THE EFFECT OF GAMMA RADIATION (^{60}Co) ON THE POSTHARVEST PHYSIOLOGY OF *Musa acuminata* COLLAR cv. DWARF CAVENDISH

by

GERHARDUS JOHANNES STRYDOM

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A voice cries out, "Proclaim a message!"
"What message shall I proclaim?" I ask.
"Proclaim that all mankind are like
grass;
they last no longer than wild flowers.
Grass withers and flowers fade
when the Lord sends the wind
blowing over them".

Isaiah 40:6–7

To

Sonja,

Gezila,

Matthys,

Jano,

parents

and parents in law

PREFACE

The work described in this thesis was carried out in the Department of Botany, University of Natal, Pietermaritzburg, under the supervision of Professor J van Staden and Dr M T Smith.

The study represent original work by the author and has not been submitted in any form to another university.

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Listen to what is wise and try to understand it. Yes, beg for knowledge; plead for insight. Look for it as hard as you would for silver or some hidden treasure. If you do, you will know what it means to fear the LORD and you will succeed in learning about God.

Proverbs 2:2 to 5

ABSTRACT

Bananas are climacteric fruits and show climacteric patterns with respect to both their ethylene production and respiration rates during the ripening process. Treatment with ethylene accelerated the ripening process, evidenced by colour changes. Chlorophyllase apparently synthesised chlorophyll in the 0.2 kGy irradiated fruit during the post–climacteric period. Gamma irradiation of green bananas with doses of 0.4 kGy or greater resulted in extensive tissue damage, undesirable colour development, and drastic changes in respiration and ethylene production. Irradiation at 0.2 kGy, did however, effectively delay ripening (without causing any phytotoxicity) of the fruit apparently by decreasing its sensitivity to its own endogenous ethylene for a period up to 28 days when kept at room temperature, provided that the relative humdity was maintained at 75 % to prevent desiccation. Irradiation at this dose did not prevent the onset of ripening when subjected to high concentrations of exogenous ethylene.

Ultrastructurally total cell collapse due to cell wall, middle lamella and membrane damage was evident at doses of 0.4, 0.6 and 1.0 kGy. Irradiation with a dose of 0.2 kGy did not cause cell wall softening and membrane breakdown and thus provided a banana fruit of excellent postharvest quality with respect to ultrastructural features.

Glucose and fructose occurred probably directly from sucrose that may not accumulate because it is transient after the banana fruit is harvested. The accumulation of sucrose

glucose and fructose was not stimulated by ethylene in non–irradiated and irradiated fruit. These results indicate a clear temporal separation between those biochemical processes that appear to be stimulated by ethylene (respiration, fruit softening and colour change) from those that are not (sugars). Pectin methyl esterase resulted in the demethylation of pectin–like material in the cell walls of ripening bananas.

CONTENTS

CHAPTER 1

INTRODUCTION

The first reference to bananas was made in Indian writings in about 500 B.C. Geographic, climatic and genetic studies indicate that bananas have been used as food since man's arrival in South–east Asia. Linnaeus, a famous Swedish botanist of the 18th century, named the banana *Musa sapientum* L. "fruit of the wise men" because certain sages of India chatted beneath the shade of the banana plant, while they feasted on the food. Bananas were planted in Africa in about 500 A.D., in the year 1000 in Polynesia and in the 15th and 16th centuries in the Americas. Significant banana exports were recorded worldwide late in the 19th century (PALMER, 1971).

Bananas belong to the family Musaceae in the order Gingiberales. The Musaceae consists of two genera, i.e. *Musa L.* and *Enseta* Horan (PALMER, 1971). Most of the edible parthenocarpic bananas are derived from *Musa acuminata L.* (2n = 22, A genome), or the hybrids of the above and *Musa balbisiana L.* (2n =22, B genome), both of these in the section Eumusa (SIMMONDS, 1966). The economically most important cultivars belong to the triploid group (3n = 33) of *Musa acuminata L.* (SIMMONDS, 1966). In this group the cultivars Cavendish and Gros Michel are by far predominant in world commerce. The Cavendish varieties have replaced Gros Michel as the principal export banana, largely because Cavendish cultivars (AAA Group, Cavendish subgroup) are resistant to *Fusarium* wilt disease (PALMER, 1971).

Bananas of the Dwarf Cavendish and Williams cultivars are the most important types cultivated in South Africa. The total area under bananas increased from 9 626 to 13 057 ha from 1982 to 1989, with a tonnage of 168 424 for the 1988–1989 harvest (MARITZ – Banana Board, 1990).

During the summer months and especially during December and January there is an oversupply of bananas to the markets, resulting in cost fluctuations. Efforts to solve the problem have been made by storing consignments at 13 °C immediately after despatch, preferably 13 to 14 days prior to ethylene treatment. However, under these storage conditions the bananas change colour and soften within 10 days. This is due to ethylene ingress from adjacent ripening chambers which results in stimulation and acceleration of ripening (MARITZ – Banana Board, 1990).

The problem can be overcome by treating the bananas with gamma rays at an exposure of 0.6 kGy and then ripening them subsequently by applying 1 000 $\mu\ell$ ethylene gas ℓ^{-1} air at 17 °C and at a relative humidity of 75 % for 24 hours. This prolongs senescence and suppresses handling damage (BRODRICK and STRYDOM, 1984; BRODRICK, THORD–GRAY and STRYDOM, 1984). However, it seems that the physiology of irradiated bananas differs from that of non–irradiated fruit under normal transport and storage conditions, as:

(i) Accelerated browning occurs in the form of localized spots over the entire surface of the peel of the irradiated banana, when stored or transported at temperatures lower than the prescribed 13 °C (Figure 1).

(ii) Localized and accelerated spot browning as well as softening over the entire surface of the peel of irradiated bananas occurred when stored or

