CHAPTER I

INTRODUCTION
Studies concerning ripening of fruits have been in progress since the past few decades. Since its inception, this area has always kept the plant biologists fascinated for a variety of reasons. Some regard fruits, especially those which undergo drastic changes with ripening, as a model system to study senescence and its regulation. Others have viewed it from the angle of hormonal and chemical control of ripening, so that a new technology may be developed for effective storage of fruits. Still others have examined this aspect with an ultimate goal of controlling microbial spoilage of fruits.

Through its life span a fruit (which is the matured ovary) passes through a series of changes including cell division, cell enlargement, maturation, ripening and finally, senescence. On the basis of their development, fruits are classified as simple and aggregate types. Simple fruits are those which develop from a single ovary e.g. mangoes, bananas, avocados, tomatoes, etc; while aggregate fruits are those which either develop from a number of ovaries of a single flower e.g. strawberries and raspberries, or from a number of ovaries originating from different flowers, as in the case of pineapples.
The diversity in origin of fruits is reflected in their growth and development patterns and also ripening processes. Many fruits exhibit a growth pattern which follows a single sigmoidal growth curve. This process is generally divided into three stages. The first stage is a phase of slow growth which is characterized by active cell division. An increase in size, fresh and dry weight combined with cell expansion is observed in stage II while in the final stage, there is a decline in the growth rate and ripening is generally initiated at this point (1). Mangoes, bananas, apples, avocados and tomatoes exhibit a single sigmoidal growth pattern. In addition to this, there are fruits which show double (1,2,3) and even triple sigmoidal growth patterns (4). Grapes, figs, plums and peaches are a few examples of fruits which exhibit double sigmoidal growth patterns, while the Chinese gooseberry exhibits a triple sigmoidal growth pattern.

THE BIOCHEMISTRY OF FRUIT RIPENING:

Dilley defines fruit ripening as a process by which a physiologically mature fruit undergoes transformation with respect to texture, colour, flavour and aroma (5). These changes are brought about by variations in activities of different types of enzymes. Table 1
illustrates some of the visible changes that occur during fruit ripening and the metabolic pathways which are likely to be responsible for these changes.

Table 1: Metabolic pathways associated with different changes occurring during fruit ripening.

<table>
<thead>
<tr>
<th>Change</th>
<th>Pathways/Metabolic Reactions</th>
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<tbody>
<tr>
<td><strong>Colour</strong></td>
<td></td>
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<tr>
<td>(a) Loss of green colour</td>
<td>Lipolysis.</td>
</tr>
<tr>
<td></td>
<td>Proteolysis.</td>
</tr>
<tr>
<td>(b) Formation of red/blue colours</td>
<td>Carotenoid and Phenylpropanoid synthesis.</td>
</tr>
<tr>
<td><strong>Flavour</strong></td>
<td></td>
</tr>
<tr>
<td>(a) Loss of Acidity</td>
<td>Tricarboxylic acid cycle and decarboxylation reaction.</td>
</tr>
<tr>
<td>(b) Increase in sweetness</td>
<td>Starch hydrolysis.</td>
</tr>
<tr>
<td>(c) Production of flavour volatiles</td>
<td>Biosynthesis of alcohol esters, etc.</td>
</tr>
<tr>
<td><strong>Texture</strong></td>
<td></td>
</tr>
<tr>
<td>(a) Softening</td>
<td>Pectolytic activity and cellulose breakdown</td>
</tr>
</tbody>
</table>

Source: Rhodes, M.J.C.(1983) (Ref.6)
Metabolic changes occurring during fruit ripening have been studied extensively. These have been reviewed exhaustively by Rhodes (7) and Sacher (8).

Kidd and West were the first to observe that the rate of respiration of detached apple fruits decreased initially and after reaching a minimum, suddenly increased rapidly, peaked and thenceforth declined (9). This sudden increase in respiration during ripening was termed as the "climacteric". Subsequently, it was shown that this phenomenon occurred during ripening of other fruits also (7,10,11).

In the study of lemons, Biale and Young observed a steady decline in the respiration rate of the fruit when kept in air at 15°C (12). Similar observations were made with oranges (13). Thus, Biale divided fruits into climacteric and non-climacteric groups, based on whether their respiration increased or gradually declined during ripening (10). Table 2 shows some fruits, representative of both groups.

In this table a number of changes have been made and other fruits have been added to the original list proposed by Biale (14). The cantaloupe melon (15), honey dew melon (16) and fig (17) are now considered climacteric fruits.
Table 2: Classification of fruits on the basis of their respiratory activity.

<table>
<thead>
<tr>
<th>Climacteric</th>
<th>Non-Climacteric</th>
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<tbody>
<tr>
<td>Apple</td>
<td>Cherry</td>
</tr>
<tr>
<td>Apricot</td>
<td>Citrus fruits</td>
</tr>
<tr>
<td>Avocado</td>
<td>Grape</td>
</tr>
<tr>
<td>Banana</td>
<td>Olive</td>
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<tr>
<td>Cantaloup Melon</td>
<td>Pineapple</td>
</tr>
<tr>
<td>Fig</td>
<td>Strawberry</td>
</tr>
<tr>
<td>Honey Dew Melon</td>
<td>nor-Tomato</td>
</tr>
<tr>
<td>Mango</td>
<td>rin-Tomato</td>
</tr>
<tr>
<td>Peach</td>
<td></td>
</tr>
<tr>
<td>Pear</td>
<td></td>
</tr>
<tr>
<td>Plum</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td></td>
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</table>

The list of non-climacteric fruits includes two tomato mutants viz. nor-tomato and rin-tomato. They differ from common cultivars in that they lack a climacteric cycle and show negligible ethylene production (18,19). Based on the study of Maxie et al., the olive has also been included in the non-climacteric class of fruits (20).
The increase in the respiration rate associated with the ripening of climacteric fruits has been variously attributed to a decrease in "organization" resistance of the cell (21), presence of natural uncouplers (22), enhancement of protein synthesis (23) and the synthesis of specific ripening enzymes (7).

