CHAPTER III
ROLE OF ABSCISIC ACID DURING RIPENING OF MANGOES.
Ripening in a number of fleshy fruits involves the process of softening, coloring and sweetening. The decline in acidity and astringency as well as an increase in aromatic compounds are also associated with the process of ripening (1). Ripening involves complex events which lead to ripeness. The ripe stage of the fruit is considered best edible (1). In order to obtain a fruit with maximum eating quality, the state of maturity of fruit and the completion of all chemical changes are essential (2).

Ripening of mango (Mangifera indica L. Var. Alphonso) is marked by the characteristic physico-chemical changes (2). The green, unripe fruit turns yellow at the ripe stage losing its firmness. The characteristic unripe flavour is lost and the fruit develops an aroma. It becomes sweet due to synthesis of free sugars with a simultaneous loss of organic acids. The pH of the pulp increases from pH 2.0 to 5.5. These changes can be accelerated by keeping fruits at a higher incubation temperature (25-30 C) (2).

The biochemical analyses of Alphonso mango was first attempted by Leley et al (3) and later the results obtained were confirmed by Modi and Reddy (4). Some of the important observations of their study were as follows: a) the starch content was 14% and total sugars 7g% of the total fresh weight at harvest, b) at the end of ripening period, starch content decreased to 0.3% and total sugars increased to 17g%, and c) sucrose content increased from 5.0 to 14.0g% of the total fresh weight.
Mango is an important crop of India. Studies related to the regulation of the ripening process are essential as a better understanding of this phenomenon and to minimize the wastage resulting due to improper handling and storage conditions. In other words, the knowledge of ripening process can ultimately yield substantial economic gains. The naturally occurring plant hormones are known to regulate the process of ripening (5). Ethylene, a key hormone is known to trigger ripening in fruits such as apples, pears and bananas (6,7). Indole acetic acid (IAA) delays the onset of climacteric in pears and bananas (8,9), while gibberellic acid (GA) and cytokinins have been reported to delay the ripening of tomatoes and mangoes (5,10). Abscisic acid (ABA) has been shown to promote ripening in tomatoes (11), mangoes (5,12) and grapes (13,14). However, the exact mechanism by which these hormones exert their effect is still unclear.

The present investigation deals with the effect of one of the hormone, Abscisic acid (ABA) on the various biochemical processes occurring during ripening of mangoes. Since ABA is known to trigger the process of ripening of mango, it will be interesting to study various physico-chemical changes brought about by its treatment and at the same time evaluating the improvement in the quality of mango by its presence. Studies were carried out with mature, unripe fruits and some biochemical changes were followed for the ripening period of eight days. The day '0' indicates the day at which mature, unripe fruits were brought to the laboratory and the hormone treatment was given. A concentration of 10 M of the hormone was found to enhance the
Ripening of mango (15) and was used throughout the present study. Ripening of climacteric fruits is accompanied by a characteristic respiratory behaviour (16). The rate of respiration remains low during development of fruit, attains a peak value as ripening commences and then decline during late ripening stage. Fig. 1 shows the respiratory pattern of untreated and ABA treated alphonso mangoes. Mature, unripe fruits were allowed to ripen at 0°C and the amount of carbon dioxide evolved was monitored during ripening period of eight days. After an initial decline (pre-climacteric minimum), the carbon dioxide evolution was maximum on the 2nd day of ripening, followed by a decrease in the rate of respiration for another 24 hours. The carbon dioxide evolution again increased after 3rd day of ripening to attain another peak value on the 6th day of ripening. This was followed by a decrease in the respiration rate till 8th day of ripening. Thus, the respiratory pattern of alphonso mangoes showed two peak values, viz. 2nd day and 6th day of ripening process. A similar pattern was also observed during ripening of Langra variety of mango (Fig. 2). The amount of carbon dioxide evolved on the 6th day was nearly half of that evolved on the 2nd day of ripening. The 1st peak of respiration was due to the climacteric rise whereas the 2nd peak may be attributed to the accumulation of gene products or due to the permeability changes. Tucker and Laties (17) have observed two phases of respiration, viz. first phase which was linked to the high energy demand for protein synthesis, and the second phase was possibly associated with the expression of new genes and accumulation of the
Legend to Fig. 1: Rate of respiration during different days of ripening of control (○—○) and ABA (▲—▲) treated alphonso mangoes.
Legend to Fig. 2: Rate of respiration during different days of ripening of Langra variety of mangoes.
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Amount of CO$_2$ evolved

