

Table 3: Effect of ethrel on the polyphenoloxidase changes during the ripening of off-season fruits of *Mangifera indica* L. var. Neelum (Values are Mean ± SE of 7 samples expressed in units/mg protein/min)

Days	Peel				Pulp			
	Control	100 ppm	200 ppm	300 ppm	Control	100 ppm	200 ppm	300 ppm
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
1	0.056 ± 0.004	0.059 ± 0.005	0.064 ± 0.005	0.062 ± 0.005	0.031 ± 0.002	0.032 ± 0.003	0.032 ± 0.003	0.033 ± 0.003
3	0.065 ± 0.005	0.067 ± 0.005	0.072 ± 0.005	0.069 ± 0.005	0.038 ± 0.003	0.041 ± 0.003	0.042 ± 0.003	0.041 ± 0.003
5	0.072 ± 0.004	0.075 ± 0.005	0.081 ± 0.005	0.079 ± 0.005	0.045 ± 0.003	0.048 ± 0.003	0.056 ± 0.003	0.051 ± 0.003
7	0.080 ± 0.004	0.084 ± 0.004	0.092 ± 0.005	0.088 ± 0.004	0.054 ± 0.003	0.057 ± 0.003	0.063 ± 0.003	0.059 ± 0.003
9	0.091 ± 0.007	0.095 ± 0.008	0.105 ± 0.008	0.098 ± 0.008	0.061 ± 0.005	0.065 ± 0.005	0.072 ± 0.006	0.068 ± 0.005
11	0.104 ± 0.007	0.108 ± 0.008	0.116 ± 0.008	0.113 ± 0.008	0.072 ± 0.005	0.076 ± 0.005	0.082 ± 0.006	0.079 ± 0.006
13	0.108 ± 0.006	0.113 ± 0.007	0.139 ± 0.008	0.122 ± 0.007	0.084 ± 0.005	0.087 ± 0.005	0.112 ± 0.007	0.092 ± 0.006
15	0.115 ± 0.006	0.123 ± 0.006	0.144 ± 0.007	0.136 ± 0.007	0.101 ± 0.005	0.110 ± 0.006	0.132 ± 0.007	0.123 ± 0.006

SE – Standard Error ppm – Parts per million

Table 4: Effect of ethrel on the catalase changes during the ripening of off-season fruits of *Mangifera indica* L. var. Neelum (Values are Mean ± SE of 7 samples expressed in units/mg protein/min)

Days	Peel				Pulp			
	Control	100 ppm	200 ppm	300 ppm	Control	100 ppm	200 ppm	300 ppm
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
1	0.043 ± 0.003	0.045 ± 0.004	0.046 ± 0.004	0.045 ± 0.004	0.057 ± 0.005	0.058 ± 0.005	0.062 ± 0.005	0.066 ± 0.005
3	0.048 ± 0.003	0.052 ± 0.004	0.054 ± 0.004	0.053 ± 0.004	0.063 ± 0.004	0.065 ± 0.005	0.067 ± 0.005	0.071 ± 0.005
5	0.054 ± 0.003	0.064 ± 0.004	0.070 ± 0.004	0.072 ± 0.004	0.067 ± 0.004	0.071 ± 0.004	0.075 ± 0.005	0.073 ± 0.004
7	0.062 ± 0.003	0.073 ± 0.004	0.098 ± 0.005	0.086 ± 0.004	0.075 ± 0.004	0.088 ± 0.004	0.094 ± 0.005	0.102 ± 0.005
9	0.079 ± 0.006	0.085 ± 0.007	0.116 ± 0.009	0.099 ± 0.008	0.081 ± 0.006	0.092 ± 0.007	0.099 ± 0.008	0.108 ± 0.009
11	0.094 ± 0.007	0.098 ± 0.007	0.121 ± 0.008	0.114 ± 0.008	0.091 ± 0.006	0.096 ± 0.007	0.112 ± 0.008	0.116 ± 0.008
13	0.106 ± 0.006	0.118 ± 0.007	0.136 ± 0.008	0.127 ± 0.008	0.098 ± 0.006	0.102 ± 0.006	0.118 ± 0.007	0.126 ± 0.008
15	0.118 ± 0.006	0.129 ± 0.006	0.146 ± 0.007	0.138 ± 0.007	0.103 ± 0.005	0.112 ± 0.006	0.123 ± 0.006	0.129 ± 0.006

SE – Standard Error ppm – Parts per million

References

Aziz, A.B.A., Abdul Wahah, F.K., and Elghandour, M.A. (1976). Effect of different storage temperature on phenolic compounds in banana and mango fruit. *Hort. Sci.* **4**, 309 – 315.