Although climacteric fruits differ from one another in several ways, they do share a few properties in common, namely, their ability to produce ethylene and their response to exogenous ethylene treatment. Ethylene evolution may precede the respiratory increase as observed in the case of bananas, avocados (chequettes) and guavas (yellow strawberries) (24-26). In the case of apples (Cox's orange pippin) (27), pears (Anjou) (28), and apricots (Moorpark) (29), the rise in ethylene production coincides with the increase in respiration rate. In mangoes (Haden) (24), papayas (Solo) (30), plums (Wickson) (31) and tomatoes (Rutgers) (37), ethylene production or evolution lags behind the onset of the climacteric rise in respiration. On the other hand, non-climacteric fruits (for instance grapes) exhibit a constant, low level of ethylene during the inception of ripening (33). Exogenous ethylene treatment brings about a shift in the respiratory peak in the
case of climacteric fruits. These fruits are able to respond to exogenous ethylene only if it is applied in the preclimacteric stage. In non-climacteric fruits however, ethylene is able to stimulate the respiration rate at any stage during the post-harvest life (34). Removal of ethylene gas after the burst in respiration has occurred, does not result in reversion of the respiratory shift in the case of climacteric fruits. Contrarily, in non-climacteric fruits discontinuation of ethylene treatment at any time during storage results in reduction of respiration levels to those of the untreated controls.

Disappearance of chlorophyll marks the beginning of the process of ripening in many climacteric fruits. Chlorophyllase has been implicated in the degradation of chlorophyll (35,36). Concomitant to the disappearance of the green color, the synthesis of carotenoids begins in certain fruits such as mangoes (37). A seven fold increase in \(\beta\)-carotene content has been observed during the ripening of alphonso mangoes (37). The levels of various precursors of carotenoid synthesis, such as geraniol and mevalonic acid were also reported to increase progressively with ripening (38). Mango tissue slices have been shown to utilize these precursors
for carotene production (39). Bean and Todd (40) and Bean et al (41) have reported the loss of chlorophyll and the appearance of carotenoids in citrus fruits. Gibberellic acid treatment was found to retard carotene production while abscisic acid and ascorbic acid hastened the same (42,43). Though carotene synthesis and breakdown is independent of temperature, lycopene synthesis and breakdown in tomatoes has been shown to be dependent on temperature (44,45). Temperatures between 60-70°F were optimum for lycopene synthesis.

Tissue softening generally accompanies fruit ripening. Softening is due, at least in part, to the dissolution of cell walls which results from ripening associated changes occurring in this cellular component (46,47). The pectin content has been reported to decrease with the process of ripening (48,49). Enzymes like polygalacturonase and pectin methyl esterase have been suggested to play a prominent role in the softening of fruit tissues (50,51). The levels of these enzymes generally increase with the process of ripening (52,53). An elevation in the levels of amylase and cellulase with the advancement of ripening has also been observed and this correlates well with increased softening of fruit.
tissues (54, 55). Recently, Grierson et al. have purified and characterized two isozymes of polygalacturonase in Potentate and Alisa Craig varieties of tomatoes (56).

Extensive studies have been carried out on the production of volatiles in fruits. Nursten has reviewed fruit volatiles depending on their nature and biosynthesis (57). He has listed 160 different volatile compounds for apples, 56 for bananas, 155 for oranges and 143 for strawberries.

Bahdyopadhyay and Gholap have tried to correlate the changes in the glyceride content and fatty acid composition of five different varieties of mangoes with their aroma and flavour (58, 59). The glyceride content was found to increase with the process of ripening. At the same time, there was a decrease in saturated fatty acids and an increase in unsaturated fatty acids. They concluded that the ratio of palmitic to palmitoleic acid could be taken as an index for measuring aroma. Depending on whether the ratio is greater than or less than one, mangoes possess a mild or strong aroma respectively. Further, with the aid of labelled acetate and palmitic acid they have investigated fatty acid biogenesis in ripening alphonso mangoes. $\left[2^{14}C\right]$ acetate was found to be maximally incorporated into palmitic acid.
and to a lesser extent into palmitoleic acid. \[^{14}C\]palmitic acid was recovered in the hydroxy fatty acids. It is suggested that fatty acids may be involved in the development of the characteristic aroma in the ripe fruits (64).

Most of the aromatic compounds in plant tissues are either acids, esters, carbonyl ethers or acetals. Acetals are formed from aldehydes and alcohols. Organic acids are formed from other organic acids and sugars. Ester formation seems to be a more complex reaction. Salunkhe and Do have reviewed the biogenesis of aroma constituents of fruits and vegetables (60).

