CHAPTER VI

GLYOXYLATE IN IMMATURE, DEVELOPING

AND RIPENING MANGO
The accumulation of one or the other acid in fruits at maturity is a general phenomenon. In any tissue, the amount and the types of acid accumulated are genetically controlled, though the phase of development, total period of storage and ripening, nature of the controlled atmosphere storage and the environmental conditions such as temperature and humidity also play very important role.

Hulme and Rhodes showed malate to be accumulating in the apple fruit during the early stages of growth on the tree and then slowly declining, while succinate was found only in fruits stored in controlled atmosphere under relatively high CO₂ conditions. Oxalic acid has always been found in small amounts in apples.

Malate and citrate accumulate predominantly in apple and banana, and tartarate in grapes. The main organic acid of grape is tartaric with some amount of succinic, fumaric, pyruvic, oxoglutaric, glyceric, glycolic, dimethyl-succinic and quinic acids.
Oxalic acid accumulates in certain species of plants such as spinach, woodsorrel and begonia. While citric is the major acid of citrus endocarp, citrus peel contains exalic, malic and malonic acids along with citric, quinic, tartaric, benzoic and succinic acids.

Fruit tissues are able to use organic acids as respiratory substrate, since they show higher respiratory quotient with acids than that with sugars. Besides, they have specific functions in fruit metabolism like auxin metabolism, solubilization of insoluble pectic substances, and development of taste and flavour. As the tomato fruit ripens from mature green to red, acidity increases to a maximum extent and then decreases. Malate concentration is found to decrease as the tomato ripens, while citric acid increases up to the green yellow stage of ripeness and then either decreases or shows no significant change.

The mango fruit is highly acidic in the unripe stage mainly due to the presence of citric and malic acids. The main other acids present in mango fruit are oxalic, malonic, succinic, pyruvic, galacturonic, glucuronic, mucic and tartaric. Fang found the presence of glycolic, oxalic, malic, citric and
tartaric acids in Kent and Hsaing-Ien mangoes of Taiwan, along with traces of unidentified acids probably the volatile organic acids. 444

There is a paucity of information regarding the presence of glyoxylic acid in mango fruit. Present investigation therefore deals with the studies on glyoxylate accumulation, metabolism and role in different developing and ripening stages of mango.

Glyoxylic acid was found to be present in mango, the concentration of which was found to be increasing (figure-1) during development to about 29 fold and decreasing again during ripening.

The concentration of glyoxylate at any stage in mango may be directly related to the relative rates of its synthesis, catalysed by isocitrate lyase and malate lyase, degradation, catalysed by glyoxylate reductase and glyoxylate dehydrogenase, and/or transamination, catalyzed by l-alanine-glyoxylate aminotransferase, at respective stages.

Glyoxylate, produced by glycolate oxidation, is reported to be directly decarboxylated to \( \text{CO}_2 \) and formate in spinach chloroplast during photorespiration in cucumber, marrow and malon fruits, storage tissues and wheat germ. 445 Glycolic acid oxidase, a flavoprotein
which oxidized glycolate to glyoxylate in the presence of oxygen,\textsuperscript{446,447} has been suggested to have a role in the respiration\textsuperscript{285,447} and photosynthesis.\textsuperscript{448}

### Table-1

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>0.0</td>
</tr>
<tr>
<td>NAD</td>
<td>1.7</td>
</tr>
<tr>
<td>NADP</td>
<td>4.9</td>
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</tbody>
</table>

Glyoxylate can also be oxidized to oxalic acid by an enzyme which has been reported in tobacco leaves.\textsuperscript{449} Glyoxylate dehydrogenase from \textit{Pseudomonas oxalaticus}\textsuperscript{283} was optimally active at pH 8.6 and required NADP as a cofactor. Glyoxylate dehydrogenase from mango also preferentially required NADP as compared to NAD as a cofactor (Table-1). The enzyme was optimally active at pH 7.5 (figure-2a).

