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GLYOXYLATE METABOLISM AND FATTY ACID OXIDATION IN MANGO FRUIT DURING DEVELOPMENT AND RIPENING

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Key Word Index—*Mangifera indica*; Anacardiaceae; mango; fruit ripening; fruit development; fatty acid oxidation; glyoxylate metabolism; acid metabolism; isocitrate dehydrogenase; isocitrate lyase; malate lyase; malic dehydrogenase; malic enzyme; glyoxylate reductase; glyoxylate dehydrogenase; alanine: glyoxylate aminotransferase; mitochondria.

Abstract—Throughout the development (maturation) of mango fruit the contents of citric and glyoxylic acids increased steadily. As the fruit matured the levels of isocitrate lyase, malate lyase and alanine: glyoxylate aminotransferase increased and reached maximum values prior to the time of harvesting. At and after harvest the levels of malate lyase and alanine: glyoxylate aminotransferase began to decrease but that of isocitrate lyase remained high until after the harvest when it decreased. The level of glyoxylate reductase was highest in the early developmental stage but declined as the fruit matured and ripened. As the fruit ripened, after harvest, the amounts of citric and glyoxylic acids decreased concomitant with a considerable increase in the levels of isocitrate dehydrogenase, malic dehydrogenase, malic enzyme and glyoxylate dehydrogenase.

Fatty acid oxidizing capacity of mitochondria isolated from immature (developing) and postclimacteric fruit pulps was much less than that observed with mitochondria from preclimacteric and climacteric fruit. Glyoxylate stimulated the oxidation of caprylic, lauric, myristic and palmitic acids and inhibited the activity of isocitrate dehydrogenase *in vitro*.

INTRODUCTION

The sudden upsurge in respiration in the climacteric fruits on ripening is well documented [1, 2]. Most of these fruits are particularly rich in organic acids in the preclimacteric stage. During ripening after harvest these acids are lost [2, 3] and this loss has been attributed to a concomitant increase in the enzymes metabolising them [2, 4]. However, the exact nature of accumulation and source(s) of organic acids in a developing fruit is not clear. Nitsch [5] suggested that acids could be translocated from leaves or roots to the fruit [3, 5]. However, evidence on the formation of acids from carbohydrates in apple fruit is available [6] and the presence of high citrate synthase levels in mature and immature (developing) mango fruit demonstrated [7]. Earlier we reported [7] changes in the mitochondrial enzymes in mango fruit during development and ripening. In these studies a marked difference was observed in the content of glyoxylic acid of the immature and mature fruit. In the present study we have measured changes that occur in the levels of enzymes leading to and away from glyoxylate during fruit development and maturation and suggest that such changes could be related to acid metabolism and the ability of mitochondria from fruits at these stages to oxidize different fatty acids.

RESULTS

Figure 1 shows the changes in the contents of citric and glyoxylic acids and increase in average weight of mango fruit, during different stages of fruit development. A trend of continuous increase in both these acids is

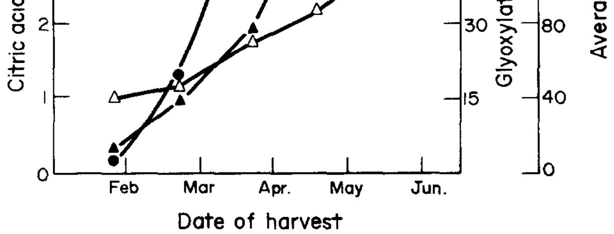

Fig. 1. Citric acid (△) and glyoxylic acid (▲) levels and average fr wt. (●) During maturation of mango fruit on the tree. (Testing was started from 28 Feb. 1974, *ca* 4 weeks after the fruit set, and continued up to 25 May 1974).

evident. These changes coincide with increases in fruit growth and development in terms of both fr wt and fruit diameters. Ripening of the fruit after harvest is

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accompanied by decreases in fr wt (not shown) and in the contents of citric acid [2] and glyoxylic acid. The concentration of glyoxylate decreased (per 100 g fresh pulp) from 107 mg at preclimacteric to 38 mg at climacteric and 19 mg at postclimacteric.

Figure 2 shows the changes in the levels (in terms of enzyme activity per mg protein) of enzymes leading to

