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MALIC ENZYME ACTIVITY AND RELATED BIOCHEMICAL ASPECTS DURING RIPENING OF γ-IRRADIATED MANGO FRUIT

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Abstract—The increase in malic enzyme (L-malate $NADP^+$ oxidoreductase (oxalacetate-decarboxylating) EC 1 1 1 40) activity, usually observed during the ripening process of mango fruit, was significantly diminished, but not delayed by γ-ray irradiation with 0 75 kGy Irradiated fruit showed higher total titratable acidity values, lower pH-values and had a smaller % physiological mass loss per day than control fruit These effects became more pronounced as ripening progressed The respiratory pattern of irradiated fruit seems to result from an initial stress response and a reduced, but not delayed, climacteric It seems that irradiation does not cause a true delay in the onset of ripening of fully mature mango fruit, but rather distorts certain biochemical processes to result in a delayed senescence

INTRODUCTION

The γ-radiation-induced delay in ripening of fruits and the accompanying advantages, such as shelf-life extension and disease control, have been reported by several authors for several different types of fruit [1] The literature on the subject was frequently contradictory with regard to the optimum irradiation doses and their resulting effects This can probably be attributed to differences in the time interval between harvesting and irradiation, and maturity of the fruit at harvest, post-irradiation storage conditions and different fruit varieties [1] The most important aspect, however, appeared to be the physiological status of the fruit at the time of irradiation [2]

The processes of growth and maturation are separated by the respiration climacteric from the onset of the essentially irreversible changes of ripening and senescence [3] With fruit belonging to the climacteric class, the position of the fruit in the climacteric sequence plays a pivotal role in the response thereof to irradiation [1, 2] Ripening is the final phase in the development of the fruit and appears to be a coordinated process of biochemical differentiation [4] It is a period of metabolic reorganization accompanied by enhanced ethylene, RNA and protein synthesis and of increased respiratory activity New enzymes are synthesized to catalyse the ripening process [4]

There are many uncertainties concerning the mechanism by which ripening is initiated [3] Malic enzyme is synthesized *de novo* during the climacteric phase of ripening [5], and it offers to participate in the ripening process of some climacteric fruits [5–7]

γ-Radiation alters the biochemical balance and leads to a delay in the onset of ripening or senescence in fruit tissues [1, 2] Unfortunately no data exist on these biochemical changes

RESULTS AND DISCUSSION

Malic enzyme activity

An increase in the respiratory quotient during the early stages of ripening, the development of the capacity to decarboxylate malate (the malate effect), as well as the development of the respiratory climacteric in the whole fruit, has been correlated with the increased malic enzyme levels during ripening of apple fruit [6–8] The role of the enzyme in the physiology of the fruit is only partially understood [8] It appears to be involved in the increasing mobilization of malic acid from the vacuole which contributes to the respiration of the fruit This mobilization of malate may then contribute to the supply of reduced NADP for synthetic purposes and the supply of respirable substrate for enhanced mitochondrial activity during the climacteric rise The characterization of malic enzyme from mango fruit has been reported elsewhere [9, 10]

Changes in the extractable levels of NADP-malic enzyme in the control group of mango fruit can be seen in Fig 1 The enzyme activity developed gradually during the climacteric rise, reached a maximum slightly ahead of the peak in respiration (Fig 5) and then diminished again The irradiated fruit showed only a slight increase in activity without a definite peak being reached It indicates that irradiation did not cause a 'delay' in the onset of the development of the malate decarboxylating system in fully mature fruit, but rather diminished such a development This result seems to be very important in interpreting the effect γ-radiation has on the ripening process

The diminished levels of malic enzyme in irradiated fruit could be the result of a general reduced capacity for protein synthesis [11] A population of damaged, modified and unaltered enzymes may result from radiation