Bray, H.G., and Thorpe, W.V. (1954). Analysis of phenolic compounds of interest metabolism. *Meth. Biochem. Anal.* **1**, 27 – 52.

Burnette, F.S. (1977). Peroxidase and its relationship to food flavor and quality: a review. *J. Fd. Sci.* **42**, 1-6.

Calderon, A.A., Zapata, J.M., Munoz, R., and Barcelo, A.R. (1993). Localization of peroxidase in grapes using nitrocellulose blotting of freezing – thawing fruits. *Hort. Sci.* **28**, 38 – 40.

Chan, H.T., Tam, S.Y.T. and Koide, R.T. (1978). Isolation and characterization of catalase from papaya. *J. Fd. Sci.* **43**, 989-991.

Chandramohan, D., Purushothama, D., and Kothandaram, R. (1973). Soil phenols and plant growth inhibition, *Pl. and Soil.* **39**, 303 – 308.

Cisneros – Zevallos, L. (2003). The use of controlled post-harvest abiotic stressed as a tool for enhancing the nutracentical content and adding – value of fresh fruits and vegetables. *J. Fd. Sci.* **68**, 1560-1565.

Da Silva, E., Lourenco, E.J. and Neves, V.A. (1990). Soluble and bould peroxidases from papaya fruit. *Phytochem.* **29**, 1051-1056.

Desai, B.B., and Deshpande, P.B. (1978). Effect of storage maturity on some physiological and biochemical constituents and enzyme activities of banana (*Musa paradisica* L.) fruit. *Mysore J. Agri. Sci.* **12**, 193 – 201.

Dilley, R.D. (1970). Enzymes. In:, The Biochemistry of fruit and their products, (ed.A.C. Hulme), Academic Press, London and New York, pp. 179 – 204.

Espin, J.C., Garcia – Ruiz, P.A., Tudela, J., Varon, R., & Garcia – Canovas, F. (1998). Monophenolase and diphenolase reaction mechanisms of apple and pear polyphenol oxidases. *J. Agric. Fd. Chem.* **46**, 2968-2975.

Frenkel, C. (1972). Involvement of peroxidase and indole 3-acetic acid oxidase isozymes from pear, tomato and blueberry fruit in ripening. *Pl. Physiol.* **49**, 757–763.

Galeazzi, M.A., Sgarbieri, V.C., and Constantinides, S.M. (1981). Isolation, purificaiton and physio-chemical characterization of polyphenoloxidase from banana. *J. Fd. Sci.* **46**, 150 – 155.

Gordon, A.R., and Alldridge, N.A. (1971). Cytochemical localization of peroxidase. A in developing stem tissues of extreme dwarf tomato. *Con. J. Bot.* **49**, 1487 – 1496.

Gruz, J., Ayaz, F.A., Torun, H., Srnad, M. (2011). Phenolic acid content and radical Scavenging activity of extracts from medlar (*Mespilus germanica* L.) fruit at different stages of ripening. *Fd. Chem.* **124**, 271-277.

Haard, N.F. (1973). Upsurge of particulate peroxidase in ripening banana fruit. *Phytochem.* **12**, 555-560.

Haard, N.F. and Tobin, L. (1971). Patterns of soluble peroxidase in ripening banana fruit. *J. Fd. Sci.* **36**, 854-857.

Haard, N.F., and Tible, D. (1973). Chilling injury in green banana fruit: changes in peroxidase isozyme in soluble and particulate pools. *J. Fd. Sci.* **38**, 642 – 645.

Hakkinen, S., and Torronen, A. (2000). Content of flavonols and Selected phenolic acids in Strawberries and Vaccinicium Species: Influence of cultivar, cultivation site and technique, *Fd. Res. Int.* **33**, 517-524.