Glyoxylate reductase has been studied in plants\textsuperscript{285,447} and algae.\textsuperscript{450} A reversible glyoxylate reductase from spinach leaf had pH optima between 6 to 6.5 of the reductive reaction and 8.9 of the oxidative reaction.\textsuperscript{451}
Fig. 1 GYCOXAL LEVELS DURING DEVELOPMENT AND RIPENING OF MANGO. "FEB" TO "JUN" INDICATES IMMATURE STAGES WHILE "UR", "PR" AND "RF" INDICATES URSIDE MATURE, PARTLY RIFE AND RIFE STAGES RESPECTIVELY.

Fig. 2 pH DEPENDENCY OF (a) GLYCOXAL DEHYDROGENASE AND (b) GLYCOXAL REDUCTASE FROM MANGO.
Table-2

Specificity of glyoxylate reductase for NADH

<table>
<thead>
<tr>
<th>Cofactor</th>
<th>Specific activity (Units/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>Nil</td>
<td>0.0</td>
</tr>
<tr>
<td>NADPH</td>
<td>0.9</td>
</tr>
<tr>
<td>NADH</td>
<td>5.8</td>
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Zelitch\textsuperscript{285} crystallized the NADH requiring glyoxylate reductase from tobacco leaves which had pH optima from 6.3 to 6.6. A photosynthesizing algae, \textit{Scenedesmus},\textsuperscript{450} also contain both NADH and NADPH glyoxylate reductases. Tolbert \textit{et al}.\textsuperscript{452} and Zelitch and Gotto\textsuperscript{453} also reported a NADPH specific glyoxylate reductase from chloroplasts. Glyoxylate reductase was also found to be present in mango fruit which was more specific for NADH as compared to NADPH (Table-2) and having pH optima of 8.0 (figure-2b).

Plant transaminases remain as an inadequately defined group, particularly with regard to their amino and keto acid specificities.\textsuperscript{454} The formation of glycine from glyoxylate and the formation of hydroxypyruvate from serine-aminotransferase reactions between glyoxylate and serine, or between glyoxylate and alanine or between glyoxylate and glutamic acid have been reported in
extracts from oat leaves. Smith showed that kidney bean leaf homogenates catalyzed aminotransferase reactions between the serine glyoxylate, alanine-glyoxylate, glutamic-glyoxylate and serine pyruvate.

The aminotransferase operating with glyoxylate as acceptor was also studied from mango tissues. L-Alanine glyoxylate aminotransferase from mango did not require pyridoxal phosphate (Table-3). The glyoxylate aminotransferase rates were not affected by exogenous pyridoxal phosphate.

<table>
<thead>
<tr>
<th>System*</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>With pyridoxal phosphate (5 umoles)</td>
<td>2.92</td>
</tr>
<tr>
<td>Without pyridoxal phosphate</td>
<td>2.90</td>
</tr>
</tbody>
</table>

* System contained in umoles; L-alanine, 300; glyoxylate, 300; and potassium phosphate buffer (pH 7.4), 750; in a final volume of 12.5 ml.
phosphate in human systems also, while pea transaminase showed 50% decrease in activity when pyridoxal phosphate was omitted from the system. Mango transaminase showed broad pH optima between 7.2 to 7.6 (figure-3). The pH optima for pea transaminase was reported to be 8.5, while it was 8.2 for glyoxylate transaminase from wheat leaves.

Isocitrate lyase, forming glyoxylate and succinate from isocitrate, was also found to be present in mango and was optimally active at pH 7.0 (figure-4a). The isocitrate lyase from *Pseudomonas aerogenosa*, *Neurospora crassa*, and *Chlorella pyrenoidosa* has been reported to have pH optima of 8.0, 6.0, 6.8 and 7.6 respectively.

