C H A P T E R III.

SOME BIOCHEMICAL CHANGES ASSOCIATED WITH THE CARBOHYDRATE METABOLISM IN RIPENING MANGOES

Many of the fruits after picking show prolonged independent existence even though the nutrient supply to it from the plant is stopped. During this phase important modifications characteristic of ripening occur. Thus the physiology of the harvested fruits is chiefly the physiology of ripening and senescence.

Over the past forty years, the manifold physiological and biochemical changes accompanying fruit ripening have been extensively studied.[2-18] The studies have provided qualitative and quantitative information on many of the important constituents undergoing changes during ripening.

Ripening of fruit involves a dramatic increase in the activities of a number of enzymes.[44,46,58-60,127,133-138] Thus, in the ripening fruit, marked changes in texture, sweetness, flavour and colour occur. Evidences of an increase in the rate of protein synthesis,[191,192] in the protein content[7,193-195] and of a changed pattern of nucleic acid synthesis[196] early in the ripening phase indicate that some of the changes associated with ripening are controlled at the level of transcription or translation of the genome. Frankel et al[197] have concluded that the ripening process in pome fruit involves a direct synthesis of new enzymes.

In fruits the most prominent chemical changes have been observed in the carbohydrate

fractions.[5,7,28,30,31,33] The material insoluble in alcohol comprises mostly of polysaccharides with a very small amount of protein.[7] The polysaccharide material consists of pectic substances, starch, hemicellulose and cellulose. In banana about 60% of the loss in dry matter during ripening has been shown to be due to the disappearance in the total carbohydrates.[15,198] Catabolism of starch is most marked, lasting about nine days at 70°-80° F resulting in the formation of reducing sugars (glucose and fructose); the same authors further report that in the post climacteric stage the relative reduction in sucrose content is more marked than the drop in glucose and fructose.

Kidd et al[25,26] have shown that on removal of fruits from the tree no further starch synthesis occurs but most of it is catabolised. The importance of starch as a guide line for harvesting fruits has been verified.[7,293]

Ripening of mango is marked by fascinating physico-chemical changes. The fruit changes colour from green to yellow loses weight due to transpiration and respiration and also loses its characteristic flavour and develops an aroma. Due to the synthesis of sugars and disappearance of acids the fruit becomes sweet in taste, it loses firmness and becomes soft to touch. The pH of the pulp increases from 2.0 to 5.5. These changes take place quicker when the fruits are kept at high temperature (25°-30° C).

Some of the chemical changes associated with the ripening of banana are also found to occur in mangoes. Leyley et al[148] attempted a biochemical study of the Alfanso mango. They found that at harvest the starch content was 14% of the fresh weight, and total sugars 7%. During the 11 day ripening period, starch content was reduced to 0.3% and total sugars increased to 17% sucrose content rose from 5.0 to 14.2%, dry matter declined from 22.2 to 20.2% and pH increased from 3.0 to 5.2 due to a ten fold drop in titrable acidity.

Modi and Reddy[65] reported results that are in accordance with Leyley et al's[148] work. Krishnamurthy and Subramanyam[210] have also further confirmed these results on pairi variety of mango.

As an approach to correlate the sugar metabolism in mangoes with polysaccharides attempts were made to study the qualitative and quantitative changes occurring in this fraction during ripening. Results in Table 1 show that in mangoes ripening is marked by an increase in the alcohol soluble matter accompanied by a decrease in the insoluble material. The changes observed are not as drastic as in banana[22] or sapote.[211] The starch and cellulose contents were found to be highest at harvest decreasing to a minimum during the 11 day ripening period (Table 1). This result supports the above observation on the decrease in alcohol insoluble material as the fruit ripened.

TABLE I.

Changes in Alcohol Soluble and Insoluble Material Starch and Cellulose content during ripening of Mangoes*

Stage of fruit	Alcohol insoluble material	Alcohol soluble material	Total dry weight	Starch	Cellulose
Unripe	13.50±2.64	20±2.3	33.5±4.94	12.0±2.64	4.92±1.05
Partly ripe	8.0±0.9	30±1.65	38±8.55	2.59±0.59	2.0±1.5
Ripe	7.0±0.65	32±2.00	39±2.65	1.08±0.9	1.12±0.2

* All the values are in g.% on fresh weight basis. Values are average of four determinations.