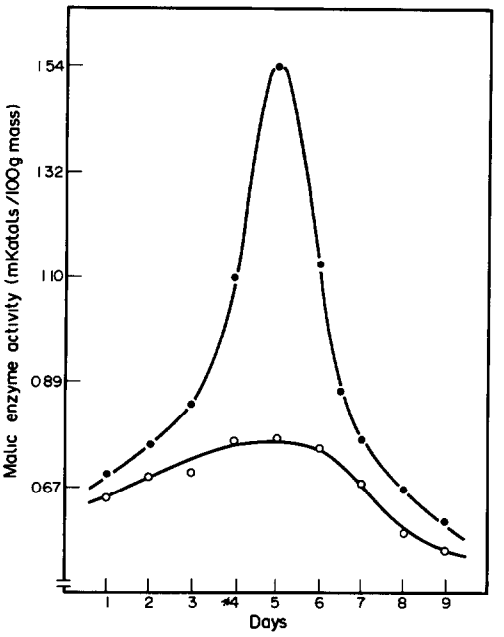

Fig 1 Changes in malic enzyme activity during ripening in γ-irradiated (O——O) and control (●——●) Haden mangoes at 25°

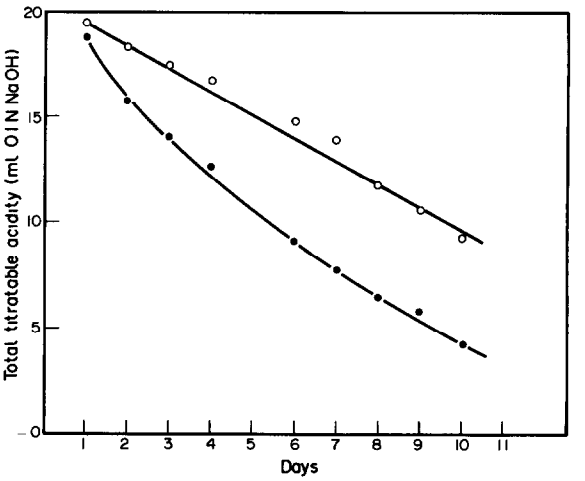

Fig 2 Decrease in total titratable acidity during ripening of γ-irradiated (O——O) and control (●——●) Haden mangoes at 25°

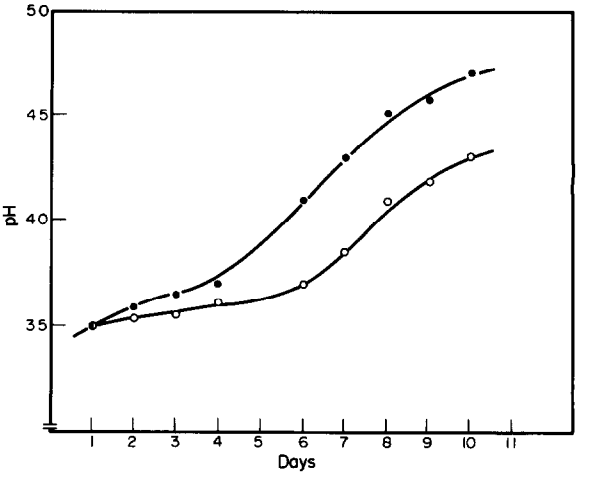

Fig 3 Changes in fruit pH during ripening of γ-irradiated (O——O) and control (●——●) Haden mangoes at 25°

treatment [12] Loss of allosteric control mechanisms, normally more sensitive to destruction by irradiation [13] may be of even more importance

Total titratable acidity (TTA) and pH

In general fruits show a decrease in acidity and an increase in sweetness during ripening [3] Malic acid and citric acid are predominant in mango fruits and the total acidity expressed as malic acid can vary from 4 to 5% in green fruit, to 3% at the time of harvest and to 0 5–0 1% in the ripe fruit [14]

As the climacteric progressed, an exponential decrease in the total titratable acidity of the control group of fruits was observed (Fig 2) The significance of the higher TTA of the irradiated group became more pronounced as the investigation period extended due to the slower decrease (linear) in TTA of the irradiated fruits Similar results were obtained for Zill-mangoes with the TTA-levels of irradiated and control fruits converging at the end of the ripening period [15]

A change in pH from 2 4 to 4 0 during ripening was reported for 'Pairi' mango fruit [16] We observed an increase in the pH values from 3 5 to 5 0 during the experimental period The variation in pH-values is shown in Fig 3 Both groups of fruit showed sigmoidal increases in pH-values as the fruits ripened, but compared to the control the increase in the pH-values of the irradiated fruits were somewhat delayed and not so pronounced

The observed higher total titratable acidity and lower pH-values of the irradiated fruit could be the result of the diminished malate decarboxylating activity which has been reported to play a role in the decrease in acidity during ripening [3] as already discussed