Generally speaking, the quality of the fruit depends upon the amount of sugars present. As the fruit ripens, there is normally an increase in the levels of sugars. During the ripening of mangoes, starch was found to be completely hydrolysed leading to the formation of sucrose (39, 61). A five fold increase in total pentose content, along with an increase in glucose and fructose content, was also observed in ripening mangoes (39). Citric acid was shown to be the predominant organic acid present in the unripe mangoes. Malic acid and ascorbic acid are also present (39). With the advance in the ripening process the acid content decreases, malic acid being metabolized first followed by citric acid.
There are numerous reports regarding changes in enzyme activities associated with ripening. In general, the levels of various hydrolytic enzymes have been observed to increase with the process of ripening. These include amylases from mangoes and tomatoes (55, 62), cellulase from tomatoes (54), lipase and lipoxidase from apple peel (36, 63) ribonuclease from apple and banana peel (8, 36) and phosphatases from mangoes (65), bananas (66) and apples (36). An increase in acid phosphatase activity has been suggested to control carotenogenesis in mangoes (65).

A significant elevation in the levels of oxidative enzymes viz. catalase and peroxidase has been reported in ripening alphonso mangoes (61, 65). Though several roles have been assigned to peroxidases, the reason for the increase in activity of these enzymes is not clearly understood. The levels of several glycolytic enzymes viz. glucose-6-phosphate isomerase, phosphofructokinase and aldolase, were found to be considerably enhanced with the progress of ripening in mangoes (61). The HMP shunt enzymes such as glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase also increased with ripening in mangoes (39). The rise in the levels of HMP shunt enzymes along with the increase in
malic enzyme is suggested to regenerate reduced NADP which is required for the synthesis of lipids (39). Earlier, Tager and Biale have reported that during the climacteric rise in bananas, there was a shift from the HMP pathway to the glycolytic pathway (67). Surendranathan & Nair have confirmed this observation (68). They reported that in bananas, the levels of glucose-6-phosphate dehydrogenase (a HMP shunt enzyme) decreased as ripening progressed. A close correlation between the fall in enzyme activity and the increase in respiratory activity was demonstrated. Further, the activity of phosphofructokinase was stimulated with the process of ripening. In keeping with these reports, an 18-20 fold increase in the levels of fructose-1,6-diphosphate (a glycolytic intermediate) has been observed in bananas (68,69) and avocados (70).

The ripening trends in non-climacteric fruits such as grapes, citrus fruits, pineapples and strawberries are strikingly different from those observed in mangoes, bananas and apples (71). The major carbohydrates present in grapes and pineapples are glucose, fructose and sucrose (72,73). The inception of ripening in the grape berry is called "veraison" and marks the beginning of sugar accumulation, loss of acid, softening and skin
coloring (74). The levels of certain enzymes like glucose-6-phosphate dehydrogenase, malic enzyme, pyruvate decarboxylase and phosphopyruvate carboxylase were found to decrease with ripening in grapes (75,76). On the other hand, the activities of enzymes involved in sugar metabolism such as invertase, sucrose synthetase, sucrose phosphate synthetase and sucrose phosphatase were found to be elevated at the time when sugars accumulated (75).

The higher levels of malic enzyme and pyruvate decarboxylase observed during the climacteric rise in apples have been suggested to be due to a high respiratory quotient and an increased rate of oxidation of added malate (77). Meynhardt (78) has shown that mature grapes contain phosphopyruvate carboxylase and malic enzyme which may be involved in the dark fixation of carbon dioxide, a process known to occur in grapes (79). However, these enzymes may also be involved in the decarboxylation of malic acid and subsequent decarboxylation of pyruvate as suggested in the case of apples (77).

Though extensive studies have been carried out to understand the ripening process in climacteric and non-climacteric fruits, it is still not clear how
the ripening events are co-ordinated and regulated. Two hypotheses have been put forth to understand the ripening phenomenon in a climacteric fruit. According to one, the process is regulated at the level of transcription - translation, indicating that de novo RNA and protein syntheses are involved. The second suggests that all the key enzymes required for the process of ripening are present and need only be activated. Evidence available favours both the theories in spite of the fact that no conclusive evidence for either theory has yet been advanced. It is likely that ripening may entail the participation of both these processes.

A change in the membrane permeability of cells during the ripening process has been suggested to occur in pears (80), avocados (81), apples (82) and bananas (83). Such changes could allow the access of ions, substrates or cofactors to the inactive enzymes, thereby inducing the enzymes to become active. Evidence for the transcription-translation theory comes from studies which indicate increased incorporation of radioactive precursors into proteins and nucleic acids (84). Further, inhibitors of RNA and protein syntheses have been shown to interfere with the process of ripening(85,86).
Lastly, new mRNA species have been found in the poly A+ RNA fraction in tomatoes (87) and avocados (88).