The increase in the sugars during the ripening of mangoes can be directly attributed to the hydrolysis of starch and cellulose. Evidence has accumulated to show that the polysaccharides associated with the cell wall (cellulose, hemi-cellulose and pectin) are continuously involved in the respiration of a fruit.[189,200]

The enzymes responsible for the breakdown of starch cellulose and pectin have been studied (Table II). A four fold increase in amylase accounts for the steep decrease in starch. Amylase has been shown to increase in ripening pears[201] and has been shown to be the cause of starch breakdown in leaves.[212]

In mangoes the reduction in cellulose is due to the increase in cellulase activity which provides additional sugar (Table II). The increase during the partly ripe stage is relevant to the observation that cellulose loss is also greater during this period than during partly ripe to fully ripe stage. Presence of cellulase in pumpkin[202] and the increase in this enzyme activity in ripening tomatoes[58] have been reported earlier.

Pectic enzymes (polygalacturonase and pectin methyl esterase) are generally considered to be the cause for the change in texture of fruits during ripening. In ripe tomatoes[203] the level of pectin methyl esterase activity has been found to be 23 to 27 times more than polygalacturonase activity. Ghosh et al[213] have shown an increase in the soluble pectin and a decrease in the parent compound protopectin during ripening of bananas. In mango fruit also a decrease in pectin content has been reported earlier.[149] A tendency towards the increase in the activity of pectin esterase accounts for the decrease in pectin content (Table II).

The pH optima for amylase and cellulase were found to be 7.0 and 5.5 respectively (Fig.3). Amylase and cellulase from mango have pH optima similar to that reported from other sources.[58,201,202] While pectinesterase from mango shows a pH optimum of 7.0 unlike the enzymic preparations from tobacco leaf and tomato fruit enzyme with a

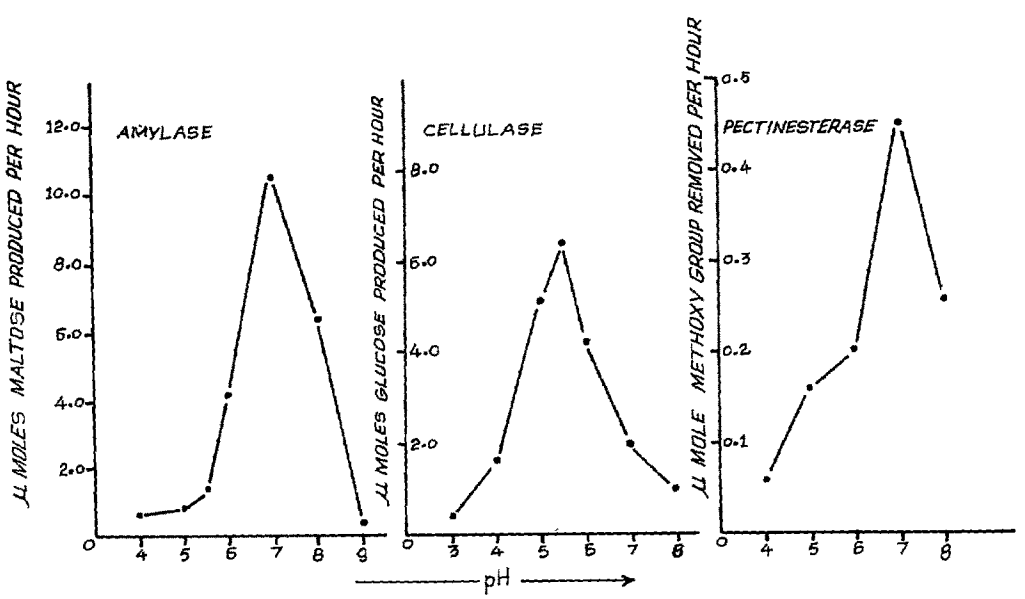

FIG. 3 – EFFECT OF pH ON AMYLASE, CELLULASE & PECTINESTERASE

pH optimum of 9.0.[214,215]

Catabolism of sugars during ripening in mangoes follows the glycolytic pathway,[153] through Kreb's cycle[210] and also the HMP shunt.[65] In addition to these in the present inquiry the activities of the key glycolytic enzymes hexokinase and phosphofructokinase were also found to increase 6.5 and 1.8 times during ripening; pyruvate kinase activity remained the same (Table II). Earlier, Mattoo[153] had reported increases in the activity of phosphoglucose isomerase and aldolase during ripening. These results show that glycolysis operates at a considerable rate during ripening. The enzymes of the Kreb's cycle viz. isocitrate dehydrogenase and α-ketoglutarate dehydrogenase also show increased activity during this phase (Table II). NADP dependent isocitrate dehydrogenase was not detected in the unripe extract where as the NAD dependent one was found to be present. AMP had no effect on the NAD dependent enzyme unlike the yeast enzyme.[204] These results indicate that glucose breakdown via glycolysis is followed through TCA cycle in mango fruit.