$\text{gm CO}_2$/kg/hr
corresponding proteins. According to Sacher (18), the second phase may be associated with the permeability changes. ABA treatment to mangoes resulted in the increased rate of respiration compared to the untreated fruits. However, the pattern of respiratory behaviour was found to be the same as observed for the untreated alphonso mango (Fig. 1) (Data not shown for Langra mango). In contrast, a shift in the respiratory peak has been observed upon ethylene treatment of the fruits, although the shape of the respiratory peak remains similar (19).

To obtain a better insight into the nature of the 2nd respiratory peak, mangoes were treated with [C]-chlorella protein hydrolysate along with the hormone and evolution of labelled carbon dioxide was monitored. As indicated in Fig. 3, maximum evolution of labelled carbon dioxide was observed on the 6th day of ripening which coincide with the 2nd respiratory peak. (Fig. 1). Minimum radioactivity was obtained in the carbon dioxide evolved on the 2nd day of ripening. This is possibly because fruits at this stage are in a state of high metabolic activities resulting in channelization of the isotope to metabolic processes other than CO evolution. The lower amount of labelled carbon dioxide in ABA treated mangoes could be due to higher rate of metabolic activities in these fruits compared to their untreated counterparts. Moreover, higher decompartmentalization of the cellular components upon ABA treatment could be responsible for higher isotope dilution. On the other hand, cycloheximide treated fruits evolved higher labelled carbon dioxide on the 6th day of ripening (B.Amin. and V.Modi., unpublished observation), suggesting
Legend to Fig. 3: Amount of $^{14}C$O evolved during different days of ripening of alphonso mangoes treated with (C)-Chlorella protein hydrolysate in absence (O—O) and in presence (▲—▲) of abscisic acid.
that cycloheximide facilitates accumulation of already synthesized proteins either by preventing degradation or turnover. Cycloheximide was reported to inhibit ripening of pear but not the respiratory climacteric (20), indicating that climacteric rise was independent of protein synthesis. Thus, these results support the hypothesis that 2nd respiratory peak may be due to the accumulation of proteins.

Another hypothesis states that the 2nd respiratory peak may be due to the permeability changes (18). In order to study the possible validity of this hypothesis, microsomal membranes were isolated from both, control and ABA treated mangoes and total lipid content was estimated. The alterations in the membrane structure at the onset of ripening leads to changes in membrane permeability which results in the decompartmentalization of cellular components and increased leakage of solutes leading to eventual senescence (21). The major change in the composition of the membrane was observed in phospholipid : sterol ratio (21). Fig. 4 shows that lipid content of microsomal membrane of the control fruit declines with the onset of ripening, whereas that of ABA treated fruit, after an initial decline, increases upto 6th day of ripening. The lipid content of the microsomal membrane of the ABA treated mango on the 6th day was about 2 fold higher than that of the control fruits. This suggests that lipid composition of the membrane undergoes some change on the 6th day of ripening which in turn might affect the membrane fluidity and thereby membrane permeability. The change in lipid composition of the membrane was evident during banana ripening (22). This observation
Legend to Fig. 4: Estimation of total lipids from isolated microsomal membrane during different days of ripening of control (θ—○) and ABA (▲—▲) treated mangoes.
indirectly supports the hypothesis that the 2nd respiratory peak may be due to the permeability changes. Thus, from the evidences available (Fig. 3 and 4), the possibility of either protein accumulation or the membrane permeability changes associated with the 2nd respiratory peak can not be ruled out. It may also be due to the combination of both the processes.