Physiological mass losses

Physiological losses in mass are an indication of the total moisture loss during ripening which results in desiccation and a shrivelled appearance of the fruit This phenomenon is primarily due to respiration and transpiration processes [14] The variation in physiological mass losses during the ripening process are presented as a % of the values at the start of the experiment (Fig 4) The mass of the control group diminished to an greater extent compared to the irradiated fruit The differences between the daily losses increased as ripening proceeded, which amplified the eventual result The slopes of the lines depicting the mass losses that occurred changes around day four for both the irradiated and control groups This might be due to the deceleration in the rate of respiration which occurs between the climacteric rise and the climacteric peak

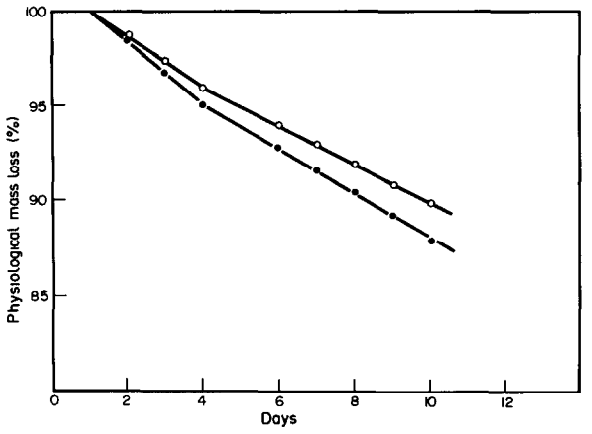

Fig 4 Physiological mass losses during ripening of γ-irradiated (O——O) and control (●——●) Haden mangoes at 25° Wt expressed as % of original wt

CO_2-*production and liberation*

The respiratory patterns of mango fruit have been classified into four distinct phases based on the note of CO_2-release The preclimacteric phase, the climacteric rise, climacteric peak and post-climacteric have been correlated with observable changes such as colour, texture, taste and odour [14] The graph of CO_2 release of the control group is representative of a typical respiratory pattern although the intensity and form of the peak may be influenced to a great extent by the stage of maturity at harvest [14]

The peak in malic enzyme activity precedes that of the respiratory climacteric by a few hr (compare Fig 1 and Fig 5) This confirms the results obtained with other fruits

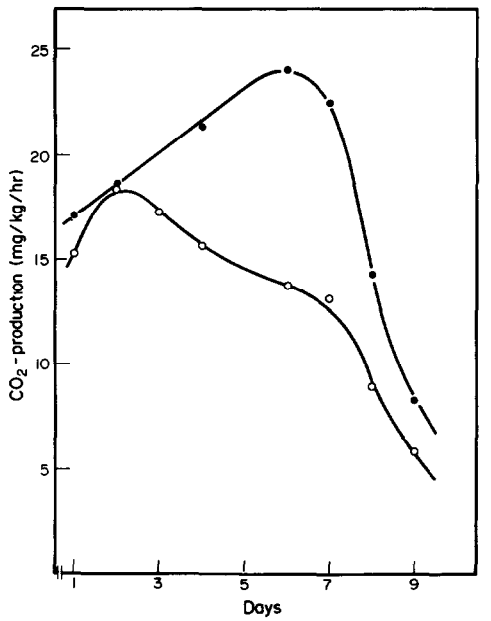

Fig 5 CO_2-respiratory patterns during the climacteric phase of ripening of control (●——●) and γ-irradiated (O——O) Haden mangoes at 25°

[7, 17] and has been used to explain the connection between the development of a malate decarboxylating system, the malate effect and the increase in CO_2 liberation during the climacteric as discussed before However, the view has been expressed that the respiration climacteric may be neither dependent upon, nor integrated with other aspects of ripening [18], and the results should be interpreted with care

The respiratory pattern of the irradiated fruits (Fig 5) seem to be a composite of two separate events, an initial rise in respiratory activity, possibly as a stress response [2] to the radiation treatment, and a normal but reduced respiratory pattern Increases in respiration and ethylene production immediately following irradiation, as well as a delay in the onset of the respiratory climacteric, has been reported for a number of fruits [1, 2] The latter seems to result from irradiation of partially mature fruit The irradiation of fully mature fruit—as was used in this experiment—does not lead to such a delay [1] and can even cause a reduced respiratory climacteric as was found in this study Thus the respiratory rate of the whole fruit serves as a measure of the metabolic response induced by irradiation