<table>
<thead>
<tr>
<th>Table-4</th>
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<tr>
<td>Requirement of ATP and CoA for malate lyase reaction</td>
</tr>
<tr>
<td>System</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Complete system*</td>
</tr>
<tr>
<td>Without ATP</td>
</tr>
<tr>
<td>Without CoA</td>
</tr>
</tbody>
</table>

* Complete system consisted of (in μmoles): tris-HCl buffer (pH 7.4), 100; 1-malate, 20; ATP, 5.0; MgCl₂, 10; CoA, 1; phenylhydrazine hydrochloride, 3 and mercaptoethanol, 20 with enzyme in 2.0 ml. final volume.
Fig. 3 pH dependency of L-Alanine glyoxylate aminotransferase.

Fig. 4 Effect of pH on (a) Malate lyase and (b) Isocitrate lyase activities.
Malate lyase, catalyzing the production of glyoxylate and acetyl CoA from malate was found to be active in mango. It required CoA and ATP as the essential components of the system (Table-4). When CoA was omitted about 97% activity was lost while omission of ATP also reduced the activity considerably.

The presence of ATP, CoA and Mg\textsuperscript{++} dependent malate cleavage enzyme had been described by Tuboi and Kikuchi\textsuperscript{286} and was also reported by Cox and Zatman\textsuperscript{463} in methylotrophically grown bacteria. Malate lyase has also been studied in Rhodopseudomonas spheroides,\textsuperscript{286,464} Pseudomonas sp.\textsuperscript{465,466} and rabbit liver.\textsuperscript{467} This enzyme in R. spheroides is suggested to supply glyoxylate for glycine and consequently porphyrin synthesis.\textsuperscript{286} The pH optima of the mango enzyme was 7.2 (figure-4b).

The rise and fall in glyoxylate and citrate concentrations (figure-1 and Chapter-5) during maturation and ripening of mango fruit may indicate the changes in the levels of different enzymes metabolizing these acids. The enzymes metabolizing glyoxylate, glyoxylate reductase, glyoxylate dehydrogenase and l-alanine glyoxylate aminotransferase were studied throughout maturation and ripening stages of mango (figure-5a). The concentration of glyoxylate reductase was high in
the immature stage, rising to a considerable extent during maturation and finally decreasing during ripening. Glyoxylate dehydrogenase activity was found to be low in the early developmental stages, which was increasing during maturation and again decreasing during ripening. L-Alanine glyoxylate aminotransferase activity was also found to be increasing during maturation, showing a peak at mature stage and decreasing during ripening (figure-5a).

The concentration of isocitrate lyase and malate lyase on the other hand, were found to be increasing during maturation continuously up to the mature stage and then decreasing during ripening (figure-5b) becoming almost undetectable in the case of completely ripe mango. The concentrations of both these enzymes showed a peak at the mature harvest stage.

Malic enzyme and isocitrate dehydrogenase activities were remarkable in the early developing stages (figure-5c), but decreased during maturation and showed a continuous increase throughout ripening. However, malic enzyme activity showed a fall again during late ripening stages. The increase in malic enzyme is in accordance with the high amount of lipids$^{264}$ and NADPH$^{421}$ during ripening. The increase in isocitrate dehydrogenase
Fig. 5 The activities of enzymes during maturation and ripening of mango. (a) L-alanine glyoxylate aminotransferase, glyoxylate reductase and glyoxylate dehydrogenase, (b) Fumarate lyase and Isocitrate lyase and (c) Isocitrate dehydrogenase, malic enzyme and malic dehydrogenase. FEB to JUL indicates the immature stages while 1 to 6 indicates ripening stages.
and malic dehydrogenase activities throughout ripening, along with high oxidation of TCA cycle intermediates (Chapter-5) by mitochondria suggest high respiration and an active participation of Krebs cycle in these stages.