The change in firmness of the fruit during ripening is also an important event contributing to the better quality of the fruit. Softening during ripening is accompanied by solubilization of pectins (23,24). Pectic substances get accumulated during development of mango and these substances get solubilized and depolymerized as ripening commences (2). Degradation of mango pectin was brought by pectin solubilizing enzymes. Activity of one such enzyme, pectin lyase, was measured during ripening of control and ABA treated mangoes. ABA treated fruits showed 30% higher activity as compared to their untreated counterparts (Fig.5). This suggests that ABA treatment enhances softening of mangoes. Earlier observations from this laboratory (5,25) have shown that ABA treatment increases the levels of hydrolytic enzymes, viz. amylase and cellulase, during ripening of mango and thereby causing softening of the fruits. Cellulase activity has also been correlated with the softening of avocados (26,27). Beside these enzymes, polygalacturonase has also been reported to be involved in softening of mangoes (28).

Due to solubilization of pectins and hemicellulosic substances, looseness of cellulosic fibrillar structure occurs during ripening (29). This leads to structural changes in the cell
Legend to Fig. 5: Activity of pectin lyase during different days of ripening of control (O—O) and ABA (▲▲) treated mangoes. Values expressed are a mean of results obtained from five different fruits.
wall and eventual softening. To obtain a better insight into the structural changes taking place in peel and pulp of the control and ABA treated mangoes, microscopic examination was carried out. 1μ sections of peel and pulp were cut on an ultramicrotome (JEOL, Japan), which were later stained with Toluidine Blue O (pH 7.2) and observed under light microscope at 100x magnification. As ripening proceeds, elongation of cells and widening of intercellular space occurs, resulting in the loss of intercellular binding and thereby cell integrity which could also be the cause of overall softening of the peel (Fig. 6). Mangoes treated with ABA showed significant enhancement of these changes.

The cells in pulp of a mango are parenchymatous and large in size. These cells were also found to elongate upon attaining the ripe stage along with cell wall hydrolysis, ultimately resulting in the loss of cell integrity (Fig. 7). These changes are found to be significantly accelerated upon treatment of fruits with ABA.

The studies on structural changes taking place in cells of peel and pulp of mangoes revealed the presence of abundant polysaccharides which consists mainly of starch as confirmed by iodine staining procedure (data not shown). The level of starch decreases during ripening of mango with a concomitant increase in the amylase activity, leading to increased degradation of starch (25). Fig. 6 and 7 confirm the enhanced degradation of starch upon ABA treatment.

The biochemical and structural changes induced by ABA treatment suggest that it enhances softening and possibly
Legends to Fig. 6: 1 μ sections of peel of control and ABA treated mangoes during different days of ripening.

a) Control 0 day, x 133,  b) Control 2nd day, x 133,
c) Control 6th day, x 133,  d) Control 8th day, x 133,
e) ABA 1st day, x 133,  f) ABA 6th day, x 133,
g) ABA 8th day, x 133  s - starch
Legends to Fig. 7: 1μm sections of mesocarp of control and ABA treated mangoes during different days of ripening.

a) Control 0 day, x 133.  
b) Control 2nd day, x 133.  
c) Control 8th day, x 133.  
d) ABA 1st day, x 133.  
e) ABA 4th day, x 133.  
f) ABA 6th day, x 133.  

s- starch.
sweetening (starch conversion to sugars) of mangoes during ripening.