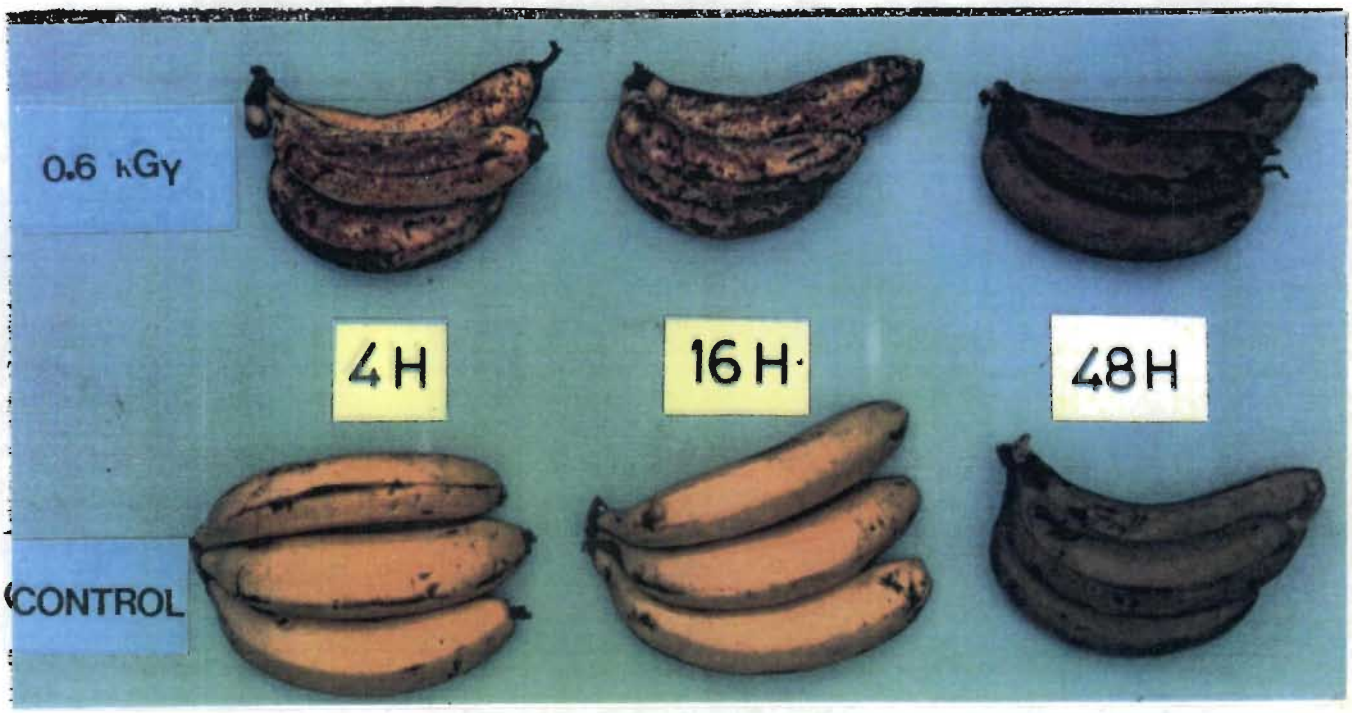


Figure 1: Browning of the entire surface of the peel of 0.6 kGy irradiated bananas, stored or transported at temperatures lower than 13 °C. The non–irradiated fruit and 0.6 kGy irradiated fruit were stored for 4, 16 and 48 hours respectively at 0 °C.

Figure 2: Aggravated spot browning and softening of the peel of 0.6 kGy irradiated bananas when stored or transported at temperatures above 25 °C.

transported at temperatures above 25 °C (Figure 2). Non–irradiated fruits aged normally but very rapidly under the same conditions. Associated with this was browning of the banana peel, and therefore this treatment could not serve as an adequate control for the irradiated fruit.

The browning, blackening and softening of the 0.6 kGy irradiated fruits are indicative of tissue damage.

The ripening of green bananas by treatment with a 1 000 $\mu\ell$ ethylene gas ℓ^{-1} air for 24 hours at 17 °C and a relative humidity of 75 %, or with ethylene releasing agents, is common practice in the industry. By using this technique, bananas can be ripened to a specific colour stage and ripe bananas can be provided according to a predetermined schedule. This study was undertaken to investigate the effect of gamma irradiation on certain aspects of the postharvest physiology, ultrastructural changes of the peel, and changes in sugar and specific enzymes of Dwarf Cavendish bananas in an attempt to determine the maximum tolerable irradiation dose that will effectively delay ripening at room temperature without causing phytotoxicity but still allow ripening by means of treatment with exogenous ethylene at a later stage.

CHAPTER 2

LITERATURE SURVEY

Fruit ripening

Fruit ripening is associated with a wide spectrum of biochemical and physiological changes that are either degradative or synthetic in nature (RHODES, 1980; BIALE and YOUNG, 1981). These metabolic processes listed in Table 1 are closely linked to the transformation of the mature, unripe fruit into an over–ripe and senescing fruit. Fruits are divided into two categories, climacteric and non–climacteric. Climacteric fruits are characterized by a surge in respiration and ethylene evolution during ripening whereas non–climacteric fruits ripen without a concomitant increase in both respiration and ethylene production (LESHEM, HALEVY and FRENKEL, 1986). Fruits displaying a climacteric include the banana, apples, avocado and tomato. All show a climacteric rise in respiration and an increase in ethylene production associated with comparatively rapid ripening. Non–climacteric fruits show a more gradual progression from growth to 'ripening' without sharp changes in respiration and their ethylene production remains low (BIALE and YOUNG, 1981).

The precise mechanisms by which the processes associated with fruit ripening are co–ordinated and controlled remain unknown. Two hypotheses have been proposed to explain the ripening processes of climacteric fruit (BLACKMAN and

Table I: Metabolic Changes Taking Place during Ripening of Climacteric Fruits

1 DNA and RNA SYNTHESIS

2 COLOUR

Destruction of chlorophyll
Revelation of carotenoids (orange and yellow colours)
Synthesis of carotenoids (red colour of tomato)
Synthesis of anthocyanins (red and blue colours)

3 RESPIRATION RATE

To satisfy the energy requirements of other changes
To reflect deterioration of rate controls
In response to excess ethylene
In relation to new enzyme activities

4 RATE OF ETHYLENE PRODUCTION

To initiate ripening
Excess ethylene production as a phenomenon of senescence.

5 CELL MEMBRANE PERMEABILITY

6 SOFTENING–CHANGE IN PECTIC COMPOSITION

7 CARBOHYDRATE COMPOSITION:

Starch converted to sugar
Interconversion of sugars

8 PROTEIN

Quantitative and Qualitative enzymes syntheses

9 PRODUCTION OF FLAVOUR VOLATILES

10 DEVELOPMENT OF WAX ON SKIN

11 ORGANIC ACID

Absolute changes in amounts present
Relative–change in flavour relative to sweetness

PARIJA, 1928; SACHER, 1973; RHODES, 1980). One hypothesis postulates that the organizational resistance of the cell changes during ripening (BLACKMAN and PARIJA, 1928). This would lead to changes in the permeability of cell membranes, which permits access of ions, substrates or co–factors to the pre–existing constituent enzymes and consequently to their activation (BLACKMAN and PARIJA, 1928; SACHER, 1973). Experimental evidence cited in support of this postulate includes the observed increases in volume of the apparent free space (BAUR and WORKMAN, 1964; SACHER, 1973) and membrane permeability (BAUR and WORKMAN, 1964; LEWIS and MARTIN, 1969; BRADY, O'CONNELL, SMYDZUK and WADE, 1970) during ripening. Although the permeability of cellular membranes is known to change during senescence, it has not been ascertained whether this is a result rather than a cause of senescence.

BRADY, O'CONNELL, SMYDZUK and WADE (1970) demonstrated that banana slices treated with ethylene, had an increased rate of respiration whereas membrane permeability was not altered until ripening was at an advanced stage. BURG (1968) and VICKERY and BRUINSMA (1973) pointed out that changes in cell leakage often reflect changes in total concentration of the solute available for leakage rather than changes in membrane permeability. Banana cells retain their normal osmotic properties throughout ripening. An alternative hypothesis concerning ripening, initially proposed by KIDD and WEST (1930), attributes the onset of ripening to an increase in enzymes synthesized **de novo** early in the climacteric rise, which in turn catalyse key changes associated with ripening (FRENKEL, KLEIN and DILLEY, 1968). The synthesis of new protein was

suggested by an earlier experiment showing that ripening is accompanied by an increase in enzymes in some fruits (HULME, 1948; LI and HANSON, 1964; WADE, O'CONNELL and BRADY, 1972) while not in others (WADE O'CONNELL and BRADY, 1972), and by the specific increase in the synthesis of alcohol dehydrogenase in banana and new isozymes in pear (FRENKEL, KLEIN and DILLEY, 1968). The increase in amino acid incorporation into proteins at the onset of ripening (HULME, RHODES, GALLIARD and WOOLTORTON, 1968), the incorporation of RNA (ribonucleic acid) precursors into RNA (RICHMOND and BIALE, 1967; KU and ROMANI, 1970) including ribosomal RNA (ROMANI , 1975) as well as the production of new messenger–RNA species in ripening tomatoes (RATTANAPANONE, SPEIRS and GRIERSON, 1978), suggest that transcriptional and translational processes are associated with the onset of ripening. McGLASSON, PALMER, VENDRELL and BRADY (1971) and FRENKEL, KLEIN and DILLEY (1968) showed that the inhibition of these processes prevented many of the ripening processes in pear and banana. Collectively the above data suggest that fruit ripening is associated with, and dependent on, enhanced transcriptional and translational activity. Therefore the production of new protein species represents the effective enzymes envisaged by KIDD and WEST (1930).