Fruit ripening is known to be affected by treatment with plant hormones or plant growth regulators (89). Ethylene, abscisic acid, indole acetic acid, gibberellic acid and kinetin are amongst the important hormones which have received considerable attention in recent years. a) Ethylene: An increase in ethylene evolution is known to be associated with the ripening process in many climacteric fruits such as avocados (25), apples (27), pears (28), and apricots (29). Non-climacteric fruits exhibit a constant low level of ethylene at all stages of ripening (33). Lieberman et al were the first to report a model system which could synthesize ethylene from methionine in the presence of ascorbic acid and Cu++ (90). Methionine was suggested to be the precursor of ethylene in apples (91), bananas (92), tomatoes (93) and avocados (94). The third and fourth carbon of methionine were found to give rise to ethylene. Besides methionine, several other compounds such as glucose, acetate, pyruvate, acetaldehyde (95), L-alanine (96), propanal (97) and methional (98) have been shown to give rise to ethylene. Recently, Yang and Adams have conclusively shown that methionine is indeed the biological precursor of ethylene in higher plants (99).
S-adenosylmethionine (SAM) and 1-aminocyclopropane 1-carboxylic acid (ACC) were found to be the intermediates of the ethylene biosynthetic pathway. Further, an enzyme which converts SAM to ACC (viz. ACC synthase) has been isolated and characterized from tomato fruit tissues. This enzyme required pyridoxal phosphate for activity and was found to be inhibited by aminoethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA) (100,101). The conversion of SAM to ACC and ACC to ethylene appear to be the rate limiting steps in ethylene biogenesis. Under anaerobic conditions, ACC accumulates and can be further converted to ethylene only in the presence of oxygen (99). Recently, Mcrea et al presented evidence suggesting the involvement of the superoxide radical in the conversion of ACC to ethylene by microsomal membranes from etiolated pea seedlings. Formation of ethylene from ACC was dependent on the availability of oxygen, was heat sensitive, was inhibited by the radical scavanger n-propyl gallate and was blocked by superoxide dismutase and catalase (102). The enzyme system catalysing the conversion of ACC to ethylene has also been isolated from citrus fruits (103). The $K_m$ for ACC was 2.8 mM. It exhibited IAA oxidase activity, but had no guaicol peroxidase or NADH oxidase activity.
Studies conducted during the past years have indicated the involvement of ethylene in the ripening of fruits such as apples (104), avocados (105), and tomatoes (106). Burg and Burg reported that bananas, when stored at hypobaric pressure, did not evolve significant amounts of ethylene (107). Moreover, the ripening process was delayed. Lieberman et al observed that rhizobitoxin could specifically inhibit ethylene production from methionine, in the case of apples (104). In Penicillium digitatum, ethylene evolution was not inhibited by rhizobitoxin indicating the operation of an alternate pathway in this fungus.

The mechanism by which ethylene concentrations shoot up during ripening is not very well understood. However, two theories have been put forward to explain it. According to one, treatment with ethylene induces an autocatalytic type chain of reactions, probably triggered by the formation of unique isozymes of peroxidase. This enzyme is suggested to play a role in ethylene production (108). This theory is supported by the observation that one of the ethylene biosynthetic enzymes isolated from citrus fruits exhibits indoleacetic acid oxidase activity (103). The second hypothesis states, that membrane permeability changes with ripening thus leading to more contact between the substrates and enzymes involved in ethylene biogenesis (109).
Besides being regarded as the hormone which initiates the process of ripening in climacteric fruits, ethylene is of importance in that it directly promotes the progress of some ripening changes (110-113). The process of ripening (in bananas) was found to be completely inhibited when fruits were stored in an atmosphere containing 1-5% oxygen (114). This effect could be reversed if ethylene was introduced into the atmosphere of low oxygen concentration. Earlier studies from this laboratory have indicated the presence of proteinic inhibitors in unripe mango fruits. These inhibitors were found to mask the activities of enzymes such as peroxidase, catalase and amylase (55,65,115,116). Ethylene could inactivate the inhibitors leading to an increase in the activities of these enzymes. It was suggested that ethylene produced during ripening might inactivate these inhibitors in vivo also, thereby explaining the increase in the levels of these enzymes upon ripening. Many studies have demonstrated a rise in some specific metabolic activities in response to ethylene treatment. In some cases this enhancement was prevented by cycloheximide and actinomycin D suggesting that ethylene acted by regulating gene
expression (118,119,120). In a recent report, Christoffersen and Laties, have observed that ethylene treatment to carrot roots could stimulate respiration and increase the polysome content (121). In vitro translation experiments with poly A^+ RNA (mRNA) isolated from carrot roots showed that ethylene treatment resulted in the appearance of new species of mRNA^2s. This provided direct evidence for the theory that ethylene regulates gene expression.

b) Abscisic Acid: Of all the plant hormones, ethylene has received the maximum attention of plant biochemists, in view of its involvement in the senescence of climacteric fruits. Sufficient evidence has now accumulated to suggest that ethylene might not be the only factor involved in ripening and the process may be the result of the interaction of this gas with other hormones (122). One such hormone, capable of stimulating the process of ripening, is abscisic acid (ABA). This diterpene has been reported to be synthesised from mevalonate (123,124). Studies with plants, to date, have not revealed much details regarding the intermediates involved in its biosynthesis. A fungus Cercospora rosicola has been shown to produce large amounts of ABA (125).
organism may prove valuable for elucidating the pathway of ABA biosynthesis. 1-deoxy abscisic acid has been isolated from cultures of C. rosicola and reported to be efficiently converted to ABA (125).

ABA has been shown to accumulate during ripening in climacteric fruits such as apples (126,127), pears (128) and avocados (129). The increase in ABA either coincides with, or follows the rise in ethylene production. ABA levels have also been reported to be elevated during ripening in non-climacteric fruits such as oranges (130,131), strawberries (132), grapes (133) and cherries (134). In the case of peach fruits, the increase in ABA tends to precede the rise in ethylene evolution associated with ripening (135). Similar observations were made in tomatoes, in which system it was observed that ABA accumulated much before the onset of ethylene evolution (136).

It has been proposed that ABA may be involved in the initiation of increased ethylene production, which accompanies ripening in climacteric fruits (5,8,122). Conversely, it is possible that higher levels of ABA are a result of the rise in ethylene production as observed in the case of avocados (129).
Studies conducted with tomatoes imply that the first proposition may hold true (136). Such studies however, do not suggest an independent mode of action for ABA, which has been found in senescing oat leaves (137). ABA slightly stimulated ethylene evolution in oat leaves, but its senescence promoting effect in light was only partially antagonized by Ag⁺, Co²⁺ or aminoethoxyvinylglycine (AVG) which are the known inhibitors of ethylene production and action. This study thus indicated that both ethylene and ABA independently controlled leaf senescence (137).

