CHAPTER VIII

CONCLUSIONS
The present study focuses attention on two aspects. The first one deals with the effect of plant hormones on the ripening of mangoes and grapes and understanding the mechanism of action of one such hormone, namely abscisic acid (ABA). The second aspect deals with the spoilage of mangoes by a fungus Penicillium cyclopium and the methods by which decay of fruits by this isolate can be controlled.

The mechanism by which the process of ripening is coordinated and regulated is uncertain. Two theories have been propounded to explain this phenomenon. According to one, fruit ripening involves de novo synthesis of RNA and proteins. The other theory suggests that activation, or the release of preexisting enzymes, might play a dominant role in the process of ripening. Earlier studies reported from this laboratory lend support to the latter theory. Unripe mangoes were found to contain proteinic inhibitors which masked the activity of ripening enzymes such as peroxidase, catalase, amylase and invertase. Ethylene was found to inactivate these inhibitors in vitro thereby explaining the increase in the levels of these enzymes upon ripening (1-3).

The present study, though not ruling out the latter theory, supports the first theory. Evidence has been put forth suggesting the involvement of RNA and protein syntheses during the ripening of mangoes and grapes. The levels of total soluble proteins and RNA were found to
increase with the process of ripening in mangoes. However, in the case of grapes, total soluble protein levels did not change significantly with the process of ripening, while there was a 20% increase in the RNA content upon maturation. Sweetening resulted in a 50% decrease in the amount of RNA. An increase in the levels of total proteins and RNA has been reported in the case of apples (4, 5) but it is not a feature of ripening in other fruits such as avocados (6), bananas (7), and grapes (as observed in the present study). Acidic and neutral protease activities were maximum in the partly ripe mango fruits, while alkaline protease and RNase activities were higher in ripe mangoes as compared to unripe and partly ripe ones. The gel patterns of soluble proteins also showed changes with the process of ripening in mangoes. This suggests that protein and RNA turnover may be involved in the ripening of mangoes. An increase in the RNA and RNase content has been observed in the case of ripening apples (5, 8). The authors suggested that the enzyme RNase was sequestered in the vacuoles and released only at later stages of ripening. It is possible that a similar phenomenon occurs in mangoes as this study has shown enhancement of both RNA and RNase during the ripening of these fruits. In the case of grapes, the increase in the RNase content was correlated with a decrease
in the level of RNA. Furthermore, the incorporation of $[^{14}\text{C}]$ leucine and $[^{3}\text{H}]$ uridine into proteins and RNA respectively was found to be enhanced with the process of ripening in mangoes. These results are in keeping with earlier observation made in various other climacteric fruits, such as apples (9), avocados (10,11), bananas (12), pears (13,14) and tomatoes (15). The levels of total polysomes (measured as polysomal RNA) increased with ripening in mangoes while in the case of grapes, the polysome content rose on maturation but declined upon sweetening. These essential differences between mangoes and grapes might be a reflection of the fact that they belong to two different classes of fruits.

Plant hormones have been shown to affect ripening. Ethylene and abscisic acid generally hasten this process in the climacteric class of fruits while indole acetic acid, gibberellic acid and kinetin are known to delay the same (16-20). In the case of mangoes, ABA enhanced ripening as judged by the levels of total sugars, acids, sugar : acid ratio, carotene content, hydrolytic and gluconeogenic enzymes. Cycloheximide, a protein synthesis inhibitor was found to interfere with the progress of ripening and also with ABA action, suggesting that protein synthesis was essential both for normal ripening and ripening mediated by abscisic acid. However, actinomycih D, at a concentration
of 20 μg/ml, did not inhibit the ripening process nor did it affect ABA action. It is likely, that the concentration of the antibiotic used was not able to block RNA synthesis effectively, as the RNA content was found to increase with the process of ripening. Studies with [3H] labelled uridine establish the fact that RNA synthesis does play an important role in the ripening of mangoes.