Ethylene and senescence

Unripe banana fruits show a constant, but low level of ethylene production until the day before the onset of ripening (BURG and BURG, 1962; 1965a). The respiration rate of unripe banana fruit is also low (MAPSON and ROBINSON,

1966). At the onset of ripening ethylene production increases and this is followed by a rise in the rate of respiration (BURG and BURG, 1962, 1965b). During ripening at 20 °C peak ethylene production is reached while the rate of respiration continues to increase (McMURCHIE, McGLASSON and EAKS, 1972; BIALE AND YOUNG, 1981). As the rate of ethylene production in the banana fruits begins to decline, the rate of respiration reaches its maximum, and then decreases slightly, but remains at a relatively high level (PALMER, 1971). In climacteric fruit increasing concentrations of applied ethylene progressively accelerate the onset of the respiratory climacteric but do not significantly alter the magnitude of the respiratory upsurge. However, in non–climacteric fruit comparable ethylene applications elicit an increasingly larger respiratory response (BIALE and YOUNG, 1981). The biosynthesis of ethylene during banana fruit ripening follows a sequence of reactions from methionine (MET), to S–adenosyl methionine (SAM) and 1–Aminocyclopropane–1–carboxylic acid (ACC) as shown in Figure 3 (YANG and HOFFMAN, 1984). The increase in respiration involves glycolysis, the pentose phosphate pathway, the Krebs cycle, the cytochrome–mediated terminal electron transport system (MARRIOTT, 1980) and the emergence of cyanide insensitive respiration (LANCE, 1981).

Ripening would seem to be a genetically programmed phase of development in the life history of fleshy fruits (COOMBE, 1976; HOBSON, 1979) involving a co–ordinated set of metabolic changes, which for the consumer, may lead to the conversion of the fruit from an inedible to an edible state (Figure 4). The interrelationships between the changes which occur during ripening in bananas and other climacteric fruits, are not well understood. Available evidence indicates that many of the changes in metabolism are brought about through the

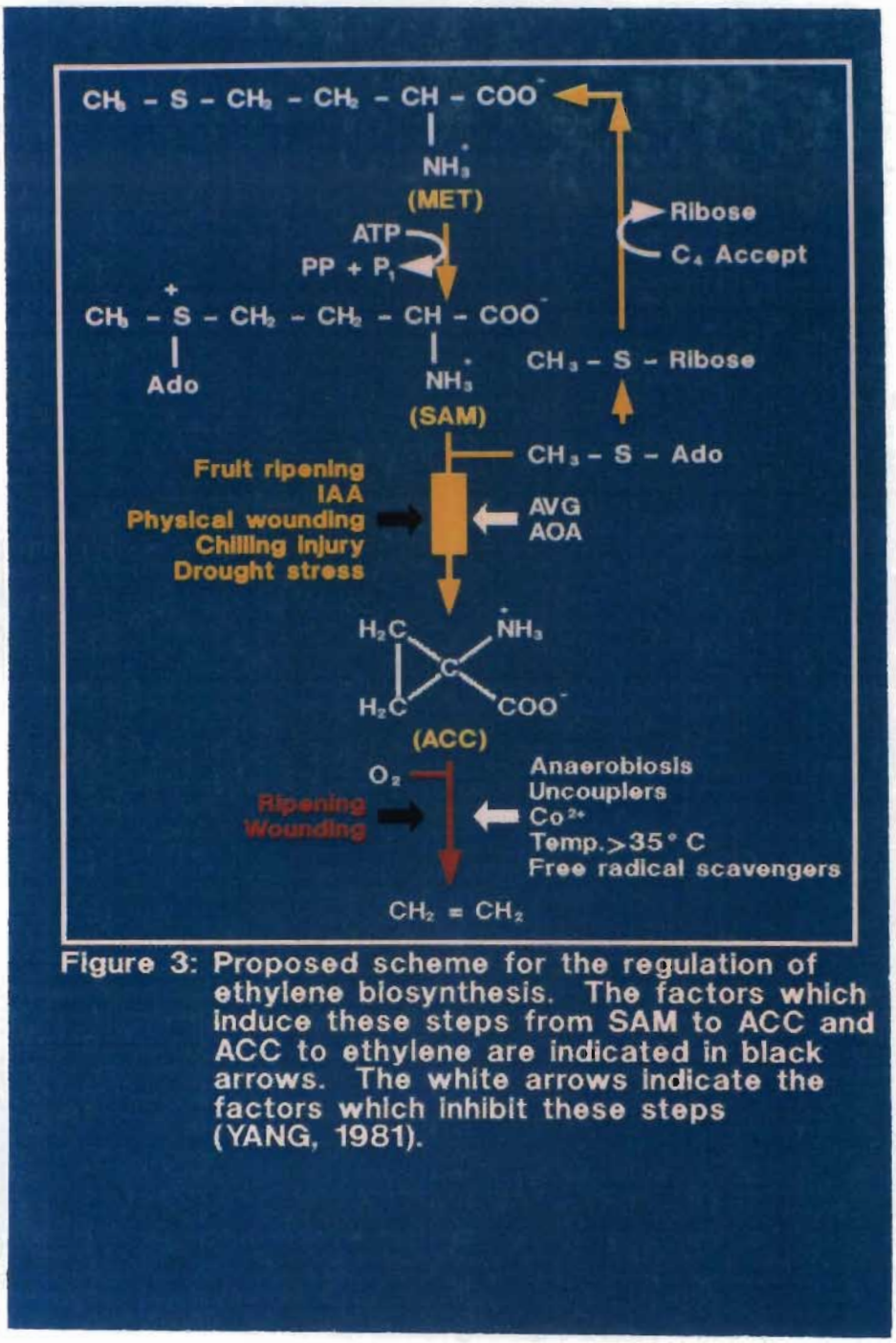


Figure 3: Proposed scheme for the regulation of ethylene biosynthesis. The factors which induce these steps from SAM to ACC and ACC to ethylene are indicated in black arrows. The white arrows indicate the factors which inhibit these steps (YANG, 1981).