Jang, J.H., and Moon, K.D., (2011). Inhibition of polyphenol oxidase and peroxidase activities on fresh-cut apple by simultaneous treatment of ultrasound and ascorbic acid. *Fd. Chem.***124**, 444-449.

Jarret, R.L. and Litz, R.E. (1986). Isoenzymes as genetic markers in bananas and plantains. *Euphytica*, **35**, 539-549.

Joshi, P.R., and Shiralkar, N.K. (1977). Polyphenolases of local variety of mango. *J. Fd. Sci. Technol,* **14**, 77 – 79.

Ketsa, S., Phakawatmongkol, W., and Subhadrabhandhu, S. (1999). Peel enzymatic activity and colour changes in ripening mango fruit. *J. Plant Physiol.* **154(3)**, 363–366.

Kubo, I., & Matsumoto, A. (1984). Mollusciscides from Olive Olea europea and their efficient isolation by counter current chromatogrphies. *J. Agri. Fd. Chem.* **32**, 687-688.

Kumar, K.B., and Khan, P.A. (1982). Peroxidase and polyphenoloxidase in excised ragi (*Elusine carocana* Cv PR 202) leaves during senescence. *Ind. J. Exp. Bot.* **20**, 412 – 416.

Kuroda, M., Ozawa, T., and Imagama, H. (1990). Changes in chloroplast peroxidase activities in relation to chlorophyll loss in barley leaf segments. *Plant Physiol.* **80**, 555 – 560.

Lakshminarayana, S., and Subramanyam, H. (1966). Physcial, chemical and physiological changes in sapota fruit (*Achras sapota* L.) during development and ripening. *J. Fd. Sci. Technol.* **3**, 151 – 54.

Lakshminarayana, S., Subhadva, N.V., and Subramanyam, W. (1970). Some aspects of developmental physiology of the mango fruit *J. Hort. Sci.* **45**, 133 – 42.

Lal, B.L., Gunjate, R.T., and Salvi, M.J. (1985). Effect of post–harvest ethephon dipping of fruits on occurrence of spongy tissue disorder in Alpheniso mango. *Ind. J. Plant Physiol.* **28**, 85 – 87.

Lazan, H., Ali, Z.M., Lee, K.W., Voon, J., and Chaplin, G.R. (1986). The potential role of polygalacturonase in pectin degradation and softening of mango fruit. *ASEAN Fd. J.* **2**, 93 – 98.

Lazan, H., Ali, Z.M.A., Lee, K.W., Voon, J. and Chaplin, G.R. (1986). The potential role of polygalacturonase in pectin degradation and softening in mango fruit. *ASEAN Fd. J.* **2**, 93-95.

Leopold, A.C., and Kriedmen, P.E. (1975). (ed.) *Plant growth and development*. McGrow Hill Publication, London.

Lima, M.A. C. De., Alves, R.E., Assis, J.S. De., Filgueiras, H.A.C. and Costa, J.T.A (2000). Quality, phenols and oxidative enzymes of 'Italia' grape under calcium influence, during ripening. *Pesquisa Agropecuaria Brasileria*, **35(12)**, 2493 – 2499.

Macheix, J.J., Flewriet, A., & Billot, J.(ed) (1990). *Fruit Phenolics*. Boca Raton, Florida: CRC Press.

Martinoia, E., Dalling, M.J., and Matile, P.H. (1982). Catabolism of chlorophyll: Demonstration of chloroplast localized penomidnt and exidative actuvutues. *Z. Pflamzen Physiol.* **107**, 269 – 279.

Masia, A. (1988). Superoxide dismutase and catalase activities in apple fruit during ripening and post-harvest and with special reference to ethylene. *Physiol. Pl.* **104,** 668-672.

Mattoo, A.K., Modi, V.V., and Reddy, V.V.R. (1968). Oxidation and carotenogenesis regulating factors in mangoes. *Ind. J. Biochem.* **5**, 111-114.

Mayer, A.M., and Harel, E. (1981). Polyphenoloxidases in fruit–changes during ripening. In *Recent Advances in the Biochemistry of fruits and vegetables*, (eds. J. Friend and M.J.C. Rhodes), Academic Press, London and New York. pp. 159 – 177.