The gluconeogenic process in grapes was also found to be enhanced in the presence of ABA as judged by the higher levels of total carbohydrates and the lower acid content. The activities of glucose-6-phosphatase, fructose-1, 6-bisphosphatase and cytosolic malate dehydrogenase were also elevated upon treatment with abscisic acid. Cycloheximide was found to block the abscisic acid mediated increase in the levels of sugars and gluconeogenic enzymes. The decrease in the levels of organic acids and an increase in the levels of total carbohydrates was blocked when RNA synthesis was interrupted by either actinomycin D or rifampicin suggesting that the ABA effect, at least in the case of grapes, is mediated via both RNA and protein synthesis. The incorporation of [14C] labelled leucine into grape proteins was enhanced by about 80% in the presence of ABA, thus confirming that the action of the hormone entails protein synthesis. Cycloheximide has been reported to block all the processes of ripening in bananas (21) and pears (13).
and both cycloheximide and actinomycin D have been shown to interfere with the induction of phenylalanine ammonia lyase in pea seedlings (22) and swede and parsnip root tissues (23). Since cycloheximide has been reported to inhibit protein synthesis (24-26) the specificity of cycloheximide is doubted (27). The conclusion that ABA involves protein synthesis is not entirely based on studies with the antibiotic. The change in the gel patterns of proteins after treatment of grapes and mangoes with ABA, the levels of polysomes, and studies on the incorporation of radioactive leucine into proteins in grapes upon ABA treatment, lend support to the above conclusion. It is not clear at the moment whether ABA enhances the synthesis of any particular enzyme(s) directly or whether the synthesis of some other protein(s) is affected which may influence the activities of the various ripening enzymes. Future studies can be focused on this aspect.

It has been suggested that the control of ripening is dependent on the balance between ethylene and other plant growth regulators (6,28,29). In order to ascertain whether ABA mediated enhancement of the ripening process in mangoes was connected with ethylene or not, studies were performed to examine the levels of ethylene during various stages of ripening and after treatment of mangoes with ABA. Ethylene
could not be detected either from ripening mangoes or from ABA treated mangoes. Failure to detect ethylene could also be due to a higher rate of ethylene metabolism occurring in mangoes as compared to other fruits like bananas and apples. Ethylene treatment to unripe mangoes did enhance the process of ripening. However, the process was not as well coordinated as observed in the case of abscisic acid treated mangoes. Thus, it appears that ABA might be the hormone which triggers the ripening process in mangoes. However, a detailed study on the effect of the inhibitors of ethylene synthesis and action on the ripening of mangoes, and the influence of ABA on the action of these inhibitors, is required before one can conclusively state whether ABA has an independent action or not.

Studies conducted to detect the levels of ABA during various stages of ripening in mangoes revealed that it was present maximally in the unripe fruit and its content declined with the process of ripening. The level present in the unripe fruit was approximately equal to $10^{-4}$ M (10^{−4} M) μmoles/kg fr. wt. tissue). That the ripening process was not stimulated even though the ABA content was high in unripe fruits, could be due to the presence of other hormones like gibberellic acid, indole acetic acid and kinetin all of which have been shown to have an effect opposite to that of abscisic acid (30-31). Since ABA was also found to enhance
the gluconeogenic process in sour grapes, it was felt that sour grapes are those fruits which are probably deficient in this hormone. In order to test this theory, the ABA content in sweet and sour grapes was examined. It was observed that, indeed, sweet fruits did contain a higher content of ABA than the sour ones.

On the basis of studies performed, a scheme for abscisic acid action in mangoes and grapes has been proposed. In grapes, ABA enhances the gluconeogenic process, while in mangoes ABA enhances polysaccharide degradation as well as the gluconeogenic process.
Prolonged storage and ripening leads to spoilage as a result of which the market value of the fruit is lowered. Several organisms have been isolated in this laboratory from spoiled mangoes. These include *Rhizoctonia bataticola* (33), *Aspergillus flavus* (34), other *Aspergillus* spp. (35), *B. megaterium*, *B. cereus* and *B. marcerans* (36,37). In the present study, a mold identified to be *Penicillium cyclopium* was isolated from a spoiled, ripe alphonso mango. The degree of infection was found to increase with the process of ripening. Several factors that could render an unripe mango more resistant, or a ripe mango more susceptible, to infection by this mold were examined. These included carbohydrates, organic acids and phenols. Increasing concentrations of glucose, fructose and starch had a stimulatory effect on the growth of this isolate in *vitro*. However, sucrose at a 1-10% concentration, had no significant effect on its growth. Organic acids such as citric and malic acids, at concentrations which are known to occur in unripe fruits, had an inhibitory effect on the growth of the fungus. Similarly, phenols like benzoic and ferulic acids were also found to inhibit the growth of the fungus. Thus, a low content of sugars coupled with higher levels of organic acids and phenolic compounds might be responsible for the low degree of susceptibility of unripe fruits to infection by this mold. Biochemical analyses of healthy and *P. cyclopium*
infected fruits revealed that the content of total carbohydrates was less in the infected mango. However, the levels of total reducing sugars were elevated upon infection. The fructose and sucrose content declined upon infection while the amount of glucose rose after infection. Infected mangoes exhibited higher levels of amylase, cellulase and invertase. Since the organism was unable to utilize sucrose under in vitro conditions, it appears that some factor(s) produced by the fungus in the tissues, or present in the fruits itself, was (were) responsible for raising the activity of invertase.

Infection resulted in a fall in the RNA and protein levels, corresponding to an increase in the activities of acid, neutral and alkaline proteases and RNase. The fungus appears to produce cyclopiazonic acid imine which is a toxic metabolite.

Various methods by which the decay of fruits (by this isolate) can be controlled were examined. Spores of Penicillium cyclopium were found to be resistant up to a 500 krad dose of $\gamma$-radiation. The $D_{10}$ value was found to be 50 krad. Thus, 25 krad, which is normally used to prolong the shelf life of mangoes, could not protect the fruits against infection. Higher doses cannot be used to control decay as they stimulate the infection process. Similar results were obtained by Padwal-Desai et al in the case of spoilage of grapes by Aspergillus spp. (38).
Bavistin (a systemic fungicide) and aureofungin (an antifungal antibiotic) were the chemical agents employed for checking possible methods of controlling the mold infection. Bavistin was found to be more effective as compared to aureofungin both in *in vitro* and under *in vivo* studies performed with *Penicillium cyclopium*. A combination of Bavistin and γ-radiation was examined for the control of spoilage. It was observed that the fungicide alone was most effective. A combination of fungicide and γ-radiation did lower the incidence of spoilage, but the degree was high as compared to the fruits which were treated only with the fungicide.

Plant hormones like kinetin had an inhibitory effect on the growth of *P. cyclopium*, while ABA was found to stimulate the same. An earlier report from this laboratory established that kinetin could delay the process of ripening in mangoes (16). Since it can also inhibit the growth of *P. cyclopium*, this hormone can serve a dual purpose of prolonging the shelf life of mangoes and checking the decay of fruits by *P. cyclopium*.

Low temperature also had a profound effect on the growth of *P. cyclopium*. The growth of the organism was inhibited by about 80-100% when the incubation temperature was lowered from 35°C to 10-20°C. No visible signs of spoilage could be detected on fruits stored at the above mentioned temperatures.
Since these temperatures are well above that which causes chilling injury in mangoes (5°C), mangoes will remain free of this physiological disorder if stored between 10-20°C. Low temperature appears to be most effective in controlling decay. However, under conditions where low temperature facilities are not available, Bavistin can be employed to protect mangoes against spoilage by Penicillium cyclopium.

**Future Prospects** : Future studies can be performed to understand:

(a) Whether abscisic acid treatment leads to enhanced synthesis of various ripening enzymes or some other proteinic factor(s) which influence(s) the activities of these enzymes. Such studies can be performed with the aid of radioactive amino acids to check for their incorporation into specific proteins. This will also give an idea as to whether the increase in the levels of various enzymes observed during the process of ripening is due to de novo synthesis or merely due to release or activation of them.

(b) Whether abscisic acid has an independent effect on the ripening of mangoes. Ripening can be studied in the presence of inhibitors of ethylene synthesis and action such as Co++, Ag+, Co2, amino-ethoxy-vinyl-
glycine and the effect of abscisic acid on the action of these inhibitors can be seen. Such a study would clearly indicate whether abscisic acid has an independent action or whether it acts via the production of ethylene.

(c) Whether the mRNA profile changes with the process of ripening and whether abscisic acid has any effect on the same. mRNA isolated from different stages of ripening can be translated with the aid of in vitro translation systems. Such a study would conclusively indicate whether abscisic acid regulates gene expression or not.
References