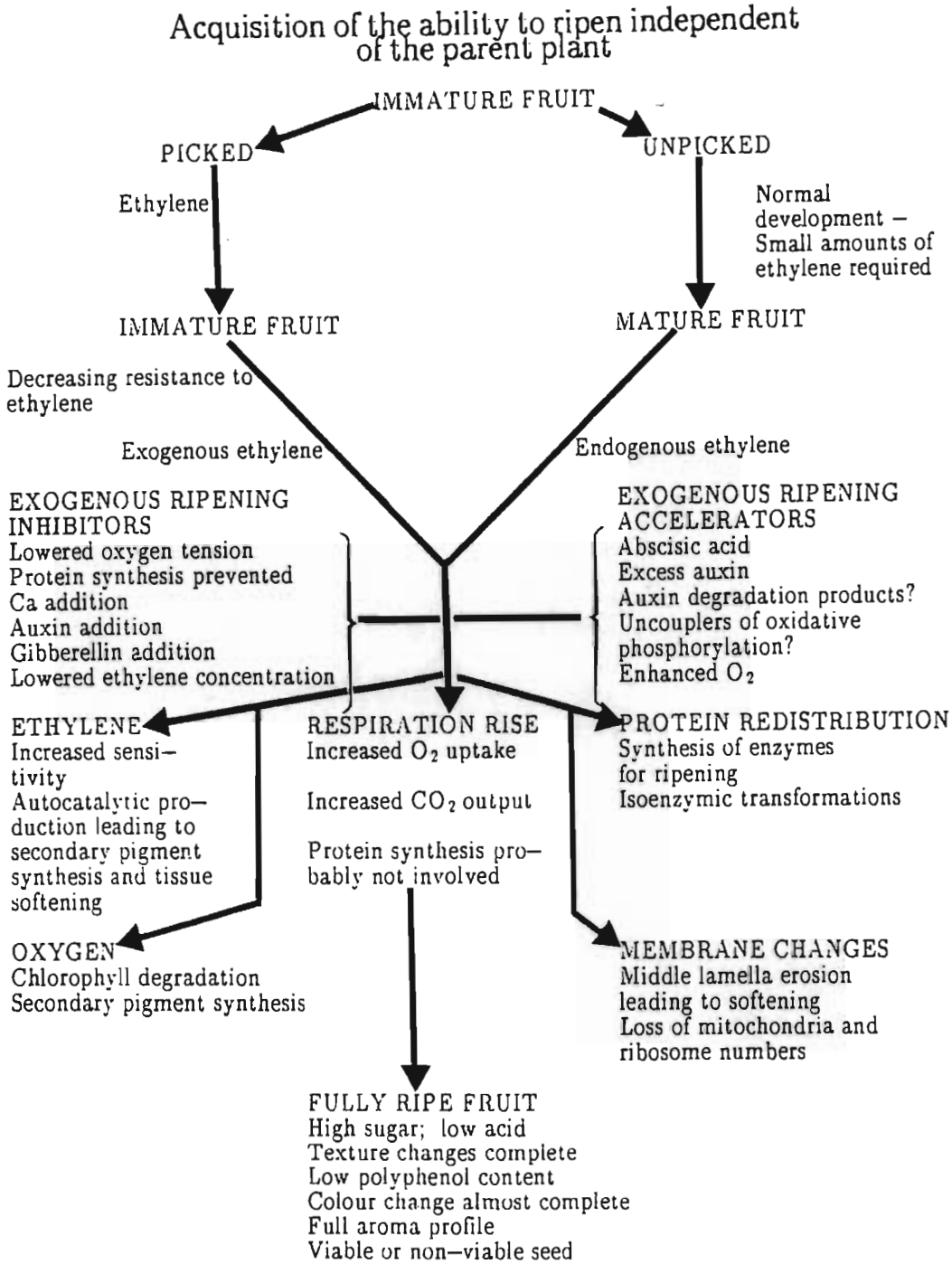


Figure 4: Tentative mechanism for the ripening of climacteric fruit (HOBSON, 1979)

synthesis or activation of specific enzymes, and that some changes which occur during ripening are stimulated and co–ordinated in some way by the action of ethylene (PRATT and GOESCHL, 1969; MARRIOTT, 1980; GRIERSON, TUCKER and ROBERTSON 1981; GRIERSON and TUCKER, 1983). It is generally accepted that ethylene is involved in the initiation of fruit ripening. If bananas are stored at low pressure, ripening is delayed even when the partial pressure of oxygen is raised to approximately that of air. Application of ethylene to fruit stored under these conditions reverses the inhibition of ripening (BURG and BURG, 1966). In pear fruits treated with aminoethoxyvinylglycine, ethylene production and ripening are delayed. This inhibition of ripening is overcome by exogenous ethylene (NESS and ROMANI, 1980). Bananas held at 21 °C with a continuous application of concentrations of ethylene gas as low as 0.015 $\mu\ell\ \ell^{-1}$ air can shorten the preclimacteric period, while exposure to 1 $\mu\ell$ ethylene gas ℓ^{-1} air for 24 hours will ensure prompt initiation of ripening (LIU, 1976). The onset of ripening in bananas appears to be determined by a change in sensitivity to endogenous ethylene during maturation (BURG and BURG, 1965b; PEACOCK, 1972). Control of the time of onset may also be related to the capacity to produce ethylene autocatalytically as is apparently the case in avocado (EAKS, 1980). The factors controlling the sensitivity of fruits to ethylene or the capacity for ethylene biosynthesis are not known. However, many fruits, including the banana, enter the climacteric phase soon after harvest, whereas left on the plant they may remain unripe for a longer period (BURG and BURG, 1965a and 1965b). These observations suggest that while attached to the parent plant an inhibitory factor may be supplied to the fruit, which determines the time of onset of ripening (BURG and BURG, 1965a; ADATO and GAZIT, 1974). There is some evidence that auxin and perhaps other growth regulators may be

involved in these effects although this role is not definitely established (MARRIOTT, 1980; BURTON, 1982). Ethylene certainly appears to be required for initiating ripening, but bananas and other climacteric fruit produce large quantities of ethylene during subsequent ripening. The role of this 'excess' ethylene is not well understood. HESSELMAN and FREEBAIRN (1969) found that if bananas were exposed to a 100 $\mu\ell$ of ethylene gas ℓ^{-1} air for 12 to 16 hours, and then stored at low levels of oxygen, the rate of ripening was reduced in comparison to the controls held in air. Furthermore, when ethylene was applied to the storage environment, low oxygen suppressed ripening of the banana fruit in comparison to those held in air (KANELLIS, SOLOMOS and MATOO, 1989). It has been reported that ethylene biosynthesis is suppressed at low levels of oxygen (MAPSON and ROBINSON, 1966). HESSELMAN and FREEBAIRN (1969) suggested that their results indicate a requirement for high levels of endogenous ethylene during ripening. NESS and ROMANI (1980) reported that when endogenous ethylene biosynthesis was inhibited in pears using aminoethoxyvinyglycine, fruit exposed to ethylene gas at 10 $\mu\ell$ ℓ^{-1} air for 24 hours ripened more slowly than those exposed continuously to a similar concentration of ethylene. They noted, however, that since ethylene production by ripening fruit in response to the 24 hours exposure to ethylene did not appear to be higher than that of non–ripening fruit treated with aminoethoxyvinylglycine–treated fruit and not exposed to ethylene, only very low levels of ethylene appeared to be necessary for completion of the ripening process. In banana the peel has a low capacity for ethylene production and evidence suggests that changes in the peel observed during normal ripening depend on high levels of ethylene production by the pulp (VENDREL and McGLASSON, 1971). Chlorophyll breakdown and maximum

stimulation of respiration in bananas requires a longer period of exposure to ethylene than the conversion of starch to sugar (VENDRELL and McGLASSON, 1971). Ethylene is therefore not only required for the initiation of certain ripening events but it also plays a role in bringing events during ripening to a rapid and co–ordinated conclusion. However, COOK and VAN STADEN (1988) while studying the cut carnation flower came to the conclusion "that ethylene does not initiate the senescence process but accelerates the irreversibility thereof": It has also been proposed that ethylene and high oxygen concentrations generate active forms of oxygen, namely peroxides, which in turn enhance senescence (BRENNAN and FRENKEL, 1977).

