C H A P T E R IV.

SUCROSE BIOSYNTHESIS IN RIPENING MANGOES

Sucrose, the most abundant disaccharide in higher plants was first synthesised *in vitro* from α-D-glucose-1-phosphate and D-fructose by a bacterial enzyme from *Pseudomonas saccharopila*.[222] The equilibrium of this reaction was found to favour the breakdown of sucrose rather than its synthesis. Various attempts by a number of investigators[222,223] to synthesise sucrose by a plant enzyme from α-D-glucose-1-phosphate and D-fructose failed. Later Leloir *et al*[150,151] and a number of others[176-179] found that in this reaction the donar was not α-D-glucose-1-phosphate but the sugar nucleotide, UDP-D-glucose.

The synthesis was shown to take place by the separate enzymes, one utilising D-fructose (1) and the other D-fructose-6-phosphate (2)

UDP - glucose + D-Fructose ---------→ Sucrose (1)

UDP - glucose + D-Fructose-6-phosphate -------→ Sucrose phosphate (2)

The first reaction is catalysed by sucrose synthetase (UDPG-D-fructose-2 glycosyl transferase) and the second reaction by sucrose phosphate synthetase (UDPG-D-fructose-6-phosphate 2 glycosyl transferase). The sucrose phosphate formed has been shown to be hydrolysed by a phosphatase to sucrose.[180,181] Evidence presented make it appear that photo synthetic leaves use the latter reaction exclusively[181-183] whereas Rorem *et al*[184] have shown the presence of both the enzymes in sugar beet leaf. Bean[185] showed

that fresh green peas contained both the enzymes for the synthesis of sucrose.

Some valuable information on the mechanism of sucrose synthesis in apple tissue has been provided by Axelrod and Seegmiller,[186] who infilterated C^{14}-glucose into apple discs, and found that it was incorporated into sucrose. The extent of incorporation of labelled glucose was greatly reduced by exclusion of oxygen. Free fructose was not labelled but both glucose and fructose moieties of sucrose were labelled, the labelling in glucose being higher than in fructose. This led the authors to suggest that both halves of the sucrose were not formed via UDPG.

Bean[187] has found that the soluble preparation from the juice of mature oranges catalyse the reaction between UDPG and fructose to form sucrose and that the particulate preparation from lemon catalysed the same reaction. He did not find any activity in these systems using fructose-6-phosphate and UDPG as substrates.

Hawker,[188] while working with grapes observed that about half way through their development an increase occured in the enzymes invertase, sucrose synthetase, sucrose phosphate synthetase and sucrose phosphatase. The presence of sucrose synthetase in ripe bananas has also been reported.[189]

During investigations on mango a four fold increase in sucrose content was reported with ripening,[65] in spite of a high invertase activity.[152] Mattoo and Modi[221] observed that mangoes stored below 5° C developed chilling injury and that in the injured tissues sucrose content was very low due to an increase in invertase activity. In view of these findings studies were undertaken on the biosynthesis of sucrose during ripening in mangoes.

While studying the sucrose synthesising ability of the mango tissue it was found that the cell free extracts prepared from fruits, (at different stages of ripening), could synthesise sucrose from the substrates fructose and fructose-6-phosphate (Table III).

TABLE III

Activity of Sucrose Synthetase, Sucrose Phosphate Synthetase and UDPG content in Ripening Mangoes

Stage of fruit	Units/mg. protein		UDPG** μ moles/100 g. fresh weight
	Sucrose Synthetase	Sucrose Phosphate Synthetase	
Unripe	0.20±0.03	0.22±0.05	11.4±1.4
Partly ripe	0.65±0.25	*	22.0±3.5
Ripe	1.27±0.15	0.40±0.03	20.0±2.0

* Not determined.

The results are mean values ± S.D. for three determinations and ** for six determinations.

The increase in sucrose synthetase activity has been found to be greater between the unripe and partly ripe stage rather than the partly ripe and fully ripe stage. The higher concentration of UDPG in the partly ripe stage confirmed these observations. Fructose was found to be a better substrate since three times higher activity of the enzyme was obtained with it as compared to Fructose-6-phosphate. These results substantiate the findings of Modi and Reddy[65] that the rate of increase in sucrose is greater between the unripe and partly ripe stage although the highest concentration is in the fully ripe fruit.