Increase in the activities of the enzymes glucose-6-phosphatase and fructose diphosphatase during ripening suggests a possibility that reversal of glycolysis might be taking place (Table II).

To eliminate probable errors due to non specific phosphatases a one step purification was attempted for fructose diphosphatase. The tissue was homogenised in a precooled mortar and pestle with 0.005 M sodium lactate buffer (pH 3.5) and centrifuged at 800 x g. The supernatant obtained was incubated for 8 hours at 37° C, cooled to 4° C, immediately the pH was adjusted to 7.0 and the insoluble material was removed by centrifugation at 10,000 x g. The supernatant so obtained was used for the estimation of fructose-diphosphatase. It was observed that the one step purified enzyme had no activity towards glucose-6-phosphate, fructose-6-phosphate and β -glycerophosphate and the pH optima was 7.0 (Fig.4).

Glucose-6-phosphatase could be the non specific acid phosphatase, which is present during all stages of ripening mango. The only difference between the general acid phosphatase and glucose-6-phosphatase reported here is that the activity of the latter enzyme is detectable only in the ripe mango whereas the former is found in all the stages.

From the data reported above it is evident that significant changes take place in the carbohydrate fraction of the mango fruit accompanied by a marked increase in a number of enzymes associated with carbohydrate metabolism. The decrease of polysaccharides leads to the formation of monosaccharides, imparting sweetness to the ripe fruit.

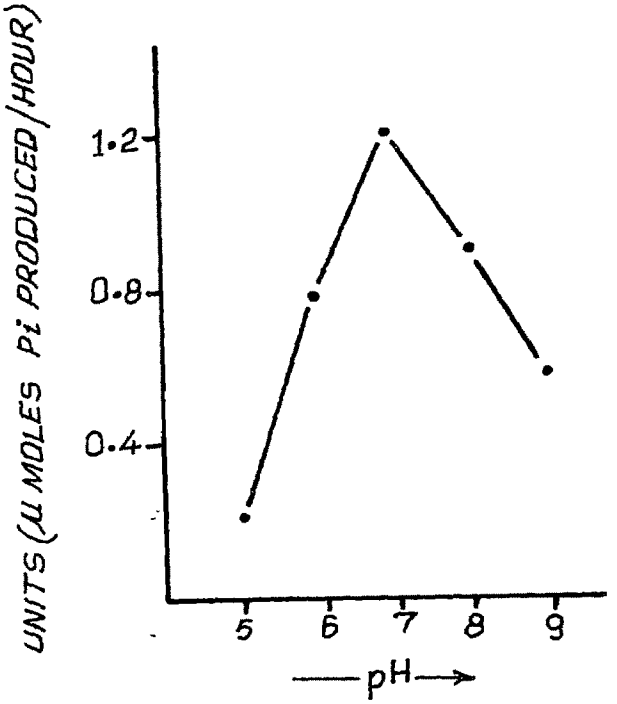

FIG.4 - EFFECT OF pH ON FRUCTOSE DIPHOSPHATASE

TABLE II.

Changes in some Enzyme Activities during Ripening

Enzymes tested	Stage of fruit		
	Unripe	Partly ripe	Ripe
Amylase	3.5±1.06	5.2±2.01	13.4±3.5
Cellulase	5.6±3.20	11.2±4.50	6.5±3.56
Pectinesterase	0.53±0.105	0.60±0.11	0.62±0.30
Hexokinase	0.38±0.02	1.60±0.30	2.50±0.35
Phosphofructokinase	0.2±0.015	0.32±0.02	0.36±0.022
Pyruvate kinase	1.08±0.25	1.00±0.30	1.1±0.40
Glucose-6-phosphatase	*	*	0.65±0.20
Fructose-1:6-di-phosphatase	*	*	1.2±0.5
Isocitratedehydrogenase:			
NAD	0.55±0.2	0.66±0.15	1.00±0.25
NADP	*	0.2±0.02	1.03±0.5
α-ketoglutarate dehydrogenase	0.7±0.3	1.06±0.23	2.05±0.33

All values are mean S.D. of 3 determinations.

* These activities could not be detected.