JEFFERY, SMITH, GOODENOUGH, PROSSER and GRIERSON (1984) found that during ripening of the tomato three classes of biochemical changes can be recognized.

1. Changes in the specific activity of citrate synthase, malate dehydrogenase, and malic enzyme, with consequent changes in citrate and malate metabolism. Metabolism of starch to equal quantities of glucose and fructose. All of these appear to occur immediately after the fruits are removed from the tomato plant at the mature–green stage. These changes may also occur in fruit ripening on the plant. They are not stimulated by ethylene and do not rely on cell wall breakdown by polygalacturonase (GOODENOUGH and THOMAS, 1981; TUCKER and GRIERSON, 1982).
2. Loss of chlorophyll, which occurs slowly when the fruits are removed from the tomato plant. Loss of chlorophyll is greatly enhanced by ethylene.
3. Formation of lycopene, polygalacturonase, and an increase in invertase,

seemingly intimately dependent on ethylene for initiation. Changes in class 1 seem to require regulation of pre–existing enzymes, i.e. post–transcriptional modification. Class 3 changes seem to require transcription and translation for the change to occur and seem to be the main target for ethylene stimulation.

Therefore, many of the changes which occur during ripening appear to result from the activation or synthesis of enzymes. Changes in protein synthesis have been reported during banana ripening (MARRIOTT, 1980). Ethylene regulation of specific messenger–RNAs has been demonstrated in avocado fruits (TUCKER and LATIES, 1984). In addition, the senescence of carnation petals has been linked to temporal changes in gene expression as evidenced by changes in protein and messenger–RNA populations (WOODSON, 1987). Increased synthesis of enzymes may be responsible for many of the changes which occur during ripening, such as stimulation of ethylene production, conversion of starch to sugar, and changes in texture. RIOV and YANG (1982) and BUFLER (1986) indicated with their work on citrus leaf tissue and peel of apple respectively, that autocatalytic ethylene production may involve increased synthesis of two enzymes in the biosynthetic pathway of ethylene; ACC synthase and the ethylene forming enzyme. Modifications in carbohydrate metabolism during ripening of slices of banana tissue have been observed on application of protein synthesis inhibitors. TERRA, GARCIA and LAJOLO (1983) reported that infiltration of tissue slices with actinomycin D or cycloheximide reduced the level of incorporation of (^{14}C) glucose–1–phosphate into sucrose, suggesting a requirement for the synthesis of enzymes involved in the production of sucrose. The activity of polygalacturonase, which is implicated in fruit softening has been reported to increase

during the ripening of tomato fruit. Studies undertaken using immunological methods and enzyme purification techniques indicate that this increase in activity is due to enzyme synthesis (GRIERSON, TUCKER and ROBERTSON, 1981). Investigations on the control of respiration during banana ripening (MARRIOTT, 1980) indicated that there was an increase in the activity of phosphofructokinase during the climacteric. This change in enzyme activity, which did not appear to be due to enzyme synthesis, was sufficient to account for the increase in respiratory activities during the climacteric. Taken together these results indicate that senescence is regulated at the level of transcription and/or translation.

The interrelationship between the changes which occur during ripening is not well understood. For instance, the significance and role of the climacteric respiration is unclear. One possibility is that the respiratory climacteric is a response to energy demands of the process of ripening. However, in tomatoes normal ripening can take place in low oxygen atmospheres in the absence of a respiratory climacteric (SACHER, 1973). Furthermore, under certain conditions, some aspects of ripening may occur without others. KANELLIS, SOLOMOS and MATOO (1989) for example, showed that sugar accumulation increased at low oxygen levels, although no increase in acid phosphatase activity was observed, suggesting differential effects of low oxygen on metabolic processes during fruit ripening. At very high temperatures (±50 ^{o}C) respiration may be stimulated in the banana, without the occurrence of other ripening changes (LIZANA, 1976). These responses to different environmental conditions may indicate a difference in metabolic control between different ripening processes, for example protein

synthesis inhibition may prevent ripening but a respiratory climacteric can still occur (MARRIOTT, 1980).

The way in which ethylene acts to bring about changes associated with ripening is not known. It has been suggested that ethylene might act on the physical state of cells or membranes bringing about alterations in intracellular compartmentation of enzymes and substrates. However, MEHARD (1969) using mitochondria and model membranes was unable to find any evidence that ethylene acted by a simple physical effect on cell membranes. Also, although SOLOMOS and LATIES (1973) reported that chloroform and bromine will induce ripening in bananas (these compounds might be expected to act on the physical structure of cell membranes), they noted that these agents evoked an increase in endogenous ethylene production, and therefore they could not establish with certainty whether these chemicals exert their effect on ripening independantly or through ethylene. Changes in tissue permeability have been reported during banana ripening, but their role is not understood at the present time (MARRIOTT, 1980). The synthesis or activation of specific enzymes during ripening suggests a degree of control which is not compatible with the simplest form of hypothesis on breakdown of intracellular compartmentation.

It is not known whether ethylene affects enzyme synthesis or activation directly. However, it may act by altering the activity of a metabolic pump resulting in changes in ionic balance within the cell. CHALMERS and ROWAN (1971) found evidence to support a proposal that redistribution of inorganic phosphate within the cell may be responsible for activation of phosphofructokinase during tomato

fruit ripening although the activation of this enzyme in banana tissue does not appear to be dependent on inorganic phosphate (SALMINEN and YOUNG, 1975). MALONE and RIDGE (1983) have also reported results which indicate that ethylene promotes growth in the aquatic plant *Nymphoides peltata* Hill, in the presence of auxin by some method involving H^+–excretion. BURG and BURG (1965a, 1967) investigated the molecular requirements for the biological activity of ethylene by testing the ability of a range of chromatographically pure gases to retard curvature and elongation in pea stem sections. The requirements for ethylene action had a marked resemblance to the rules for metal binding to alkenes, and it was postulated that ethylene and its analogues may act by binding to a metal containing receptor site within the cell. Since the work of BURG and BURG (1965a, 1967) a number of investigations have been undertaken to test for the presence of ethylene binding sites in plant tissue and to isolate and characterise these binding sites (SISLER, 1979; DODDS and HALL, 1980; SISLER, 1980; EVANS, BENGOCHEA, CAIRNS, DODDS and HALL, 1982; SANDERS, SMITH and HALL, 1986). Although it has proved possible to detect sites that bind ethylene, using ^{14}C–ethylene in the presence and absence of unlabelled ethylene, it has not proved possible as yet, to show how the interaction of the hormone with the receptor sites leads to the onset of biochemical and morphological changes within the plant. Also, there is no conclusive evidence that the binding sites detected in these experiments are the actual ethylene receptors. There is evidence to indicate that ethylene is metabolised by plant tissue. Using high purity ^{14}C–labelled ethylene, BEYER (1981) has reported that pea seedlings (*Pisum sativum L.*) will metabolise ethylene to ethylene oxide, ethylene glycol and carbon dioxide. SANDERS, SMITH and HALL (1986) proposed a metabolism–action hypothesis (Figure 5) stating that ethylene oxide