Sucrose phosphate synthetase activity increased after dialysis for six hours (Table IV) unlike the wheat germ enzyme which has been shown to be inactivated after five hour of dialysis.[151]

TABLE IV

Effect of Dialysis on Sucrose Phosphate Synthetase Activity of Mango Fruit

	Specific activity (units/mg. protein)
Undialysed	0.20
Dialysed	0.50

Using paper chromatographic technique with butanol:pyridine:water (6:4:1.5) as the solvent system the reaction mixture revealed the presence of sucrose which was absent in the blank tubes. The substance was identified as sucrose when sprayed with alkaline silver,[224] resorcinol[225] and benzidene tri-chloro acetic acid.[226]

The extracts contained a high activity of sucrose synthetase and sucrose phosphate synthetase at pH 7.0 whereas invertase and phosphatase activities were very low at this pH. Phosphatase was assayed in a manner similar to that used for FDPase with the modification that FDP was substituted with fructose-6-phosphate. It was interesting to note that ADP-glucose could not act as a substrate for the mango enzyme unlike the topioca enzyme.[190]

In order to study the reversibility of the reaction the following system was used; 0.6 μ moles UDP or ADP, 2.0 μ moles sucrose, 50 μ moles phosphate buffer pH 7.0) and the enzyme in a total volume of 0.3 ml.; the system was incubated at 37° C for one hour, the reaction was terminated by boiling in a water bath for one minute and the reducing sugar estimated by Nelson's method. It was found that when UDP or ADP was added the reaction was irreversible unlike the potatoe enzyme which was shown to be weakly reversible.[177]

The pH optima for both the enzymes were found to be 7.0 (Fig. 5) with a sharp decrease at pH 7.5 and negligible activity at pH 8.5. The mango enzyme differed from the sugar beet leaf enzyme[184] which was shown to have a pH optimum of 8.7 but resembled the wheat germ enzyme.[150,151] This result does not necessarily mean that the pH optima of sucrose synthetase in mango is 7.0 but it seems that at this pH the rate of transglycosylase is much more than the rate of invertase activity which is also found to increase by about nine fold during ripening (Table V). The optimum pH for invertase lies between 5.0 and 5.5 (Fig. 6) unlike the potato tuber,[253] carrot root[254] or sugarcane[255] invertase which exhibits a double pH optima. To see if the same enzyme has the capacity to accept both fructose and fructose-6-phosphate as substrates or two different enzymes are present a partial purification of the enzymic preparation has been attempted.

TABLE V

Activity of Invertase during Ripening in Mangoes

Stage of fruit	Specific activity (units/mg. protein)
Unripe	0.17±0.05
Partly ripe	0.44±0.20
Ripe	1.15±0.55

The results are mean values ± S.D. for six determinations.

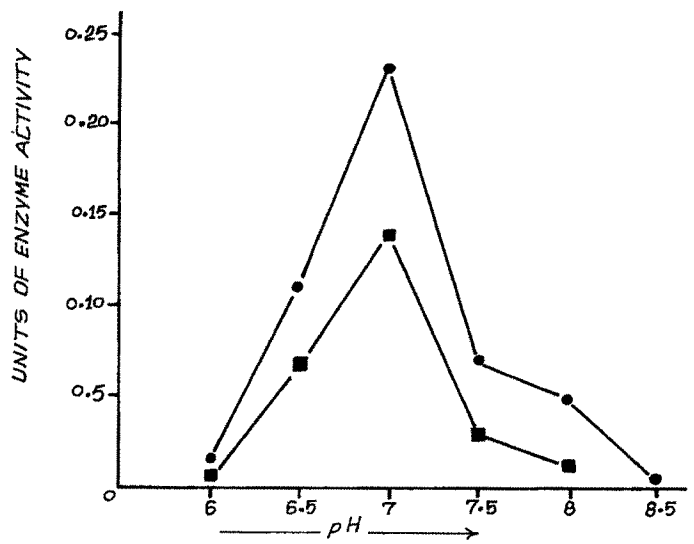

FIG. 5 – EFFECT OF pH ON SUCROSE SYNTHETASE AND SUCROSE PHOSPHATE SYNTHETASE ACTIVITY

(●—● SUCROSE SYNTHETASE)
(■—■ SUCROSE PHOSPHATE SYNTHETASE)

