REGULAR ARTICLE

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

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KEYWORDS

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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 fruit firmness and pectin decreased during ripening, both in the treated and control fruits. On the other hand, polygalacturonase, activity 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 is a complex genetically programmed process involving increases in respiration and ethylene production, changes in colour and flavor, and softening. Softening is associated with structural changes in the cell wall including reduction in the size of hemicellulose, loss of galactose sidechains, and solubilization and depolymerization of pectin (reviewed by Fischer and Bennett, 1991). Pectins are likely to be the key substances involved in the mechanical strength of the primary cell wall which are important to the physical structure of the plant (Sirisomboon, *et al.,* 2000). Their degradation during ripening seems to be responsible for tissue softening, as reported for a number of fruits including tomato (Poovaiah and Nukuya, 1979; Seymour, *et al.,*1987), Kiwi (Redgwell, *et al.,*1992), apple (De-vries, *et al.,* 1984), and bush butter (Missang, *et al.,* 2001a). The major changes in the cell wall structure are the dissolution of middle lamella and primary cell wall during ripening. Thus, elucidation of the chemical structure of pectin is essential in understanding its role in plant growth/development and during ripening of fruits (Thakur *et al.*, 1997). Plant pectin is one of the major contributors for maintaining the texture of fruits and vegetables. It is enriched in the cell middle lamella cellular junctions which are thought to largely contribute to cell-cell adhesion and tissue cohesion, while the cellulose-hemicellulose network plays a role in rigidity. As pectin is an important component for texture, the degradation of pectin would affect fruit ripening. The degradation leads to the disassembly of cellulose–hemicellulose network and accelerates the fruit softening rate (Duan *et al.*, 2008).

Most fruits soften during ripening and this is a major quality attribute that often dictates shelf-life. Fruit softening could arise from three mechanisms: loss of turgor; degradation of starch; or breakdown of the fruit cell walls. Loss of turgor is largely a non-physiological process associated with the post-harvest dehydration of a fruit, and as such can assume commercial importance during storage. Loss of water equivalent to about 5-10% of a fresh fruit fresh weight, although having little effect on the fruit's biochemistry, can render the fruit commercially unacceptable. Degradation of starch probably results in a pronounced textural change, especially in those fruits like banana, where starch accounts for a high percentage of the fresh weight. In general, however, texture change during the ripening of most fruits is thought to be largely the result of cell wall degradation. Carobohydrate polymers make up 90-95% of the structural components of the wall, the remaining 5-10% being largely hydroxyproline–rich glycoprotein (HPRG). The carbohydrate polymers can be grouped together as cellulose, hemicelluloses or pectins (Tucker, 1993). The textural changes, which occur during fruit ripening, are thought to be related to alterations in cell wall structure (Huber, 1983; Tucker and Grierson, 1987). Changes in cell wall structure during ripening have been observed under electron microscope in many fruits, including avocado (Pesis *et al.*, 1978), pear (Ben – Arie *et al.*, 1979) and tomato (Crookes and Grierson, 1983). These changes usually consist of an apparent dissolution of the pectin–rich middle lamella region of the cell wall. At a biochemical level, major changes can be observed in the pectic polymers of the wall. During ripening, there is a loss of neutral sugars; in most fruits this is predominatly galactose, but some loss of arabinose also occurs (Tucker and Grierson, 1987 and Seymour *et al.*, 1990).