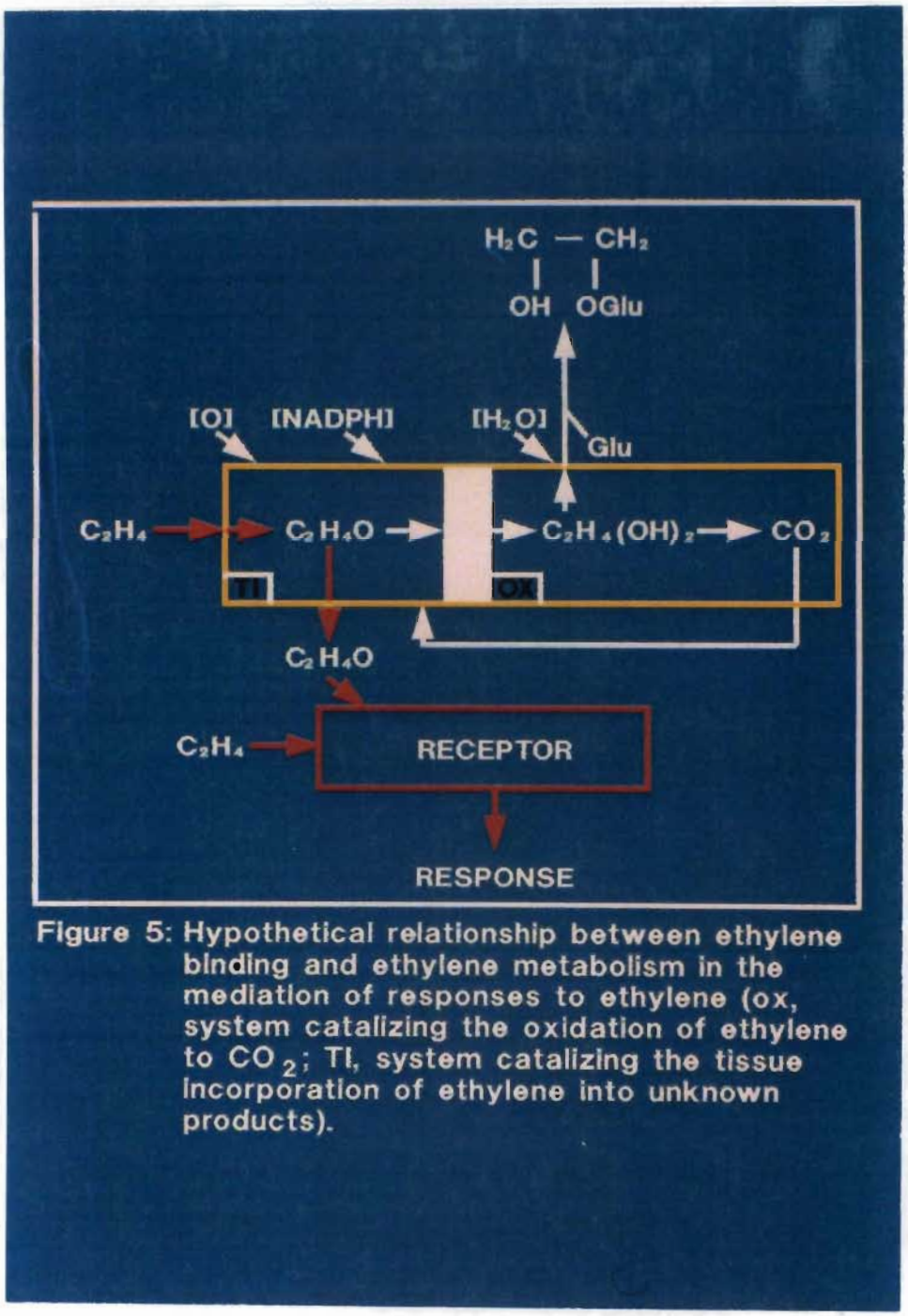


Figure 5: Hypothetical relationship between ethylene binding and ethylene metabolism in the mediation of responses to ethylene (ox, system catalizing the oxidation of ethylene to CO_2; TI, system catalizing the tissue incorporation of ethylene into unknown products).

(C_2H_4O) could interact with the binding site along with ethylene itself to bring about the appropriate biochemical response. If the CO_2–forming system is part of the same complex it may adopt a role in increasing ethylene monoxygenase activity. Glycosylation of ethylene glycol [$C_2H_4(OH)_2$] could represent an inactivation pathway for ethylene oxide. It can be concluded that ethylene is the primary hormone involved in fruit ripening, but whether it acts as the trigger, as it does in the abscission process (SAGEE, GORAN and RIOV, 1980), or only as one of the key factors accelerating a multicausal process (HOBSON, 1979) remains to be established once the many methodological pitfalls have been overcome. Whatever the outcome, the control of ethylene biosynthesis and the sensitivity of the tissue to this hormone is the main means of regulating the shelf life of harvested fruits (BRUINSMA, 1983).

Climacteric respiration

In the banana climacteric respiration is often associated with the degradation of starch to simple sugars (BIALE and YOUNG, 1981). In other fruits these processes may, however, not coincide. The released sugars are apparently an important respiratory substrate (BEN–YEHOSHUA, 1964). The sugars (as glycolytic substrates) released from starch degradation, other carbohydrate reserves, or from sugar storage pools may undergo interconversion and priming by phosphorylation as glycolytic substrates (LESHEM, HALEVY and FRENKEL, 1986). Changes in enzyme kinetics at crossover points in glycolysis may allow enhanced flux of intermediates through the pathway and ultimately the availability of respiratory substrate (LEHNINGER, 1975). Analysis of intermediary metabolites at crossover points are consistent with the suggestion

that glycolysis activity is enhanced during climacteric respiration (BIALE and YOUNG, 1971; SOLOMOS, 1983) and may partially account for climacteric respiration. SOLOMOS (1983) indicated that the available information is insufficient to suggest whether the respiratory climacteric is also regulated by the transport of storage tricarboxylic acid intermediates, or the rate of oxidative phosphorylation in the mitochondria. There is also insufficient information regarding the role of the pentose phosphate pathway in the process (LESHEM, HALEVY, FRENKEL, 1986).

BLACKMAN and PARIJA (1928) attributed the enhanced respiratory activity to the lowering in 'cellular organization resistance' to reactions which may not occur normally, due to the sequestration of reactants. The increase in membrane permeability and the subsequent availability of previously compartmentalised respiratory substrates (e.g. organic acids), may lead to unhindered enzyme–substrate interaction and may determine the rate of enzymic reactions. According to this view the respiratory upsurge, represents a loss of metabolic control. Though an increase in membrane permeability has been demonstrated in climacteric fruit (SACHER, 1967; BRADY, O'CONNELL, SMYDZUK and WADE, 1970) the relevance of the phenomenon as it applies to metabolite transport **in vivo** is not entirely clear (BURG, 1968; VICKERY and BRUINSMA, 1973). Furthermore, it has yet to be demonstrated whether the process is causal or coincidal to the respiratory rise.

LANCE (1981) provided evidence that there is the emergence of a cyanide insensitive respiration during the climacteric respiration. The cyanide insensitive respiration, by employing an alternative path to the cytochrome pathway, may offer a mechanism for circumventing control points in the mitochondrial electron

transport from the substrate to oxygen. Furthermore, cyanide and ethylene may both either activate or affect the link between the conventional electron transport chain and the cyanide–insensitive path. It is further proposed that this activation may well be the primary event leading to the rise in respiration (SOLOMOS and LATIES, 1976). It is not certain if the cyanide insensitive pathway is engaged **in vivo** (DAY, ARRON and LATIES, 1980; SOLOMOS, 1983). The role of the cyanide insensitive respiration pathway in the release of respiration from constraints awaits proof.