Evidence collected from studies on climacteric and non-climacteric fruits suggests that ABA has an important role to play during ripening and senescence. This conclusion is based on the following observations: (a) Endogenous ABA levels increase just before or along with fruit ripening (126-133) (b) Treatments that hasten or delay the ripening process have a similar effect on ABA accumulation (133). In the case of nasturtium leaves, treatments which delayed senescence, retarded the decline in endogenous gibberellins and prevented the increase in ABA content (138).
ABA treatment was found to enhance the levels of RNase and acid phosphatase in leaves of *Rhoeo discolor* and in fruit tissues (139, 140, 141). Gibberellic acid, kinetin and indoleacetic acid (three other plant growth regulators) generally exert an effect which is contrary to that of abscisic acid (139-143).

ABA seems to have a different mode of action on growing systems (a classical example being that of barley aleurone layers) as compared to systems in which the growth is completed, such as fruits. As mentioned earlier, ABA promotes senescence in both climacteric as well as non-climacteric fruits and hence, can be considered as the hormone that forms a common link between these two classes of fruits (144). In climacteric fruits, ABA treatment stimulates the activity of hydrolases e.g. amylase (89). In the case of barley aleurone layers, the secretion of amylase is controlled by two plant hormones viz. gibberellic acid (GA) and ABA (145, 146). Both hormones act antagonistically to each other. GA induces the synthesis and secretion of amylase, while ABA inhibits its synthesis (147). Recently, Mozer isolated mRNA's from GA treated barley aleurone...
layers. In vitro translation results indicated that the major product was α-amylase (148). Though ABA did not interfere with GA mediated induction of translatable mRNA, it prevented the translation of these mRNAs (especially amylase mRNA) in vivo.

Another cellular function to be affected by ABA treatment is membrane permeability, as seen in carrot root cells (149) and mango tissue slices (150).

Besides the dominant role that ABA plays during senescence of various fruit and leaf tissues, several other roles have been assigned to this hormone. It has been shown to play a decisive part in regulating stomatal aperture under stress and certain environmental circumstances (151,152,153). Water stress conditions and darkness lead to the closure of stomata, which in turn results into accumulation of ABA, or vice-versa. Cummins showed that stomata begin to reopen rapidly only when the levels of ABA decline (154). Similarly, drought resistant cultivars of maize have been shown to accumulate ABA (155,156). Accumulation of ABA then affects stomatal closure.

A role for ABA has also been suggested in flowering. It has a promoting effect on short day
plants e.g. *Chenopodium rubrum* (157) and an inhibitory effect on long day plants e.g. *Lotium tementulum* (158). The levels of ABA have been reported to rise sharply and then fall during the development of a variety of seeds. A few examples are pear (159), tomato (136), wheat (160) and soyabean seeds (161). This hormone is capable of inducing and maintaining bud dormancy (162) and exogenous treatment inhibits germination in non-dormant seeds (163). In addition to the growth inhibitory responses, there are reports suggesting that ABA may have growth promoting effects too. When applied at low concentrations, the hormone promoted the increase in frond number and fresh weight in *Lemna polyrhiza* (164). Elongation of coleoptile segments of oats and wheat was also stimulated by ABA. One of the most certain roles of ABA is in the abscission of fruits from their stems. ABA treatment to mature peach, apple and citrus fruits leads to accelerated abscission (165-168).

Ethylene and abscisic acid represent factors which enhance the process of ripening in fruits. The picture regarding factor(s) which can antagonize
these ripening promoters is not clear. The inability of certain fruits, notably avocados, to ripen on trees, coupled with a lack of response of freshly harvested fruits to ethylene treatment (169,170) has prompted several researchers to propose the existence of ripening inhibitors (10,171). Certain other plant hormones seem to be the likely candidates for these inhibitors. Indole acetic acid has been reported to delay the onset of the climacteric in bananas (172) and pears (173) and both gibberellins and cytokinins have been shown to delay the degradation of chlorophyll in ripening tomatoes (174) and orange peels (175). Few studies have been performed to examine the levels of various growth regulators in ripening fruits. Abdel-Rahman and his colleagues (176, 177) have examined the changes in the levels of plant hormones during growth and development of cherry tomato fruits. They observed that cytokinins and auxins were abundant and their content reached a peak during early development. Gibberellin levels were more prominent during the cell enlargement stage and reached a peak before the green mature stage. A decrease in indole acetic acid concentration together with the increase in IAA oxidase activity provides a basis for
the control of ripening by the destruction of endogenous IAA (178). This thereby renders the tissues sensitive to ethylene. Further, IAA breakdown products may help to hasten ripening (117).

Besides the use of hormones, which are the natural regulators of the process of ripening, various other technologies have been developed to control postharvest fruit ripening. These include storage at low temperature, controlled atmosphere storage and hypobaric storage.

Most fruits ripen normally in a very narrow range of temperatures. A major disadvantage of the use of low temperature for the storage of tropical and subtropical fruits is that these fruits develop a physiological disorder termed as 'chilling injury'. Chilling injury is marked by discoloration of the skin and the inability of the fruit to ripen normally, once it is brought back to room temperature (179). Bananas are known to get chill injured at 10°C while mangoes develop this disorder at 5°C (180).