The loss of firmness during ripening has been associated with the nature and proportion of the pectic substances, showed that total pectin increased in ripening papaya and reached a maximum value in overripe fruits. The

increase in water soluble pectin corresponds to the decrease in protopectin. The water soluble pectin level was maximum when the fruit ripened (Biwas *et al.*, 1969; the pectic material extracted from unripe pulp contained D-galactose, D-galacturonic acid L-arabinose as major constitutent sugars with a trace of rhamnose. The pectic acid was a mixture of 1→3 and 1→4 linked galacturonase. Selvaraj *et al.* (1982) observed the highest pectin content at ripe stage in papaya varieties of Coorg, Honey Dew, Sunrise and Thailand and a pre-ripe stage in varieties pink flesh sweet and Washington. In jack fruit, pulp contained very little tannins, and their concentration decreased during ripening (Selvaraj and Pal, 1989).   Misoon *et al.* (2005) studied changes in pectic substances and cell wall degrading enzymes during tomato fruit ripening. Xin *et al.* (2010) studied morphology, profile and role of chelate-soluble pectin on tomato properties during ripening. Toboada *et al.* (2010) studied isolation and characterization of pectic substances from musta (*Ugni molinae* Turcz) fruits.

The activity of polygalacturonase was studied by several workers in details (Rhodes, 1980. Goodenough *et al.*, 1982;Grierson, 1987; Lazan *et al.*, 1989; Downs and Brady, 1990; Mitcham and McDonald 1992; Tucker, 1993 , and Lazan and Ali, 1993. Ketsa *et al.*, 1998; and Sheng Jipping *et al.*, 2000;Payasi and Snawal .2003; and Misoon *et al., 2005).* The decrease in fruit firmness is a general feature that accompanies ripening of both climacteric and non-climacteric fruits. Softening is brought about by changes in cell wall constituents among which pectic substances play a major role (Pilink and Voragen, 1970). Four enzymes are involved in the pectic degradation process namely polygalacturonase, cellulase pectic methylesterase and -galctoside. Polygalacturonase is the major enzyme involved in pectin metabolism during fruit ripening and is associated with cell wall breakdown, fruit softening and loss of tissue integrity. Large amount of Polygalacturonase enzyme accumulates specifically during fruit ripening. Polygalacturonase hydrylyses the (1-4) linkage between adjacent demethylated galacturonic acid residues, whereas cellulase hydrolyses the β (1-4) linkage between adjacent glucose residues. Pectinesterase acts to remove the methyl group from the C-6 position of a galacturonic acid (Tucker, 1993). A major enzyme involved in the solubilisation of pectin is the cell-wall bound polygalacturonase (PG), which acts on de-esterified pectin molecules and attacks linkage between galacturonic acid groups in the polyuronide. Two types of polygalacturonase have been found. An exo–polygalacturonase acts by cleaning free galacturonic acid groups from the non-reducing end and an endo–polygalacturonase cleaves polyuronide chains in a random fashion, as in peaches, pears and tomatoes (Rhodes, 1980). In mangoes, the ripening which is characterized by changes in tissue softness is believed to be initiated in inner mesocarp tissue close to the seed, and to progress outward (Lazan and Ali, 1993). While pectin solubilization in inner and outer mesocarp tissues is comparable, pectin depolymerization appears to begin earlier in the inner mesocarp than in the outer mesocarp tissue (Lazan and Ali, 1993). Mitcham and McDonald (1992) studied the cell wall modification during ripening of Keitt and Totommy Atkin mango fruit. Both the varieties increased Polygalacturonase activity during ripening. Recently three multiple forms of polygalacturonase have been reported from Alphonso cultivar of mango (Prasanna *et al.*, 2006). Softening in mango has been reported to be accompanied by a decline in pectin (both water and alkaline soluble). (Roe and Bruemner, 1981). Furthermore, this decline in alkaline soluble pectin was found to be correlated with the loss of firmness of the mango fruit and was also closely correlated with the increase in PG activity during fruit ripening Payasi, and Sanwal (2003) observed that in banana the polygalacturonase activity during fruit ripening increased the maximum activity on 22nd day. Misoon *et al.* (2005) observed that in tomato during fruit ripening PG activity progressively increased. The aim of this work was to study the effect of ethrel on the softening of off-season fruits of mango.