Cytosolic oxidases may also mediate the transfer of electrons from the respiratory substrate to oxygen (PEREZ, JONES and FRENKEL, 1981). SOLOMOS (1983) observed results which support this idea in avocado slices. The metabolic function of other non–mitochondrial terminal oxidases, although of undefined nature, is intriguing and may be contributory to the overall respiratory upsurge. Thus, whereas the relationship of enhanced glycolytic activity to the respiratory upsurge is based on less controversial facts, the overall contribution of other pathways or processes is presently unknown (LESHEM, HALEVY and FRENKEL, 1986).

The respiratory metabolism may be required to support ripening processes by furnishing metabolic energy in the form of adenosine 5'–triphosphate (ATP) and intermediary metabolites for synthetic processes, and by collecting and degrading breakdown products resulting from catabolic events (SALISBURY and ROSS, 1985). The phosphorylation of intermediary metabolites, including ADP (adenosine 5'–diphosphate) conversion to ATP in fruit, is roughly ten times greater at the climacteric than the preclimacteric stage. SOLOMOS (1983) stated that 'although the available information is insufficient to calculate the

energy change the respiratory effort is apparently in excess of the required supply of metabolic energy'. RHODES (1983) came to the conclusion that the large increase in ATP does not appear to inhibit the activity of allosteric enzymes at crossover points in glycolysis, presumably because the enzymes have become desensitised to the tissues energy state. Respiration in these tissues may be mobilized and regulated by reasons other than the energy state. Of further interest, is that a respiratory burst may be obtained even when other ripening processes are arrested, e.g. by the application of protein synthesis inhibitors in pears (FRENKEL, KLEIN and DILLEY, 1968), gibberellins in tomatoes (DOSTAL and LEOPOLD, 1967) or IAA in banana slices (VENDRELL, 1969). It therefore appears that no relationship exists between requirements for metabolic energy and the increase in respiratory activity. The above suggestion is supported by the observation that, for instance in the strawberry, a non–climacteric fruit, ripening can be as rapid and intense without a respiratory upsurge and the concomitant production of metabolic energy (KNEE, SARGENT and OSBORNE, 1977). The respiratory climacteric may be tentatively regarded as a by–product of ripening in some fruit tissue. The rise in respiration during senesence of fruits, leaves and cut flowers may not reflect the energy requirements of the senescence process but it is a facet of ethylene action and not of senescence per se (SOLOMOS, 1983). The increase in respiration is simply one of many changes underlying the reorganization of metabolism which eventually leads to ripening (RHODES, 1970).

The vacuoles of many fruits and vegetables have large reserves of organic acids that can be mobilized for use in the mitochondria as oxidisable substrates in the tricarboxylic acid cycle. The complete oxidation of malate, for example,

generates more carbon dioxide (CO_2) than the amount of oxygen (O_2) consumed, however, the oxidation of glucose (monosaccharide) generates an equal amount of carbon dioxide for the oxygen consumed. This relationship becomes very important when measuring respiration by gas exchange, in which the carbon dioxide evolved and or oxygen consumed is measured. It is therefore possible to record different values for respiration depending on which gas is monitored. Ideally both gases should be measured simultaneously (WILLS, McGLASSON, GRAHAM, LEE and HALL, 1989). The concept of respiration quotient (RQ) has been developed to quantify this variation, where:

RQ = CO_2 produced ($\mu\ell\ \ell^{-1}$)/O_2 consumed ($\mu\ell\ \ell^{-1}$).

For the complete oxidation of glucose, the RQ is 1.0, whereas for malate the RQ is 1.3. Long chain fatty acids could also be a respirable substrate. These fatty acids have much less oxygen per carbon atom than sugars and, therefore, require a greater oxygen consumption for the production of CO_2. The RQ for fatty acids in general equals 0.7. Therefore the measurement of respiratory quotient, itself can give some guide to the type of substrate that is being respired: a low respiratory quotient suggests some fat metabolism and a high respiratory quotient suggests organic acids. Changes in respiratory quotient during storage could indicate a change in the type of substrate that is being metabolized (WILLS, McGLASSON, GRAHAM, LEE and HALL, 1989).

Cell wall structure

KNEE and BARTLEY (1981) proposed that the molecular skeleton of the cell wall consists of cellulose microfibrils together with hemicellulose. The hemicellulose, consisting of xyloglucans and glucurono–arabinoxylans, is attached

by hydrogen bonds to the cellulose microfibrils. Interdispersed pectin material composed of rhamnogalacturonans is bridged to the cellulose–hemicellulose complex by neutral homogalacturonans, arabinans and galactans. McNEIL, DARVILL, FRY and ALBERSHEIM (1984) in a recent review have shown that the cell wall structure contain additional pectic and other constituents. The primary wall may also consist of up to 10 % protein (ROBINSON, 1977). Extensin (major component of the wall protein) is apparently cross–linked to the pectin and may represent a wall polymer system in addition to that of the cellulose–hemicellulose (COOPER, CHEN and VARNER, 1984). The wall proteins may represent structural components, wall enzymes, or proteins with other functions, such as disease resistance (MACNEIL, DARVILL, FRY and ALBERSHEIM, 1984). The relative abundance of wall components may vary considerably. The carboxyl groups of the uronic acids in the pectinic material may be available for cross–linkages, through multivalent cations, mainly calcium ions, for additional cell wall cohesion (KNEE and BARTLEY, 1981).

Softening associated with fruit ripening represents a complex process. MOHR and STEIN (1969) and VICKERY and BRUINSMA (1973) found that cell expansion in apples, may lead to a mechanical separation of cells at the middle lamella region which may progress further as the fruit approaches the ripening stage. Loss in intercellular cohesion resulting from the dissolution of the middle lamella in ripening avocado (PESIS, FUCHS and ZANBERMAN, 1978) may be brought about by the action of pectolytic enzymes. The loss in cohesion between cells may result from S–adenosyl methionine methylation of free carboxyl groups in the pectin material and thereby the disruption of calcium cross–linkages of adjacent polyuronides. The primary mechanism for the loss in fruit texture apparently constitutes the disruption in the intramural gross structure as well as

the degradation of the wall material. PRESSEY (1977) concluded that the disruption of the cell wall gross structure comprised of the cellulose, hemicellulose, and pectic material, is an early event that is associated with solubilisation of protopectin. The major wall components may be disengaged due to the breakdown of the connecting side chains, but the nature of the process is not clear (PRESSEY, 1977). JOSLYN (1962) proposed that protopectin may be solobulised by chain length reduction due to the action of pectin–hydrolysing enzymes of as yet undefined nature. Released cell wall components become water–soluble and may undergo degradation to varying degrees, mostly by polygalacturonase hydrolytic action. PRESSEY (1977) and PRESSEY, AVANTS and DULL (1978) found that fruit softening correlates well with the polygalacturonase–catalysed dissolution of polygalacturonic–rich pectic material. Pectic substances are known to be preferentially degraded by polygalacturonase. The pectinic material is significantly demethoxylated by pectin methyl esterase, which is present in sufficient quantities throughout the development of fruit (PRESSEY, 1977). It is furthermore possible that pectin methyl esterase isozymes (DELINCEE, 1976) catalyse demethoxylation in defined pectin domains so as to render the compound susceptible to the action of polygalacturonase. The esterification of free carboxyl groups in the pectic fraction in the middle lamella may actually be catalysed by pectin methyl esterase thereby serving as a mechanism for disrupting the calcium cross–linkages and thereafter the separation of cells. HINTON and PRESSEY (1974) and BEN–ARIE, KISLEV and FRENKEL (1979) reported that cellulase (beta – 1,4 gluconase) increased during the ripening of some fruit.