A similar set of experiments were performed on the Kent variety of mango fruit The results obtained were essentially the same, but by conducting the experiments at 18–20°, the investigation period could be extended from 11 days to 17 days Normal ripening appears to occur only in a relatively narrow range of temperatures, e g at > 25° the extent of the respiration climacteric decreases [3]

EXPERIMENTAL

Source of fruits Mature, fully developed mango fruits (cv Haden) were obtained from orchards in the Tsaneen area Fruit were selected for the experiments according to similarity in size, mass and ripening phase (pre-climacteric) and absence of disease

Radiation treatment The fruits were treated with a dose of 0 75 kGy—used for shelf life extension of South African fibre-less cultivars—in a gamma beam 650 (AECL) irradiator equipped with a ^{60}Co-source at a dose rate of *ca* 3 kGy/hr Dosimetry were performed using the Fricke dosimeter [19] Irradiation was performed in air and at room temp

Storage and sampling techniques The same set of experiments were conducted on mango fruit over a period of three seasons The control and irradiated groups contained 40 fruits each and were kept in an air-conditioned room at 25° The experiments had to be terminated after the 12th day because the control group of fruits started to collapse At that state the irradiated fruit were still reasonably firm The sampling technique as described in ref [15] was used throughout the investigation periods

The skin of the fruit in the sampling area was swabbed with 70% aq EtOH Segments in the form of pyramids were cut from the fruit with sterile knives and the hole sealed with parafilm, previously dipped in 70% aq EtOH Physical damage due to the sampling was minimal as the sample mass was small in comparison to the mass of the whole fruit It appears that ripe or senescent tissues, having achieved a state of physiological quiescence, are much less affected by most stimuli [1] and the fruit had a shelf-life equal to that of uninjured fruit stored under the same conditions

To assure representative sampling, 12 segments of ± 7 g each were cut from different fruits each day during the investigation period and used for the analyses After removal of the peel of the

fruit segments were homogenized and used for the different experimental analyses as described below The obtained values thus represent an average of 12 different fruits on a daily basis All homogenization and centrifugation steps were performed in the cold at 0–5°

Extraction and assay of malic enzyme Mango tissue (30 g) was homogenized with 60 ml of a 100 mM Tris–HCl buffer, pH 8, for 1 min at 2° in the presence of 0 3 g Polyclar AT The homogenate was adjusted to pH 7 1 and then centrifuged at 15000 *g* for 10 min at 2° The supernatant was used for determining the enzyme activity which was done by measuring the reduction of NADP at *A* 340 nm The reaction mixture contained 100 mM Tris–HCl buffer at the optimum pH of 7 1, 1 mM $MnSO_4$, 5 mM L-(−)-malate, 0 5 mM $NADP^+$ and enzyme in a total vol of 3 ml [9] The reaction was initiated by the addition of enzyme and under these conditions the relation between reaction rate and enzyme concn was linear The temp of the cell compartment was thermostatically controlled at 25° One unit of enzyme activity (1 Katal) was defined as the amount of enzyme that catalyses the conversion of 1 mol of L-(−)-malate to pyruvate and CO_2 per sec

Total titratable acidity and pH-measurements The combined mango homogenate (30 g) was again homogenized with 80 ml freshly dist H_2O, quantitatively transferred to a 200 ml volumetric flask, diluted to vol and centrifuged at 10000 *g* for 10 min at 2° The supernatant (100 ml) was transferred to a 100 ml volumetric flask The pH of the sample was measured and then titrated with 0 1 M NaOH to a final pH of 8 1 [20]

CO_2-*respiration measurements* Four mango fruits of similar size and mass were selected and used for CO_2-determinations throughout the ripening period The fruit were placed in respiration jars [21] and compressed air was continuously passed through the sealed jars The CO_2 in the air stream was removed by passing the air through 0 1 M NaOH which also served to humidify the air prior to entering the jars The effluent of the respiration jars was bubbled into a test tube containing 30 ml of 0 1 M NaOH The absorption period was 1 hr The 30 ml NaOH solns were transferred to 50 ml volumetric flasks and diluted to vol in 0 1 M NaOH The soln was then titrated with 0.1 M HCl to a pH of 8 1 and the results expressed in terms of mg CO_2/kg/hr The daily mass determinations of the above mentioned fruits were also used to measure the physiological mass losses during the investigation period

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