Sugars are an important constituents of the fruit. The maintainence of appropriate sugar to acid balance contributes mainly to the appealing flavour (30). The level of total acids during ripening decreases with a simultaneous increase in the total sugar content of mangoes. The involvement of process of gluconeogenesis is evident during ripening of mangoes (31). Cell free extracts of fruit tissue have shown to convert organic acids to sugars. Citric acid and malic acid are the major acids of mangoes (2). The metabolism of malic acid takes place faster than that of citric acid (2). As indicated in Fig. 8, the level of total acids, measured in terms of malic acid, decline during the process of ripening. ABA treated fruits showed about 20% less acid content on the 4th day of ripening as compared to the untreated controls and the overall acid content of the fruit remained low through the ripening period. The level of sucrose, glucose, fructose and total carbohydrates increase as the mango ripenes. ABA treated fruits show about 53%, 72%, 38% and 25% higher sucrose, glucose, fructose and total carbohydrates respectively, as compared to the controls. The increase in sugar to acid ratio during ripening indicates that gluconeogenic pathway is a major factor for the higher conversion of acids to sugars. ABA treated fruits showed a 2 fold higher sugar to acid ratio as compared to the control, suggesting as one of the possibilities enhanced conversion of acids to sugars and thereby rendering fruits more sweet.
Legend to Fig. 8: Estimation of a) acids, b) sucrose, c) fructose, d) glucose, e) total sugars and f) sugar-acid ratio during different days of ripening of control (○—○) and ABA (▲—▲) treated mangoes. Values expressed are a mean of results obtained from five different fruits.
The enzymes of the gluconeogenic pathway catalyze the conversion of acids to sugars. Some of gluconeogenic pathway enzyme activities, viz. sucrose synthetase, sucrose phosphate synthetase, cytosolic malate dehydrogenase (which channelizes malate into gluconeogenic pathway). malic enzyme [Forward reaction, malate dehydrogenase (decarboxylating) (NADP) \(^+\)] and malic enzyme [Reverse reaction, malate dehydrogenase (carboxylating) (NADPH) \(^-\)] were monitored.

Malic enzyme which is known to be synthesized de novo during climacteric phase of ripening has been reported to be involved in the ripening of some climacteric fruits (32). Increased decarboxylase activity of malic enzyme has been associated with an increased respiratory quotient, during ripening of apples (32). Increased malic enzyme activity has been correlated with the carbon dioxide production during pear fruit ripening (33). Fig. 9 shows that the level\(^a\) of cytosolic malate dehydrogenase was 3 fold higher during early phase of ripening (i.e. 2nd day), where as that of malic enzyme responsible for forward and reverse reaction were 1.8 and 5 fold higher, respectively during late phase of ripening (i.e. 4-6 day) in ABA treated mangoes compared to the control fruits. This suggested that utilization of malate for the synthesis of sugars occurs (by malate dehydrogenase) during early phase of ripening and possibly, formation of pyruvate from excess malate (by malic enzyme) may help in channelizing it to the TCA cycle during late phase of ripening. Malic enzyme activity was known to be higher in climacteric and post-climacteric phase of ripening of mango and
Legend to Fig. 9: Activities of a) malic enzyme (forward reaction), b) malic enzyme (reverse reaction) and c) malate dehydrogenase during different days of ripening of control (0—0) and ABA (▲–▲) treated mangoes. Values expressed are a mean of results obtained from five different fruits.
increase in the activity of this enzyme may help in regenerating reduced NADP for reductive syntheses (2). However, no correlation between climacteric and malic enzyme activity was established in the present study.

The levels of sucrose synthetase and sucrose phosphate synthetase were about 2 fold and 6 fold higher, respectively, in ABA treated mangoes, as compared to the control fruits (Fig. 10), thus confirming earlier results which showed higher synthesis of sucrose in ABA treated fruits. The activity of invertase was also found to increase upon ABA treatment during ripening of mango (15), confirming enhanced formation of glucose and fructose.

The increase in activities of various enzymes during the process of ripening indicated an involvement of protein synthesis during the process of ripening. Moreover, the inhibition of ripening process in presence of a protein synthesis inhibitor, cycloheximide (20), also supported the view that the process of ripening is associated with the process of protein synthesis. A possible involvement of protein synthesis in the ripening process was studied by monitoring the rate of incorporation of a radioactively labelled amino acid into proteins. The incorporation of (C)-Leucine into TCA insoluble fraction of control and ABA treated mangoes declined with the onset of ripening (Fig. 11). Decline in the incorporation of radiolabelled amino acid throughout the development of fruit and no increase in the radioactivity during climacteric phase has been observed in case of avocados (34), pear (20) and banana (35). Leucine is a precursor for HMG-CO A which is involved in the carotenoid
Legend to Fig. 10: Activities of a) sucrose phosphate synthetase and b) sucrose synthetase during different days of ripening of control (O—O) and ABA (▲▲) treated mangoes. Values expressed are a mean of results obtained from five different fruits.
Legend to Fig. 11: Incorporation of (C)-Leucine into proteins during different days of ripening of control (○—○) and ABA (▲—▲) treated mangoes. Values expressed are a mean of results obtained from five different fruits.
Incorporation of $[^1^4C]$ Leucine into Proteins