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 $28\pm2°C$ 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.   Fruit firmness was studying the method of Ranganna (1977).Pectin was estimated, following the method of Ranganna (1977). Hundred mg of fruit sample was dissolved in 100 ml of 0.05 N NaOH and allowed to stand for 30 minutes to deesterify the pectin. From the above solution, 2 ml was taken and made upto 100 ml with distilled water; 2 ml of the deesterified pectin solution and 1 ml of carbazole reagent was added. A white precipitate was obtained and12 ml of conc. H_2SO_4 was added with constant stirring. The tubes were closed with rubber stopper and allowed to stand for 10 minutes until the colour was developed. After 15 minutes, acid was added and absorbance was read at 525 nm Spectronic – 20. Polygalacturonase was assayed following the method of Nelson (1944) as modified by Somogyi (1952). 200 mg of fruit material was homogenized with   10 ml of 75 M acetate buffer in prechilled mortar and pestle. The homogenate was centrifuged in a clinical centrifuge, and supernatant was used as enzyme source. Enzyme assay was initiated by mixing 2.5ml of substrate stock solution and 0.5 ml of enzyme sample plus water. The mixture was incubated at 30°C and 5 ml of samples was removed from the reaction mixture at timed intervals and immediately mixed with 0.5 ml of copper reagent. Sample tubes were subsequently incubated in a vigrously boiling water bath. The sample tubes were closed by morble for 10 minutes. After the tubes have cooled to room temperature, and 1.00 ml of the arsenomolybdate reagent was added. After 30 minutes of incubation at room temperature, the assay mixture was centrifuged in a clinical centrifuge. Then the residual precipitate was removed and the supernatant was measured in a Spectronic – 20 at 500 nm. The values are expressed in absorbance unit.

Results and Discussion

The Table 1 shows the changes of fruit firmness during the different stages of fruit ripening of *Mangifera indica*. The fruit firmness gradually decreased from the initial stage to the final stage of ripening. The decrease was more in the treated fruits than in the control fruits. The percentage of loss was more in the fruits treated with 200ppm ethrel than with 100 and 300ppm ethrel treated fruits and control. Similarly in mango the firmness of untreated mango fruits (Control) showed decrease from 181.3 to 112.7N during the ripening period. In this study, the treatment with ethrel and $CaCl_2$ increased the rate of softening as compared to their respective controls. In contrast, 1-methyl cycloprophone (1-MCP), Silver nitrate ($AgNo_3$), and Gibberlic acid (GA_3) did not reduce softening compiled to their respective in delaying softening. (Singh *et.al., 2007).*

In papaya fruit firmness greatly decreased from 102.42N to 10.76N. Fruit developing on-tree lost pulp firmness dramatically after the Quarter – Ripe (QR) stage. A similar decrease in firmness was seen in post-harvest detached fruits, where average values reached 9.89N on day 5 after detaching. The firmness of the post-harvest 'Pluk Mai Lie' papaya was still acceptable even after 7 days in ambient conditions. The correlation coefficient (R^2) between the reduction of firmness and the increase in the internal ethylene concentration during fruit ripening of on-tree and postharvest fruit were 0.9545 and 0.9470, respectively. After the onset of the ethylene climacteric the fruit pulp became soft, indicating fruit ripening. (Fuggate *et.al.,* 2010). Fruit flesh firmness of the two tomato cultivars showed a progressive decline during ripening. Most of this decline occurred between the light-pink and canning-ripe stages. (Misoon *et.al.,* 2005). A similar drop in flesh firmness was reported in guava

(Bashir and Abu – Goukh 2003), banana and mango (Abu – Goukh and Abu - sarra 1993).