Controlled atmosphere storage or modified atmosphere storage is a promising method for extending the marketable life of various fruits.
In most controlled atmospheres, the carbon dioxide levels are generally raised and the oxygen levels are depressed from those normally occurring in air. Though low levels of oxygen have been reported to decrease the net respiration rates of fruits (181), their major effect lies in their ability to inhibit ethylene production, as observed in the case of bananas (182). Inclusion of ethylene into the atmosphere of low oxygen reverses the oxygen effect, and the fruit ripens normally. However, in the case of apples stored at a 3% oxygen concentration, ethylene could not reverse the arrestation of respiration and ripening (183). This suggested that the fruit requires an oxygen concentration greater than 3% in order to respond to ethylene.

Concentrations of oxygen greater than atmospheric tend to hasten the process of ripening in many fruits (184). Citrus fruits when stored between 34-99% oxygen exhibited a normal climacteric rise but ethylene evolution increased and ripening was enhanced (10).

Increasing carbon dioxide concentrations in the atmosphere around the fruit tends to delay the ripening process. Concentrations greater than 10%
have been reported to cause physiological disorders in apples (183). The role of carbon dioxide in delaying the ripening process is linked to its effect on ethylene action. Burg suggested that carbon dioxide can compete with ethylene for the receptor site (111). Anderson et al examined the combined effect of low oxygen and high carbon dioxide concentrations on the storage behaviour of peaches and nectarines (186). They found that peaches stored at 1% O₂ and 5% CO₂ at 0°C for 6 to 9 weeks and then allowed to ripen in air had a better texture, color, flavour and a lower degree of decay than those stored in air throughout this period. Further, it was observed that if the fruits were removed at 3 and 6 week intervals, kept at room temperature for two days, and then returned to a controlled atmosphere or air at 0°C, they acquired a better quality and had lower respiration rates than those stored in air (187). Mature green tomatoes, held at 55°F for 6 weeks could be stored more effectively at 3% O₂ and zero carbon dioxide, than in air (188). Goodenough et al examined the changes in color, polygalacturonase, monosaccharides and organic acids during storage of tomatoes in an atmosphere containing 5% O₂, 5% CO₂ and 90% N₂ (189).
They observed that the appearance of polygalacturonase and pigmentation was prevented in the modified atmosphere. However, the breakdown of starch to give monosaccharides and the changes in concentrations of organic acids which are associated with ripening, still occurred.

The ripening process in bananas can be completely inhibited by storage at one-fifth the atmospheric pressure in pure oxygen (107). Inclusion of small amounts of ethylene into the atmosphere reverses this effect. Burg reported that reduction of pressure led to an increased diffusion of ethylene and reduced its internal concentration (190). Mature green tomatoes could be stored for up to 100 days at 102 mm Hg at 12.8°C and subsequently ripened normally on their return to atmospheric pressure (191). Avocados can be stored up to 70 days at 6°C at a 60 mm mercury pressure (192). In the case of tomatoes, it was observed that a low oxygen tension was more important in producing the effect of hypobaric storage. This was supported by the observation that storage at 190 mm mercury pressure and normal partial pressure of oxygen, resulted in normal ripening. However, when the partial pressure of oxygen was lowered, a delay in ripening resulted (193).
Bufler and Bangerth observed that apples stored at 4°C and 6.6 KPa ($O_2$ concentration equivalent to 1.3% at atmospheric pressure) did not ripen for 4 months, neither was ACC synthase activity elevated, nor was ethylene production induced (194). However, when these fruits were ventilated either with oxygen or ethylene, ACC synthase activity was induced and rapid ethylene production was accompanied by the onset of ripening. Thus, hypobaric storage utilizes the delaying effects of low oxygen atmospheres as well as the beneficial effects of reducing internal ethylene concentrations.

**MICROBIAL SPOILAGE OF FRUITS AND ITS CONTROL:**

'Since the beginning of civilization the tiller of the soil has been robbed of some of the fruits of his labours by plant diseases'.

C.H. Gadd (Ceylon)

One of the continuing problems facing agricultural development in tropical and semitropical areas (which represent the developing areas of the world) is the identification and treatment of bacterial and fungal diseases of plants, including fruits and vegetables.
Diseases may affect the quality of fruits in several ways. They may decrease the plant stand or vigour and thereby the yield size and eating quality. They may blemish the surface, resulting in a low market value, or increase water loss and therefore decrease the keeping quality in store resulting in increased wastage. Diseases may also destroy the plant tissues by rotting them. It has been estimated that at least 40% of the unconsumed produce harvested in the world is lost through postharvest deterioration and disease. Much of the deterioration in store is pathological, resulting from either primary infection in the field or secondary infection through tissue damaged during harvesting and handling.

Eckert has listed some major postharvest diseases of fresh fruits and vegetables and their causal organisms (195). "Soft rots" of fruits and vegetables are generally caused by microorganisms such as Rhizopus, Geotrichum, Sclerotina and Erwinia Species (195,196,197). These pathogens are capable of proliferating rapidly under favourable conditions and may invade the host tissues by dissolving the cell walls. The 'brown-rot', on the other hand, is caused by Monilinia fructicola in stone fruits;
Species of *Gloeosporium* in apples; *Diplodia natalensis*, *Phomopsis citri* and *Alternaria citri* in the stem ends of citrus fruits. These organisms do not spread readily during storage and shipment, but may become a serious problem if a substantial portion of the crop is infected at the time of harvest (198,199).