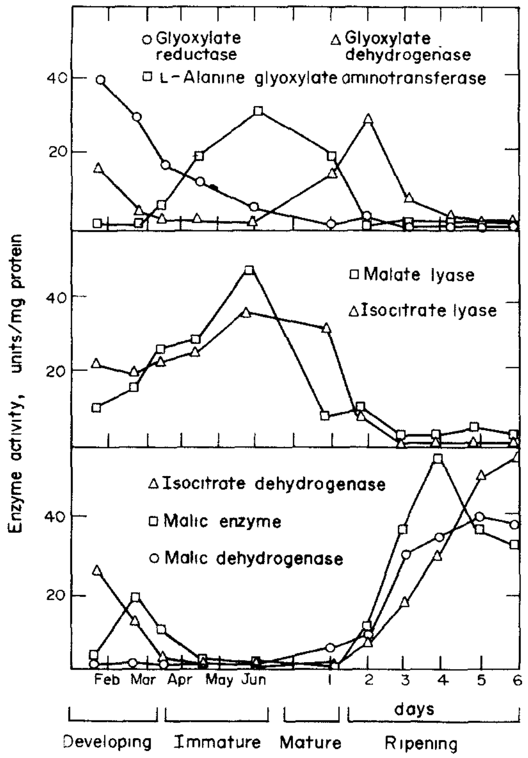

Fig. 2. Levels of some enzymes leading to and away from glyoxylate during development and ripening of mango fruit.

the formation of and the degradation of glyoxylate, during different stages of fruit development and postharvest ripening. The levels of isocitrate lyase, malate lyase and alanine : glyoxylate aminotransferase increase as the fruit develops and matures on the tree and maximum levels are found prior to the time at which the fruit is picked. Although the levels of malate lyase and alanine : glyoxylate aminotransferase decreased in the fruit at harvest that of isocitrate lyase remains high and steady until after the fruit is harvested. Glyoxylate reductase activity was detectable and high in the fruit early in the developmental stage but declined continuously as the fruit matured and ripened (Fig. 2). During the postharvest ripening of mango hardly any significant activity of these enzymes was noticeable.

On ripening the levels of enzymes leading away from glyoxylate, viz. isocitrate dehydrogenase, malic enzyme (malate dehydrogenase (decarboxylating (NADP)), malate dehydrogenase and glyoxylate dehydrogenase showed a considerable increase (Fig. 2). The level of glyoxylate dehydrogenase rises sharply in the unripe (mature) fruit and continues to rise till a few days after postharvest and then falls sharply. The rise at climacteric in the levels of isocitrate dehydrogenase and malate dehydrogenase coincides with the reported rise in mitochondrial activity at this stage [7, 8]. Although the levels of isocitrate dehydrogenase and malate dehydrogenase remain steady at the maximum levels reached at the climacteric that of malic enzyme decreases after reaching a maximum at the climacteric. It is noteworthy that malic enzyme showed an additional peak of activity early in the development of the fruit (Fig. 2).

Mitochondrial activity has been shown to increase considerably during maturation and ripening of mango fruit [7]. It is found that both saturated and unsaturated fatty acids of different chain lengths are oxidized by mitochondria isolated from immature unripe (mature, preclimacteric), climacteric and post-climacteric mango pulps, (data not given). However, the rates of oxidation are about two orders of magnitude lower than those observed with Krebs cycle intermediates [7, 8]. Fatty acid oxidizing capacity of immature and postclimacteric fruit mitochondria is much less than that observed with preclimacteric and climacteric fruit. Stearic and oleic acids are showing the largest increases in the rate of oxidation (13 and 6 fold respectively) during ripening. The postclimacteric fruit mitochondria showed considerably less ability to oxidize fatty acids compared to those from preclimacteric and climacteric fruits, however, mitochondria of these latter fruits have a very high capacity to oxidize Krebs cycle intermediates [7].

As matured (unripe) fruit contained a high concentration of glyoxylate and also had a higher capacity for oxidizing fatty acids, it was of interest to test the effect of glyoxylate on this. Glyoxylate by itself was not oxidized by the mitochondrial preparations used in this investigation. However, it stimulated appreciably the oxidation of caprylic, lauric, myristic and palmitic acids (Table 1) and exerted the most stimulating effect

Table 1. Effect of glyoxylate on fatty acid oxidation by mitochondria isolated from preclimacteric mango pulp

	μl O_2 consumed/hr/mg dry wt mitochondria			
Glyoxylate (nM)	Caprylic acid	Lauric acid	Myristic acid	Palmitic acid
0	0·25	0.42	0.44	0.39
0.66	0.25	0.44	0.49	0.40
3.33	0.35	0.50	0.53	0.45
6.66	0.43	0.52	0.57	0.49
33.3	0.47	0.53	0.58	0.50

*Each fatty acid concentration used was 33 μM.

on the oxidation of caprylic acid. The stimulatory effect of glyoxylate was concentration dependent (Table 1).

Glyoxylate (1.25 mM) was found also to inhibit isocitrate dehydrogenase by more than five-fold and this inhibition was of the competitive type.