The table–2 shows the changes of pectin content, which occur during the ripening of *Mangifera indica* fruits. The content of pectin gradually decreased during the course of ripening, both in the treated and control fruits. The pectin content was more in control than in the treated fruits. The percentage of loss was more in the 200ppm ethrel treated fruits than in the 100, 300 ppm and control. The statistical analysis on fruit firmness and pectin both in the control and treated showed a positive correlation. The correlation co-efficient values were 0.93, 0.89, 0.84 and 0.86. The observed correlation co-efficient values were significant at 1% level. The textural changes, which occur during fruit ripening, are thought to be related to alterations in cell wall structure (Huber, 1983; Tucker and Grierson, 1987). Most fruits soften during ripening and this is a major quality attribute that often dictates shelf-life. Fruit softening could arise from three mechanisms: loss of turgor; degradation of starch; or breakdown of the fruit cell walls. Loss of turgor is largely a non-physiological process associated with the post-harvest dehydration of the fruit, and as such can assume commercial importance during storage. Loss of water equivalent to about 5-10% of a fruit fresh weight although having little effect on the fruits biochemistry, can render the fruit commercially unacceptable. Degradation of starch probably results in a pronounced textural change, especially in those fruits like banana, where starch accounts for a high percentage of the fresh weight. In general, however, texture change during the ripening of most fruits is largely due to the result of cell wall degradation. Carbohydrate polymers make up 90-95% of the structural components of the wall, the remaining 5-10% being largely hydroxyproline-rich glycoprotein (HPRG). The carbohydrate polymers can be grouped together as cellulose, hemicelluloses or pectins (Tucker, 1993).

Similarly Misoon *et al.* (2005) studied changes in pectic substances and cell wall degrading enzymes during tomato fruit ripening. Xin *et al.* (2010) studied the morphology, profile and role of chelate-soluble pectin on tomato properties during ripening. Toboada *et al.* (2010) studied the isolation and characterization of pectic substances from musta (*Ugni molinae* Turcz) fruits. The decrease in the amount of pectin was also coupled with an increase in the activity of the pectic enzyme polygalacturonase. Ripening of tomato fruit has been extensively studied and a single enzyme, polygalacturonase has been implicated as a major determinant of fruit softening (Huber, 1983). Polygalacturonase has been shown to be the key enzyme, which is synthesized de novo and responsible for softening during tomato fruit ripening (Tucker and Grierson, 1987). The Polygalacturonase activity gradually increased during the course of ripening, both in the peel and the pulp of treated and control fruits. The pulp had more polygalacturonase activity than the peel throughout the ripening, and the activity of polygalacturonase was more in the treated than in the control fruits. Among the treated fruits, 200ppm ethrel treated fruits had more activity than the 100, 300ppm and control. The correlation co-efficient values are significant at 1 per cent level (Table–3). Mitcham and Mc Donald (1992) studied the cell wall modification during the ripening of Keitt and Totommy Atkin mango fruits. Both the varieties had increased polygalacturonase activity during ripening. In mangoes, the ripening which is characterized by changes in tissue softness is believed to be initiated in inner mesocarp tissue close to the seed, and to progress outward (Lazan and Ali, 1993) Furthermore, this decline in alkaline soluble pectin is found to be correlated with the loss of firmness of the mango fruit and is also closely correlated with the increase in PG activity during fruit ripening. Payasi, and Sanwal (2003) observed that in banana the polygalacturonase activity during fruit ripening increased the maximum activity on 22nd day. Misoon *et al.* (2005) observed that in tomato during fruit ripening PG activity progressively increased during ripening. During ripening, softening of fruit is caused by the conversion of protopectin, the insoluble, high molecular weight parent pectin into soluble polyuronides (John and Rey, 1986). This tightly bound protopectin is degraded into soluble pectin, which is found loosely bound to the cell walls. This phenomenon is attributed to textural softening during ripening (Gowda et al., 2001).

Table 1: Effect of ethrel on the fruit firmness changes during the ripening of off-season fruits of *Mangifera indica* L. var. Neelum (Values are Mean ± SE of 7 samples expressed in kg/cm²)