The mango fruit is susceptible to attack by *Gloeosporium mangiferae* which is known to cause anthracnose (290). *Aspergillus niger*, *Aspergillus flavus*, *Fusarium* species, *Rhizopus arrhizus* and *Rhizoctonia bataticola* are frequently associated with various fruit rots in mangoes (201,202,203,204). Among the bacteria, *Pseudomonas mangifera indica* forms necrotic spots on the fruit and stem of mangoes (205), *Erwina mangiferae* is associated with black spots (197) and various species of *Bacillus* have been identified as the causative agents for 'spongy-tissue' formation (207).

*Colletotrichum gloeosporioides* and *Dothiorella gregaria* develop latent infections in avocado fruits and become active only during ripening (200).

The banana fruit is reported to be susceptible to fungal rot. *Thielaviopsis paradoxa*, *Botryodiplodia theobromae*, *Gloeosporium musarum* and *Deichtoniella torulosa* are all capable of causing crown rot in bananas (207,208).
Anthracnose is reported to be the most common disease of the papaya fruit. The organism associated has been identified to belong to the genus Colletotrichum. Other fungi associated with fruit rot of papayas are Alternaria alternata, Botryodiplodia theobromae, Fusarium species, Rhizopus stolonifer and Phytophthora species (209,210).

A knowledge about the mechanism of microbial infection seems to be a prerequisite for developing a programme to control plant diseases. Infection occurring before harvest may either be due to the direct penetration of the organism, or its spores into the developing flower, as observed in the case of Botrytis cinerea infection of strawberries (211,212). The organism forms a latent infection and develops when the berries are harvested. Alternatively, the organism can gain entry into the host through lenticels. Lentical rotting of apples represents a major storage disease. Gloeosporium album and G.perennans develop in the lenticel of the apple fruit and remain latent until the fruit reaches a certain degree of ripeness (195).
Infections that arise during or after harvest are generally due to mechanical injuries caused on the surface of the fruit. Such infection usually arises at the site where the fruit gets injured. Stem end rots of mangoes (213), papayas (214) and avocados (205) are a few examples.

Several factors may be responsible for influencing the resistance/susceptibility of fruits to attack by microorganisms. These include the presence of certain constituents in the host which may support growth and infection of the pathogen. *Colletotrichum musae* metabolises anthranilic acid present in banana fruits to 2,3-dihydroxybenzoic acid. This metabolite stimulates conidial germination of the pathogen (216, 217). Secondly, the host may contain certain inhibitors which could affect the growth, directly or via enzymes, of the pathogens. These inhibitors might be either preformed or induced by the presence of the pathogen.

3,4-dihydroxybenzaldehyde and tannins present in bananas (218) and the phenolics present in apples (219) are amongst the known preformed inhibitors. These compounds are generally present in high quantities in the unripe fruits and their levels drop with the
process of ripening. Plants are known to synthesize phytoalexins (which possess antimicrobial activity) in response to microbial attack. The presence of *Rhizopus stolonifer* or *Botrytis cinerea* induces carrots to produce 6-methoxymellein (220,221). Since the ability of the carrots to produce this compound is lowered upon storage, the susceptibility to infection increases (222). Phytoalexins have also been implicated in the latency of *Colletotrichum musae* in bananas. These compounds disappear on ripening and ripe fruits therefore become susceptible to infection (223).

Perishable fruits generally have a high water content (about 50-90%). This property, along with the nutrients present in the fruit, makes them susceptible to microbial attack. A slight dessication reduces the susceptibility of citrus fruits towards infection by *Penicillium digitatum* (224). Fruit tissues are known to be more acidic than vegetable tissues. On this account, the former are more resistant to attack by bacterial pathogens than the latter (225,226). As the fruit ripens there is a reduction in acidity, as a result of which the resistance against microbial
attack is lost. A decrease in acidity of apple tissues during storage was found to correspond to a suppression in their resistance to *Nectria galligena* infection (227).

Insoluble pectin (protopectin) found in the cell walls of fruits has been shown to resist the attack by pectolytic enzymes of pathogens (228, 229). Protopectin gets converted to either pectin or pectic acid with increasing ripening. This makes the tissues less coherent and more susceptible to microbial decay. The susceptibility of strawberries to spoilage by *Botrytis cinerea* has been correlated with the soluble pectin content of the fruit (230).

Several methods have been developed to combat pre- and post-harvest diseases. These aim either at preventing the occurrence of infection, or at eradicating established infections. Chemicals have been used extensively to reduce the amount of inoculum on the host and in the environment. Formaldehyde and nitrogen trichloride have been employed as disinfectants for lemon storage boxes. Similarly, sulphur dioxide has been recommended for picking boxes for figs (195). Hypochlorous acid or hypochlorite salts added to water used for washing fruits, reduce the microbial load in it, as well as the risk of contaminating the fruits (231). Sodium-0-
phenylphenate is added to hydrocooler water in order to prevent the infection of peaches by brown rot causing fungi (232).

In order to reduce the probability of infections due to harvest injuries, fungicides are applied either before or after harvest. It was observed that benomyl and thiabendazole sprays on orchards of pears resulted in a considerable reduction in the decay caused by *Penicillium expansum* and *Botrytis cinerea* (233,234). Systemic fungicides are widely used for the eradication of established infections. Benomyl has been shown to prevent, or at least delay, the development of latent infections of species of *Gloeosporium* on apples (235), bananas (236), and mangoes (237). The high fungicidal activity of these compounds is partly due to their ability to penetrate into the host tissues and thereby wipe out latent infections.