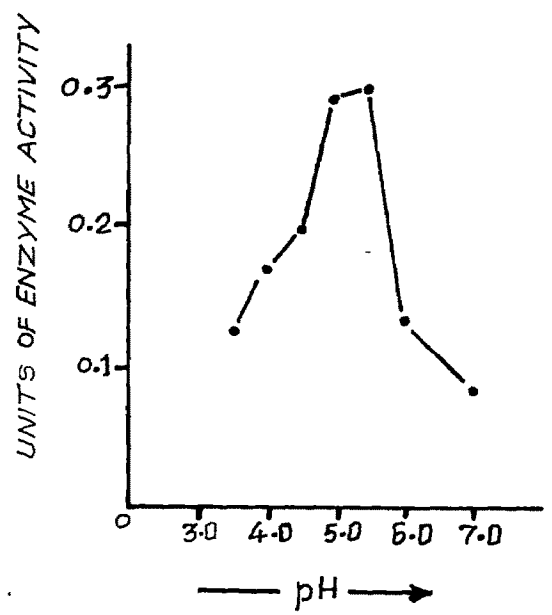

FIG. 6 — EFFECT OF pH ON INVERTASE ACTIVITY

For the purification studies 30% ripe mango extract was prepared as described in materials and methods. To the supernatant 0.016 M magnesium acetate was added to remove nucleic acids and pectins. After allowing it to stand for 20 minutes the precipitates were removed by centrifugation at 10,000 x g and to the supernatant obtained, powdered ammonium sulphate was added to bring the saturation to 20% and was kept standing for 6 hours. The precipitate formed were centrifuged and dissolved with 0.1 M phosphate buffer (pH 7.0). Further ammonium sulphate was added to the supernatant to bring the saturation to 50% and was left standing for eight hours. The precipitates formed were centrifuged and dissolved in 0.1 M phosphate buffer pH 7.0. All the fractions were dialysed against distilled water for 12 hours.

The results in Table VI indicate that the two enzymes are different and get precipitated at different ammonium sulphate saturations. A 15 fold purity of sucrose synthetase and a 19 fold purity of sucrose phosphate synthetase has been obtained.

While studying the kinetics of sucrose synthetase using UDPG as the variable substrate it was found that the Michaleis constant was 3.3×10^{-5} M (Fig. 7) whereas, that of invertase for sucrose was much higher 5.5×10^{-2}M(Fig.11). These results indicate that the affinity of sucrose synthetase is greater towards its substrate UDPG

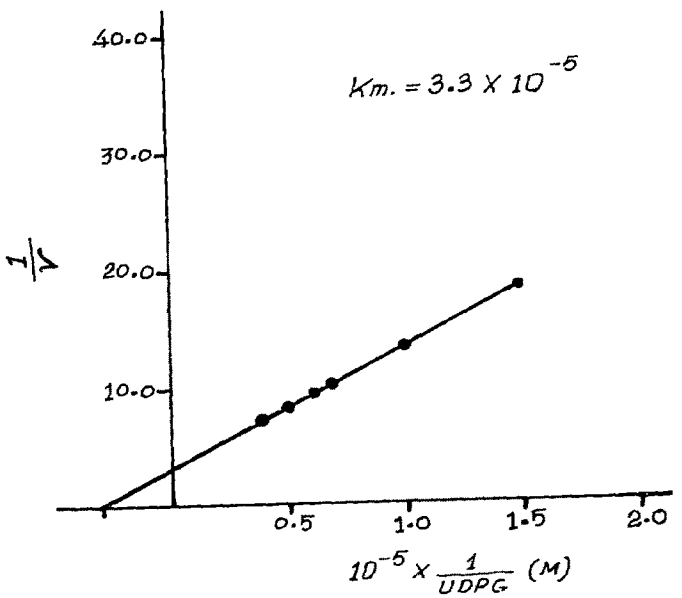

Fig. 7 LINE WEAVER-BURK PLOT OF SUCROSE SYNTHETASE

than that of invertase for sucrose. This might be one of the reasons for a high accumulation of sucrose in the fruit.

TABLE VI

Partial Purification of Sucrose Synthetase and Sucrose Phosphate Synthetase from Ripe Mango

Fractions	Volume ml.	Total units	Protein (mg/ml.)	Specific activity	Fold purity
Crude	115	34.5(25.0)	0.48	0.71(0.52)	-
0.016 M Magnesium Acetate supernatant	105	31.0(23.0)	0.305	1.0(0.73)	0.5(0.4)
0-20% $(NH_4)_2SO_4$ precipitate	4	- (3.6)	0.096	- (10.0)	(19)
20-50% $(NH_4)_2SO_4$ precipitate	6	7.0(-)	0.096	11.0(-)	15

Values in parenthesis are for sucrose phosphate synthetase.

These results indicate the presence of an efficient machinery for the synthesis of sucrose, which is not different from other sucrose synthesising plant tissues. It also suggests the operation of a cyclic pathway for the synthesis and breakdown of sucrose thus pointing towards a fine regulatory mechanism in the mango fruit.