DISCUSSION

The data presented above on the patterns of accumulation of glyoxylic and citric acids during development of mango fruit and the disappearance of these acids on ripening after harvest seem closely related to the patterns in the levels of enzymes involved in their metabolism. During development of the fruit a situation exists in which the enzymes leading to the formation of glyoxylate and citrate, viz. isocitrate lyase, malate lyase (Fig. 2) and citrate synthase [7] increase. However, on ripening after harvest the dramatic decline in glyoxylic and citric acids and of malate [9] appears to coincide with the observed increases in the activities of enzymes leading to the catabolism of these acids (Fig. 2) and in the mitochondrial respiration at these stages [7]. The changes observed in the level of alanine: glyoxylate aminotransferase suggest that some of the glyoxylate formed may in turn be converted to amino acids. A marked qualitative and quantitative change in various amino acids at the preclimacteric stage and on ripening has been observed in mango fruit [2]. These observations lead us to believe that fruit cells have the potential to bring about changes in the acid metabolism and to synthesise organic acids, although the possibility of some amount of the acids being translocated from other parts of the parent plant into the fruit still exists. Furthermore, glycollate, one of the products of photosynthesis, could arise in the early developmental phase and be transported into the fruit. As the level of glyoxylate reductase during this stage is high (Fig. 2) glycollate could be converted to glyoxylate.

The different rate at which the mitochondria oxidized fatty acids at different stages of maturity and ripening may affect the quantitative and qualitative changes in fatty acids, the formation of glyoxylate during maturation and development of the aroma of the fruit, since it has been known for some time that different aroma compounds are formed as a result of lipid degradation [10].

Stimulation of mitochondrial fatty acid oxidations by glyoxylate *in vitro* is an interesting observation. Although more than 20% stimulation in the oxidation of lauric, myristic and palmitic acids was observed at a concentration of glyoxylate which is close to its intracellular concentration in the mature fruit, the concentration of the effector required to produce one-half of the maximum rate may extrapolate to non-physiological range. However, a stimulatory effect of glyoxylate on caprylate oxidation appears to take place within the physiological concentration of glyoxylate for at 6.66 mM glyoxylate a 75% stimulation in oxidation was observed (Table 1). However, the physiological importance of this effect is difficult to assess at present.

Glyoxylate in combination with oxaloacetate is known to inhibit isocitrate dehydrogenase from animal tissues [11] and microorganisms [12–15]. The inhibition of mango isocitrate dehydrogenase by glyoxylate is of significance in this regard. A strong inhibition of the mango enzyme at concentrations much below the physiological level of the mature fruit seems to indicate that at the mature stage of the fruit, glyoxylate may regulate the activity of this enzyme. However, the possible regulation of the first isocitrate dehydrogenase *in vivo* will depend on the concentration of glyoxylate at the site of enzyme activity.

In some plant tissues specialized organelles such as microbodies (glyoxysomes, peroxisomes) are the sites of fatty acid oxidation and glyoxylate metabolism [16, 17]; it is possible that such a situation may exist in mango fruit for the mitochondrial preparations used in the present study were obtained under conditions at which microbodies might sediment.

EXPERIMENTAL

Mangoes (*Mangifera indica* L.var.Dadomia) were obtained from a local orchard. Differentiation of various stages during development and ripening, methods for cell free-extract preparations, protein assay, isolation of mitochondria, determination of enzymic activities of malic enzyme (EC 1.1.1.40 L-malate: NADP oxidoreductase), malic dehydrogenase (EC 1.1.1.37-L-malate: NAD oxidoreductase), isocitrate dehydrogenase (EC 1.1.1.42-threo-Ds-isocitrate: NADP oxidoreductase (decarboxylating)) and isocitrate lyase (EC 4.1.3.1. threo-Ds-isocitrate glyoxylate lyase), and estimation of glyoxylate have been described earlier [7]. Citric acid was determined by the method as described in ref. [18]. L-Alanine glyoxylate aminotransferase (EC 2.6.1.12. L-alanine-keto acid aminotransferase) was assayed by the method of ref. [19]. One unit of the enzyme activity is that amount of the enzyme which forms 1 μ mol of pyruvate per min. Glyoxylate reductase (EC 1.1.1.26. Glycollate: NAD oxidoreductase) was assayed by the method of ref [20]. One unit of the enzyme activity is taken as the amount of enzyme which causes the change in A of 0.01/min at 340 nm. Glyoxylate dehydrogenase (EC 1.2.1.17) Glyoxylate: NSDP oxidoreductase (acylating CoA)) was assayed by the method of ref. [21]. One unit of enzyme activity is that amount which catalyses the reduction of 1 μmol of NADP/min at 30°. Malate lyase (EC 4.1.3.2. Malate synthase) was assayed by the method of ref. [22]. One unit of the enzyme activity is the amount of enzyme catalysing an increase of 0.01 A/min at 324 nm. Fatty acid oxidation was determined by the Warburg manometer. The reaction mixture contained NAD(100 μg), fatty acid (50 m μmol) Tris–HCl buffer (100 μmol, pH 7.2) and mitochondrial preparation (0.50–0.10 mg dry wt) in a final vol of 1.5 ml. The rates were determined up to 60 min and the values extrapolated to zero time.

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