Mayer, A.M. and Harel, E. (1979). Polyphenoloxidases in plants. A Rev. *Phytochem.* **18**, 193-215.

Miesle, T.J., Proctor, A., and Lagrimini, L.M. (1991). Peroxidase activity, isoenzymes and tissue localization in developing highbush blue berry fruit. *J.Am. Soc. Hort. Sci.***116**, 827 – 830.

Miller, A.R., Dalmasso, J.P., and Kretchmen, D.W. (1988). Developmental variation of cell wall degrading enzymes from cucumber (*Cucumis sativus*) fruit tissues. *Can. J. Bot.* **67**, 817 – 821.

Miller, A.R., Kelley, T.J., and Mujer, C.V. (1990). Anodic peroxidase isoenzymes and polyphenoloxidase activity from cucumber fruit: tissue and substrate specificity. *Phytochem.* **29**, 705 – 709.

Mowlah., and Itoo, S. (1982). Quantitative changes in guava polyphenols and the polyphenoloxidase at different stages of maturation, ripening and storage. *Nippon shokuhin kogyo Gakknishi* **29**, 413 – 417.

Neish, A.C. (1964). Major pathways of biosynthesis of phenols in *Biochemistry of phenolic compounds*, (ed. J.B. Harborne), Academic Press, London, pp. 295 – 359.

Pal, D.K., and Selvaraj, Y. (1987). Biochemistry of papaya (*Carica papaya*) fruit ripening: changes in RNA, DNA, protein and enzymes of mitochondrial. Carbohydrate, respiratory and phosphate metabolism. *J. Hort. Sci.* **62**, 117 - 126.

Prabha, T.N., and Bhagyalakshmi, N. (1998). Carbohydrate metabolism in ripening banana fruit. *Phytochem.* **48(6)**, 915 – 919.

Prakash, K., and Tamilmani, C. (2002). Influence of polyethylene bag Storage on the pigment changes during the ripening of fruits of chilli (*Capsicum annuum* L). *J. A.U.*

Preiss, J., and Levi, C. (1980) Starch biosynthesis and degradation. In: "*The Biochemistry of Plants*" – A comprehensive treatise. (ed. J. Preiss), Academic Press, vol. **13**, 371 – 424.

Quesada, M.A., Sanchez – Roldan, C., Heredia, A., Valpuesta, V., and Bukovae, M.J. (1992). Peroxidase and IAA oxidase activities and peroxidase isoenzymes in the pericarp of seeded and seedless 'Redhaven' Peach fruit. *J. Plant Grwoth Regul.* **11**: 1-6.

Rao, D.V., and Chundawat, B.S. (1989). Post-harvest changes in respiration and enzyme activities in sapota (*Manikkara achras mill forsberg*). *Ind. J. Pl. Physiol.* **32**, 105 – 109.

Reddy, Y.V., and Srivastava, G.C. (1999). Ethylene biosynthesis and respiration in mango fruits during ripening. *Ind. J. Pl. Physiol.* **4(1)**, 32 – 35.

Riberio, S.M.R., Barbos, L.C.A., Queiroz, J.H., Knodler, M., and Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Fd. Chem.* **110**, 620-626.

Riberio, S.M.R., Barbosa, L.C.A. Queiroz, J.H., Knodler, M., and Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L). varieties. *Fd. Chem.* **110**, 620-626.

Robards, K., Prenzler, P.D., Tucker, G., Swatsitang, P., and Glover, W. (1999). Phenolic Compounds and their role in oxidative processes in fruits. *Fd. Chem.* **66**, 401-436.

Rothan, C., and Nicolas, J. (1989). Changes in acidic and basic peroxidase activities during tomato fruit ripening. *Hort. Sci.* **24**, 340 – 342.

Sacher, J.A. (1973). Senescence and post-harvest physiology. *Ann. Rev. Pl. Physiol.* **24**, 197 – 224.

Sapis, J.C., Machiex, J.C. and Cordonnier, R.E. (1983a). The browning capacity of grapes. I. Changes in polyphenol oxidase activities during development and maturation of the fruit. *J. Agri. Fd. Chem.* **31**, 342-345.

Sapis, J.C., Macheix, J.C. and Cordonnier, R.E. (1983b). The browning capacity of grapes. II. Browning potential and polyphenol oxidase activities in different mature grape varieties. *Am. J. Enol. Viti.* **34**, 157-162.