In addition to chemicals, hot water treatment of fruits can also result in a considerable reduction of decay of fruits. Temperatures used for treatment vary from fruit to fruit. For mangoes, the temperature employed is 54-55°C for 1-2 minutes (237). Hot water treatment was found to be more effective against *Monilinia* brown rot than against *Rhizopus* rot (238,239).
Peaches stored at low temperatures after hot water treatment may get injured. Such injury makes them susceptible to infection by *Penicillium*, *Alternaria* and *Botrytis* (239, 240).

The use of ionizing radiations for the control of established infections has not proved to be very beneficial. Deep seated infections caused by *Diplodia* and *Alternaria* are generally resistant to $\gamma$-radiation. The dose required to inactivate such pathogens may exceed that which is tolerable by the fruit tissues, thus resulting in radiation damage (195). Maxie *et al* concluded that ionizing radiations are not a good candidate for the treatment of fresh fruits and vegetables to control postharvest spoilage. The reason for this is that firstly, many fruits get damaged by the radiation doses required to delay the development of decay. Secondly, pathogens which respond favourably are as easily controlled by fungicide treatment (241).

Cold storage of fruits may result in the delay of onset of the infection (242). However, low temperature does not eradicate the pathogen and generally, only retards its development. An exception however, has been observed with germinated spores of *Rhizopus* which are killed when exposed to low temperatures (243).
Postharvest decay can be controlled by treatment of fruits with plant growth regulators which retard ripening and senescence (244). Modified atmosphere storage can also reduce the incidence of spoilage in fruits as observed in the case of strawberries stored in a 20-30% CO₂ atmosphere (245).

**Present investigation:**

Extensive studies have been conducted in the laboratory to understand the process of mango ripening at the biochemical level. Amylase and invertase levels were found to increase with the process of ripening (55). There was a three fold enhancement in invertase activity while amylase activity rose by about 2 fold. Around the same time, the levels of starch were reported to decline, with a concomitant increase in hexose and reducing sugar content. Pectin methyl esterase activity was doubled with the process of ripening (55). An increase in the levels of NADP-dependent malic enzyme, glucose-6-phosphate and 6-phosphogluconic dehydrogenase was also observed (39). A decline in the levels of citrate during ripening was correlated with a higher activity of the citrate cleavage enzyme (246). A crude fatty acid preparation from mango was found to enhance the activity of the enzyme, suggesting that lipid
breakdown products might regulate its activity in vivo. Phosphatase activity was found to increase with the process of ripening (65). β-carotene stimulated this enzyme, thus implying that carotene might regulate the phosphatase enzyme during the process of ripening.

Mitochondria isolated from immature, mature unripe and ripe mangoes were reported to actively oxidize the intermediates of the Krebs' cycle (247). The levels of malic dehydrogenase and succinate dehydrogenase increased with the onset of ripening, while those of citrate synthase were found to decrease. The fatty acid oxidizing capacity of mitochondria isolated from immature and postclimacteric fruits was much less as compared to preclimacteric and climacteric fruits (248). A four to five fold increase in the levels of catalase and peroxidase has been reported in ripening mangoes (65). Unripe mango fruits were found to contain a nin-hydrin and biuret-positive, heat-labile, non-dialysable inhibitor for both these enzymes. These inhibitors were partially purified (55, 65). Ethylene was found to inactivate them completely. Further, in unripe mango-tissue slices treated with ethylene, catalase
and peroxidase activities were stimulated (116). It was suggested that ethylene might be inactivating the inhibitors of these enzymes in the preclimacteric fruit, thereby explaining the increase in enzyme activity.

The gluconeogenic system has been established in ripening mangoes. Both acidic and alkaline fructose-1,6-bisphosphatase have been isolated and partially purified from ripening mangoes (249). Cell-free extracts were reported to convert organic acids and amino acids to sugars, suggesting the operation of the gluconeogenic pathway.

Plant hormones have been reported to affect the process of ripening (89). Abscisic acid enhances the process of ripening while gibberellic acid, kinetin and indoleacetic acid have been shown to retard the ripening process.

The mechanism of action of hormones is a neglected area of study. A recent report indicates that plant hormones may regulate gene expression (121).

The present study has been undertaken to understand the mechanism of action of one such hormone, namely abscisic acid, in a climacteric (mango) and a non-climacteric (grape) fruit. Antibiotics which inhibit
protein and RNA syntheses have been used in the past to understand the mode of action of hormones such as ethylene (118-120). Cycloheximide, a protein synthesis inhibitor and actinomycin D and rifamycin, RNA synthesis inhibitors, have been employed in the present study.

An attempt has been made to study the ripening process in mangoes and grapes at the molecular level. The levels of DNA, RNA, proteins, and polysomes during various stages of ripening have been examined. The effect of abscisic acid on the above mentioned parameters has also been studied.

Abscisic acid levels have been reported to increase with maturation and ripening of fruits (126-129). The levels of abscisic acid in ripening mangoes and sweet and sour grapes have been estimated.

Prolonged storage and ripening leads to microbial spoilage as a result of which the market value of fruits is decreased. Several organisms have been isolated from spoiled mangoes in this laboratory. Rhizoctonia batiticola, Aspergillus flavus other Aspergillus species, B. megaterium, B. cereus and B. marcerans are a few examples.
Penicillium cyclopium was isolated for the present study from a spoiled, ripe alphonso mango. The degree of infection during various stages of ripening was examined. Various factors that might confer an unripe fruit resistant to attack by this organism were studied. \( \gamma \)-radiation, Bavistin, (a fungicide) and aureofungin (an antifungal antibiotic) and their combinations were used to combat postharvest spoilage of mangoes by this isolate.
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