It is interesting to note that the levels of isocitrate lyase and 1-alanine glyoxylate aminotransferase are high between the immature to mature unripe stages, when the isocitrate dehydrogenase activity is considerably low. During the subsequent ripening stages, when isocitrate lyase and 1-alanine glyoxylate aminotransferase activities fall off, isocitrate dehydrogenase activity shoots up. These findings suggest that in the maturing stages the citrate in the tissues is directed towards the glyoxylate formation, which is then probably transaminated to form glycine and thus may help to increase amino acid pool for protein synthesis, whereas in the later stages of mango ripening, citrate is metabolized faster by isocitrate dehydrogenase leading to TCA cycle during ripening of mango fruit.

The ratio of the activities of isocitrate lyase to isocitrate dehydrogenase changed from 3.2 in the immature fruit to 15 in the unripe mature fruit and to 0.54 in the ripe mango fruit. The higher amount of glyoxylate (Table-1) and malate and the higher ratio
of the activities of isocitrate lyase to isocitrate dehydrogenase all add to the indication of the operation of the glyoxylate bypass in the developing and pre-climacteric mango fruit, possibly resulting in the conservation of carbon atom at these stages. For higher plants the operation of glyoxylate cycle could provide an explanation of the accumulation of di- and tri-carboxylic acids at the expense of acetyl units derived from carbohydrate or other food reserves.  

Similarly, the high concentration of malate lyase along with those of isocitrate lyase and l-alanine glyoxylate aminotransferase during the late developing stages suggest the liberation of glyoxylate for other synthetic processes. Increase in glycine concentration along with other amino acids during ripening of mango is in accordance with the high activity of amino transferase, converting glyoxylate to glycine during preclimacteric stages (figure-5). Malate lyase is known to liberate glyoxylate for glycine synthesis in methylotrophic bacteria.

In addition to this, the activity of glyoxylate reductase is remarkable in developing fruit, declining slowly during maturation and ripening. However, glyoxylate dehydrogenase shows a slow increase during ripening, reaching almost a peak on 2nd day and again decreasing
during ripening of mango, providing a good evidence for high oxidation of glyoxylate to oxalate in these stages of mango fruit.

At the mature unripe stage, the glyoxylate concentration increases in the cell, along with this, the activity of isocitrate lyase is highest. The steady increase in glyoxylate concentration throughout development of mango on the tree leading to its accumulation in the unripe mature stage may suggest its important physiological role in the fruit metabolism. Payes and Latties have suggested that the concentration of glyoxylate may regulate the direction of metabolic processes in the cell. Glyoxylic acid is also reported to inhibit many metabolic processes. It was shown to be an inhibitor of oxidative phosphorylation in respiration. It inhibited pyruvate decarboxylation and isocitrate lyase activity in *Brevibacterium flavum*, the Krebs cycle activity of pea mitochondria and oxidation of certain Krebs cycle acids by leaf mitochondria. Oxalomalate, produced by equimolar combination with oxaloacetate and glyoxylate inhibits isocitrate dehydrogenase (NADP) and aconitase hydratase in animal cells, the inhibition being of competitive type if the enzyme is not preincubated with the inhibitor.
Fig. 6 Line graph: Upper panel shows inhibition of dehydrogenase without (- - -) and with (---) glutamate (1.45mM).

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Oxalomalate also produces inhibition of a concerted type on isocitrate dehydrogenase from protozoa and other bacteria.

The effect of glyoxylate on isocitrate dehydrogenase activity of mango fruit was therefore studied. It was noticed that glyoxylate inhibits the isocitrate dehydrogenase activity (figure-6) non-competitively showing Ki value of 26.6 mM. The inhibition of isocitrate dehydrogenase by glyoxylate may be significant in mango at the mature unripe stage, since the glyoxylate concentration is higher in the unripe mature stage and the activity of isocitrate dehydrogenase is much lower. During ripening as the concentration of glyoxylate decreases, the activity of isocitrate dehydrogenase increases and thus there may be a shift from glyoxylate bypass to TCA cycle indicating fine regulation offered by glyoxylate during the unripe to ripe stages.