DPM/mg protein (x 100)

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biosynthesis (36). Leucine is reported to get incorporated into carotenoids in the mold *Phycomyces blakesleeanus* (37). Thus, \(^{14}C\)-Leucine might be getting channelized to carotenoids during ripening of mangoes and thereby giving lower incorporation into proteins. ABA treatment to mangoes enhances carotene formation (36), suggesting that a higher amount of leucine may possibly be getting converted to carotenoids which therefore explains the lower incorporation into the proteinic fraction. Another possibility exists that before incorporating into proteins, incoming amino acid gets mixed with the endogenous pool of amino acids due to increased decompartmentation of the cell organells. The endogenous pool of leucine increases during ripening of mangoes (2). Hence, due to dilution effect lower incorporation of \(^{14}C\)-Leucine into TCA insoluble fraction was obtained. To obtain higher amount of radioactivity in the TCA insoluble fraction, incorporation of \(^{14}C\)-chlorella protein hydrolysate containing all uniformly labelled amino acids except methionine was carried out. As observed in Fig. 12, incorporation of \(^{14}C\)-chlorella protein hydrolysate into TCA insoluble fraction increased by about 15% on the 2nd day of ripening which coincides with the respiratory climacteric and then decrined. The TCA insoluble fraction of ABA treated fruits showed 15% less incorporation on the 2nd day of ripening as compared to the control fruits, possibly because ABA treated fruits showed high metabolic activities and increased softening such that isotope might get channelized to other metabolic processes. Since, \(^{14}C\)-chlorella protein hydrolysate contains 19 amino acids, the possibility of a
Legend to Fig. 12: Incorporation of \(^{14}\text{C}\)-Chlorella protein hydrolysate during different days of ripening of control (O—O) and ABA (▲—▲) treated mangoes. Values expressed are a mean of results obtained from five different fruits.
Protein Hydrolysate into Proteins
Incorporation of $[^{14}]C$ Chlorella Protein Hydrolysate into Proteins
DPM/mg protein (x 100)

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particular amino acid channelizing for the formation of other metabolite would be higher eg. Leucine as a precursor for carotene biosynthesis, glutamic acid gets converted to γ-amino-butyric acid and then gets incorporated into TCA cycle (2,37). ABA treatment accelerates these processes, thus explaining lower incorporation of radiolabelled amino acid into proteins. Moreover, cycloheximide has been shown to inhibit the ABA induced incorporation of the isotope during ripening of langra mangoes (15), suggesting that ABA action is mediated via protein synthesis.

The question that arises from these observations is whether the respiratory climacteric is associated with other aspects of ripening? Cycloheximide, which is known to inhibit protein synthesis, inhibits ripening but not the respiratory climacteric. (B.Amin and V.Modi., unpublished observation), suggesting that climacteric rise is ‘not dependent upon or integrated with’ other aspects of ripening. These results are in accordance with those obtain by McGlasson et al (39) during banana ripening. The increase in incorporation of amino acids during the early climacteric phase may be due to the induction of a number of enzymes which catalyze respiratory climacteric and final cell breakdown (39).

Thus, these results indicate that ABA treatment enhances the ripening of mangoes without causing any deleterious effects and at the same time increases its eating quality. Although these observations concentrate on the effect of a single hormone, the fact that ABA may not be the sole agent governing the process of ripening in mangoes, can not be ruled out. It is likely
that interaction between ABA and other hormones may have a significant role to play in regulating the process of ripening in mangoes.
REFERENCES:


