

C H A P T E R   V I I I .

CONCLUSION

The present study indicates that the biochemical changes taking place in mangoes during ripening have an important bearing on the regulation of the carbohydrate metabolism in the fruit.

From the data reported in this investigation it is evident that significant changes take place in the carbohydrate fraction as the mango fruit ripens. A marked increase in the activities of a number of enzymes associated with the carbohydrate metabolism occurs.

A decrease in the polysaccharides starch, cellulose and pectin is observed throughout the course of ripening, and this can be directly attributed to the corresponding increase in the activities of the enzymes amylase, cellulase and pectinesterase. These changes in the polysaccharide fraction lead to the formation of monosaccharides which in turn impart sweetness to the fruit.

A high respiratory upsurge has been observed by other investigators during the ripening of mangoes.<sup>153,210,245</sup> This is due in part to the utilisation of carbohydrates, the major constituent of the fruit, as the carbon source. This statement receives support by the finding that the operation of glycolysis and TCA cycle are observed in mangoes. This is evident from the study with the cell free extracts of mango which show activity of the enzymes hexokinase, phosphofructokinase, pyruvatekinase, in addition to aldolase and

phosphoglucisomerase reported earlier;<sup>153</sup> and also the TCA cycle enzymes isocitrate dehydrogenase (NAD and NADP dependent) and  $\alpha$ -ketoglutarate dehydrogenase are present. Further the increase in fructose diphosphatase and glucose-6-phosphatase indicates that a reversal of glycolysis might be occurring in the ripe fruit.

The catabolism of sugars by the direct oxidative pathway has also been reported earlier.<sup>65</sup> The general trend during ripening is observed towards an increase in the activities of the above mentioned enzymes. Exception to this generalisation is pyruvate kinase which shows neither an increase nor a decrease in activity.

In spite of the possibility for a high consumption of carbohydrates, the accumulation of sugars is remarkably high in the fully ripe fruit, suggesting thereby, the operation of an efficient regulatory mechanism. In mangoes  $\beta$ -carotene and fatty acids have been shown to increase during ripening. Present studies indicate that these substances regulate the catabolism of sugars by inhibiting the initial and rate limiting enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase of HMP shunt and malic enzyme. The nature of the inhibition was found to be competitive with respect to the substrates of these enzymes, suggesting thereby, that these substances affect that form of the enzyme which combines with the substrate. The possibility of these substances acting as natural detergents in the fruit

cannot be overlooked. Further investigations in this area though laborious would certainly be rewarding.

It was found that  $\beta$ -carotene and fatty acids inhibited the above mentioned enzymes of HMP shunt from papaya and 6-phosphogluconic dehydrogenase from yeast. However, amylase and invertase, from mango and other sources and fructose diphosphatase and isocitrate dehydrogenase (NADP) from mango were not inhibited by these substances.

Sucrose the only disaccharide reported in mango fruit has been shown to increase tremendously during ripening.<sup>65</sup> The results in chapter IV indicate that the biosynthesis of sucrose follows a path common to that reported in other plant tissues. Fructose as well as fructose-6-phosphate were found to be substrates for this enzyme. Partial purification revealed that sucrose synthetase and sucrose phosphate synthetase both are present in the fruit.

Activity of invertase, an enzyme that hydrolysis sucrose, was found to increase during ripening. Kinetics of these enzymes revealed that UDPG, one of the substrates for the synthesis of sucrose, has greater affinity towards its enzyme sucrose synthetase as compared to that of sucrose towards invertase. These results indicate that a fine regulatory mechanism exists between the synthesis and breakdown of sucrose.

Earlier Mattoo and Modi,<sup>154,252</sup>

have reported that proteinic inhibitors exist in the unripe mango for the enzymes catalase peroxidase and amylase which mask the activities of these enzymes. Ethylene, a fruit ripening hormone, inactivates these inhibitor and in turn activates the enzymes.

During the present study it was found that a number of enzymes namely invertase, cellulase, amylase, glucose-6-phosphatase, phosphofructokinase, sucrose synthetase and sucrose-phosphate synthetase were inhibited by the unripe dialysed extract. The enzymes glucose-6-phosphate dehydrogenase 6-phosphogluconic dehydrogenase and pectinesterase were not inhibited. The presence of these inhibitors in the unripe fruit would be expected to mask the enzymes thus preventing them from manifesting their activities and thereby playing an important role in the metabolism of the fruit.

The qualitative nature of protein (as per acrylamide electrophoretic analysis) during ripening was also found to change. Unripe extracts gave six discrete bands whereas ripe extracts gave only four diffused bands. Two glycoprotein bands were observed throughout the ripening process.

Partial purification of the invertase inhibitor has been achieved by the chromatographic

separation using DEAE saphadex and DEAE cellulose columns. This inhibitor is non-dialysable, heat labile, ninhydrin and folin phenol reagent positive indicating its proteinic nature. Studying the kinetics of the invertase inhibitor it was found that the inhibitor competes with the substrate to bind with that form of the enzyme with which the substrate binds. Further work would throw more light in the understanding of the mode of action of this inhibitor.

Unripe banana and papaya were also found to contain inhibitor for invertase. The inhibitor from banana was found to inhibit the mango enzyme and vice-versa.

During this study it has been found that as mangoes ripen the inhibitory activity decreases and a concomitant increase in the activity of invertase occurs. These results were substantiated by the finding that the increase in reducing sugars was parallel to the increase in the enzyme activity.

Mattoo and Modi<sup>252</sup> have earlier shown that the proteinic inhibitor(s) of catalase, peroxidase and amylase were inactivated by ethylene, the fruit ripening hormone.

Similar results were obtained with invertase inhibitor. In ethylene treated mango slices the immediate inactivation of invertase inhibitor followed by the

promotion of the enzyme activity was observed. The development of softness and slight but significant pigmentation in these slices also clearly indicates the role of inhibitor and ethylene in mangoes prior to ripening. The inactivation of the purified inhibitor with ethylene was also observed. From these results it is pertinent to view that after the inactivation of its inhibitor the interaction of invertase with its substrate becomes easier.

Two possibilities exist invivo

(a) ethylene inactivates the inhibitor (b) ethylene affects the enzyme as well as the inhibitor in such a way that the enzyme inhibitor interaction is not feasible. These interactions either between ethylene and enzymes and/or ethylene and enzyme inhibitors need more detailed study to understand the mechanism of ethylene action at the level of macromolecular organisation.

Successful use of antifungal antibiotic aureofungin in prolongation of the storage life has also been reported in this study.

The unripe fruits, because of their unfavourable conditions, such as high acidity, low ethylene synthesis low respiration rate and insignificant amount of enzyme activities due to the presence of inhibitors are metabolically less active, whereas, in partly ripe and fully ripe fruit the low acidity, high ethylene content, increased

respiration, inactivation of inhibitor(s) and a considerable stimulation in enzyme activities are some of the favourable conditions for high metabolic rate. In spite of the high metabolic rate the catabolism of sugars is regulated by  $\beta$ -carotene and fatty acid; this might be one of the many reasons for imparting sweetness to the fruit.

On the basis of these observations Fig 20 and Table XXVI are drawn to indicate the post harvest biochemical changes and the factors that regulate the carbohydrate metabolism during ripening in mangoes.

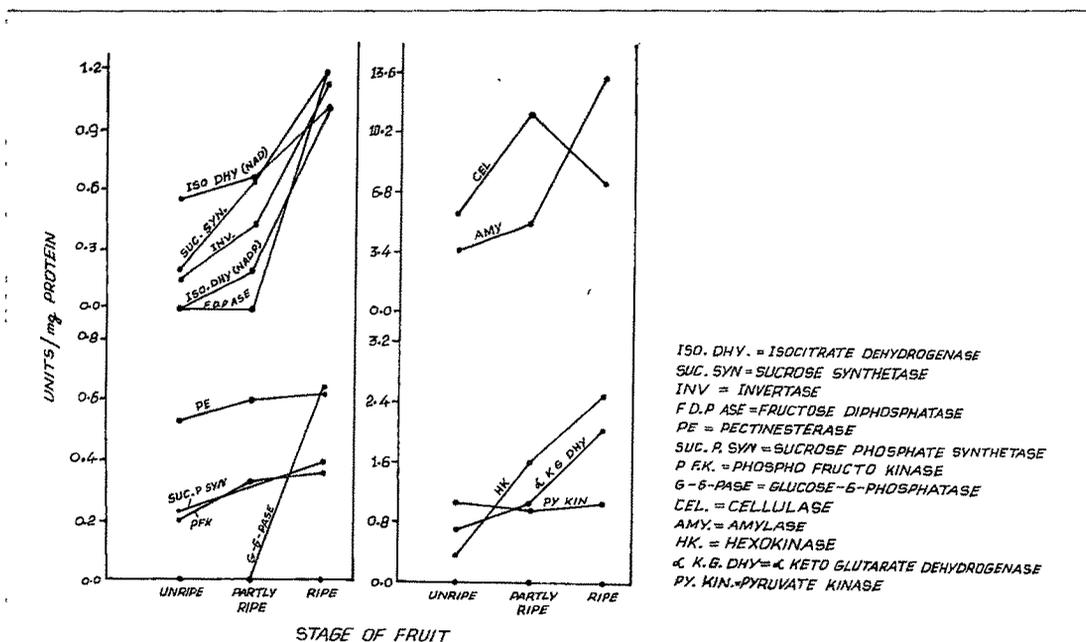


FIG. 20 - CHANGES IN ENZYME ACTIVITIES IN MANGOES DURING RIPENING

TABLE XXVI.

Some Factors Controlling Carbohydrate Metabolism in Ripening  
Mangoes

Inhibitor		Fatty acids and $\beta$ -carotene	
Enzymes affected	Enzymes not affected	Enzymes affected	Enzymes not affected
Cellulase	Pectin-esterase	Malic enzyme	Invertase
Amylase	Glucose-6-phosphate dehydrogenase	Glucose-6-phosphate dehydrogenase	Amylase
Invertase	6-phospho-gluconic dehydrogenase	6-phospho-gluconic dehydrogenase	Isocitrate dehydrogenase (NADP)
Phosphofructo kinase			FDPase
Sucrose synthetase			
Sucrose phosphate synthetase			
Glucose-6-phosphatase			