Selvaraj, Y., and Kumar, R. (1989). Studies on fruit softening enzyme and polyphenoloxidase activity in ripening mango (*Mangifera indica* L.) fruit. *J. Fd. Sci. Technol.* **26**, 218 – 222.

Selvaraj, Y., and Rajivkumar. (1989). Studies on fruit softening enzymes and polyphenoloxidase activity in ripening mango (*Mangifera indica* L.) fruit. *J.Fd. Sci. Technol.* **26**, 218 – 22.

Silva, E.D., Lourenco, J.L., and Neves, V.A. (1990). Soluble and bound peroxidases from papaya fruit. *Phytochem.* **29**, 1051 – 1056.

Singh, P., and Dwivedi, U.N. (2008). Effect of Ethrel and 1-Methylcyclopropene (1-MCP) on antioxidants in Mango (Mangifera indica Var. Dashehari) during fruit ripening. *Fd. Chem.* **111**, 951-956.

Singh, P., and Dwivedi, U.N. (2008). Purification and characterization of Multiple forms of polygalacturonase from mango (*Mangifera indica* CV. Dashehasi) fruit. *J. Fd. Chem.* **111**, 345-349.

Steup, M. (1988). Starch degradation. In: "The Biochemistry of Plants" - A comprehensive treatise. (ed. J. Preiss), Academic Press, vol. **13**, 225 – 296.

Subramanyam, H., Narayanamoorthy, N.V., Lakshmi-narayana, S., and Santha, K. (1972). Studies on harvesting transport and storage of mango. *Acta Hort.* **24**, 260 – 264.

Tamilmani, C. (2005). Phenolic Degradation during the Ripening of Bitter gourd Fruit (*Momordica charantia* L). *Biosci. Biotech. Res. Asia*. Vol.**3(2)**, 411-413.

Tan, S.C. and Weinheimer, E.A. (1976). The isoenzyme patterns of developing fruit and mature leaf of papaya (*Carica papaya* L.). *Saius Malaysiana*, **5**, 7-14.

Thomas, R.L., Jen, J.J., and Moore, C.V. (1981). Changes in soluble and bound peroxidase and IAA oxidase during tomato fruit development. *J. Fd. Tech.* **47**, 158 – 161.

Tomas–Barberan, F.A., Gil, M.I., Cremin, P., Waterhouse, A.L., Hess – pierce, B., and Kader, A.A. (2001). HPLC – DAD – ESIMS analysis of phenolic compounds in nectarines, peaches and plums. *J. Agri. Fd. chem.* **49(10)**, 4748 – 4760.

Toraskar, M.V. and Modi, V.V. (1984). Peroxidase and Chilling injury in banana fruit. *J. Agri. Fd. Chem.* **32**, 1352-1354.

Van Buren, J. (1970). Fruit phenolics in *The Biochemistry of fruit and their products*, (ed. A.C. Hulme), Academic Press, London and New York, pp. 269 – 300.

Venkaiah, B., and Babu, C.K. (1977). Polyphenols in the aqueous acetone extracts of the peel of ripening fruit. *Pl. Biochem.* **4**, 39 – 42.

Vir, S., and Grewal, J.S. (1975). Changes in the catalase of gram plant induced by ascochyta rabiei infection. *Ind. J. Phytopath.* **28**, 223 – 225.

Wang, S., and Lin, H. (2000). Antioxidant activity of fruits and leaves of blackberry raspberry and strawberry varies with cultivar and development stage. *J. Agric. Fd. Chem.* **48**, 140-146.

Williams, D.H. (1959). Beriont der wirsenschaftech technician kamission der internationalen frucht sof. *Urions*, pp. 259 – 263.

Wisseman, K.W. and Lee, C.Y. (1980). Polyphenol oxidase activity during grape maturation and wine production. *Am. J. Eno. Viti.* **31**, 206-211.

Yoshioka, T. (1979). Changes in content of carbohydrate and enzyme of carbohydrate metabolism during ripening of chill injured banana fruit. *J. Jap. Soc. Fd. Sci. Tech.* **26**, 57–64.

.

ISSN: 2220-4822