Days	Control Mean ±SE	100 ppm Mean ±SE	200 ppm Mean ±SE	300 ppm Mean ±SE
1	25.8 ± 1.54	25.5 ± 1.56	25.6 ± 1.79	25.8 ± 1.66
3	22.8 ± 1.41	20.6 ± 1.48	20.3 ± 1.42	21.3 ± 1.38
5	16.6 ± 0.99	15.7 ± 0.78	9.3 ± 0.73	12.1 ± 0.97
7	14.2 ± 0.99	10.7 ± 0.72	8.0 ± 0.48	9.2 ± 0.64
9	12.1 ± 0.87	11.0 ± 0.55	7.0 ± 0.49	8.6 ± 0.43
11	11.0 ± 0.55	10.5 ± 0.73	6.5 ± 0.33	8.2 ± 0.49
13	10.1 ± 0.61	9.4 ± 0.47	5.6 ± 0.34	7.4 ± 0.44
15	9.0 ± 0.63	8.5 ± 0.60	4.2 ± 0.29	6.0 ± 0.30

SE – Standard Error   ppm – Parts per million

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

Days	Control Mean ±SE	100 ppm Mean ±SE	200 ppm Mean ±SE	300 ppm Mean ±SE
1	0.46 ± 0.037	0.45 ± 0.036	0.48 ± 0.038	0.44 ± 0.035
3	0.44 ± 0.031	0.43 ± 0.030	0.47 ± 0.033	0.42 ± 0.029
5	0.42 ± 0.025	0.41 ± 0.025	0.44 ± 0.026	0.40 ± 0.024
7	0.38 ± 0.019	0.37 ± 0.019	0.40 ± 0.020	0.35 ± 0.018
9	0.34 ± 0.027	0.33 ± 0.026	0.36 ± 0.029	0.33 ± 0.026
11	0.28 ± 0.020	0.29 ± 0.020	0.30 ± 0.021	0.30 ± 0.021
13	0.26 ± 0.016	0.27 ± 0.016	0.30 ± 0.018	0.28 ± 0.017
15	0.23 ± 0.012	0.25 ± 0.013	0.28 ± 0.014	0.25 ± 0.013

SE – Standard Error   ppm – Parts per million

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

Days	Peel Control Mean ±SE	100 ppm Mean ±SE	200 ppm Mean ±SE	300 ppm Mean ±SE	Pulp Control Mean ±SE	100 ppm Mean ±SE	200 ppm Mean ±SE	300 ppm Mean±SE
1	0.023 ± 0.002	0.023 ± 0.002	0.023 ± 0.002	0.024 ± 0.002	0.050 ± 0.004	0.055 ± 0.004	0.051 ± 0.004	0.046 ± 0.004
3	0.029 ± 0.002	0.035 ± 0.002	0.037 ± 0.003	0.036 ± 0.003	0.078 ± 0.005	0.065 ± 0.005	0.074 ± 0.005	0.056 ± 0.004
5	0.035 ± 0.002	0.039 ± 0.002	0.048 ± 0.003	0.044 ± 0.003	0.089 ± 0.005	0.077 ± 0.005	0.085 ± 0.005	0.074 ± 0.004
7	0.042 ± 0.002	0.046 ± 0.002	0.057 ± 0.003	0.051 ± 0.003	0.099 ± 0.005	0.088 ± 0.004	0.094 ± 0.005	0.082 ± 0.004
9	0.049 ± 0.004	0.054 ± 0.004	0.066 ± 0.005	0.059 ± 0.005	0.102 ± 0.008	0.098 ± 0.008	0.105 ± 0.008	0.091 ± 0.007
11	0.056 ± 0.004	0.061 ± 0.004	0.075 ± 0.005	0.067 ± 0.005	0.119 ± 0.008	0.106 ± 0.007	0.113 ± 0.008	0.098 ± 0.007
13	0.066 ± 0.004	0.069 ± 0.004	0.089 ± 0.005	0.078 ± 0.005	0.127 ± 0.008	0.115 ± 0.007	0.121 ± 0.007	0.108 ± 0.006
15	0.074 ± 0.004	0.079 ± 0.004	0.099 ± 0.005	0.086 ± 0.004	0.118 ± 0.006	0.124 ± 0.006	0.129 ± 0.006	0.117 ± 0.006

SE – Standard Error   ppm – Parts per million

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