HOBSON (1981) suggested that the complementary action of glycosidases, glucanases and pectin methyl esterase may constitute an early and preparatory process. Rapid degradation of the wall material and loss in the wall structure may ensue with the emergence of polygalacturonase activity. Cell wall degradation may also be accompanied by the release of enzymes and proteins (TICHELAAR, McGLASSON and BUESCHER, 1978).

Pigments

The chlorophyll content of the banana cultivar 'Gros Michel' is reported to decrease from between 50–100 μg gram^{-1} fresh weight in the unripe fruit to zero in the ripe fruit during ripening while the level of banana peel carotenoids remained constant at 8 μg gram^{-1} (VON LOESECKE, 1929). Little is known about the biochemistry of chlorophyll breakdown. The enzyme chlorophyllase (E C 3.1.1.14) will split the phytol moiety from chlorophyll **in vitro** to give free phytol and chlorophyllide (SIMPSON, LEE, RODRIQUEZ and CHICHESTER, 1976). The role of chlorophyllase **in vivo** is not clear. Rises in the activity of the enzyme during ripening of bananas (LOONEY and PATTERSON, 1967) and degreening of citrus (PURVIS, 1980) were reported. There is however, evidence to suggest that chlorophyllase is involved in chlorophyll biosynthesis although a number of workers have been unable to find a consistent relationship between chlorophyllase activity and senescence (THOMAS and STODDART, 1980). HOLDEN (1967) described an enzyme system from legume seeds that converts chlorophyll into colourless compounds. In this system long chain fatty acids are peroxidised by lipoxidase and other enzymes and the hydroperoxides are then broken down by a lipoperoxidase leading to free–radical formation and oxidation

of chlorophylls. However, HOLDEN (1967) states that there is no reason to suppose that this is the only way or the usual way that chlorophyll is broken down in vivo. Evidence has been obtained that, in senescing leaves, chlorophyll degradation is catalysed by an 'oxidase' which is localised in the chloroplast lamellae and which is activated by linolenic acid (MARTINOIA, DALLING and MATILE, 1982; LüTHY, MARTINOIA, MATILE and THOMAS, 1984). Chlorophyll appears to be organized into complexes with specific thylakoid membrane proteins (HILLER and GOODCHILD, 1981). Chlorophyll degradation during fruit ripening or leaf senescence is accompanied by breakdown of the internal membrane system of the chloroplast (THOMAS and STODDARD, 1980; THOMSON and WHATLEY, 1980; BURTON, 1982). Linolenic acid is a major product of galactolipid breakdown, and unsaturated fatty acids like linolenic acid are able to induce the breakdown of membrane proteins (THOMAS, 1983). It is therefore possible that chlorophyll breakdown during fruit ripening is mediated by degradation of thylakoid lipids. The released lipid breakdown products are able to solubilise the chlorophyll–protein complexes and activate chlorophyll oxidase. Furthermore, large osmiophilic globuli within chloroplasts are the most conspicuous indicators of leaf senescence (BUTLER and SIMON, 1971). Chloroplasts normally have few plastoglobuli, and these increase in number and size as the thylakoids are degraded during leaf senescence (HARRIS and ARNOT, 1973). It is therefore assumed that plastoglobuli have a function connected with thylakoid formation or breakdown. Plastoglobuli may serve as pools for storage of thylakoid constituents especially of lipids (STEINMüLLER and TEVINI, 1985). The main components in plastoglobuli isolated from chloroplasts were triacylglycerols and lipophylic prenyl quinones, mainly plastohydroquinone and

α–tocopherol. The corresponding oxidised prenyl quinones, plastoquinine (OX), α–tocoquinone and the phylloquinone, vitamin K_1, were detected in trace amounts. Plastoglobuli isolated from chromoplasts contained large amounts of carotenoid esters. Triacylglycerols constituted two–thirds of the content of these plastoglobuli (STEINMüLLER and TEVINI, 1985). The complete composition of plastoglobuli, however, is still unknown.

Carbohydrates

Starch which in the banana forms about 20 to 25 % of the pulp (fresh weight basis) is rapidly degraded during ripening, with the concomitant accumulation of sucrose, fructose and glucose. Traces of maltose may also be present (PALMER, 1971). The predominant sugar, at least during the early stages of ripening, is sucrose (MARRIOTT, ROBINSON and KARIKARI, 1981). Its formation preceeds the accumulation of glucose and fructose. BIALE (1960b), however, found that in the banana approximately equal concentrations of glucose and fructose, together with a little sucrose, appear during starch hydrolysis at the climacteric. The mango fruit, on the other hand, shows a large increase in sucrose concentration, and a smaller proportion of reducing sugars as starch is hydrolysed. Later in storage, sucrose tends to disappear in the banana and to be replaced by an equal amount of reducing sugars (BIALE, 1960b). The unripe peel contains about 3 % starch and shows similar changes in carbohydrates as the pulp during ripening (BARNELL, 1941). Bananas ripened at 40 °C produced little sucrose but glucose and fructose still accumulated (LIZANA, 1976).

accumulated. These observations may indicate that a high proportion of the glucose and fructose results from sucrose that may not accumulate because it is transient.

There appear to be two possible routes for starch breakdown in higher plants. The hydrolytic cleavage of starch to maltose can be catalysed by *a* (1–6) glucosidase and amylases. Maltose can be broken down to glucose by maltase. Phosphorylase can split glucose from starch from the non–reducing end of the polymer in combination with inorganic phosphate to D–glucose 1–phosphate. The above enzymes have been detected in the banana (GLASS and RAND, 1982; TERRA, GARCIA and LAJOLO, 1983). Of importance however, is that the relative contributions of hydrolysis and phosphorolysis to starch breakdown during ripening is not clear. In bananas an increase in amylase activity has been reported to increase during ripening (MOA and KINSELLA, 1981). AREAS and LAJOLO (1981) found that during ripening phosphorylase showed an increase in activity prior to starch degradation and a decrease in activity during the respiratory climacteric. Sucrose can be synthesised from fructose–1–phosphate by conversion to uridine diphosphate–D–glucose. Uridine diphosphate–D–glucose, may then be involved in one of two reactions which lead to sucrose as the product (AKAZAWA and OKAMOTO, 1980).

(i) UDP–D–glucose + D–fructose $\xrightarrow{\text{sucrose synthetase}}$ sucrose + UDP

(ii) UDP–D–glucose + D–fructose–6–phosphate $\xrightarrow{\text{sucrose phosphate synthetase}}$ sucrose phosphate + UDP $\xrightarrow{\text{phosphatase}}$ sucrose + Pi.

TERRA, GARCIA and LAJOLO (1983) reported that sucrose synthetase activity increases during ripening concomitant with the rise in sucrose formation. AKAZAWA and OKAMOTO (1980) however, reported that the production of sucrose via sucrose–phosphate–synthetase is a more energetically favourable reaction and may be more important than sucrose synthetase for catalysing sucrose synthesis, while sucrose synthetase may be important in sucrose degradation.

Quality of ripening fruit

Ripening in general leads to an increase in sweetness and a decrease in the acidity of fruit. The release of simple sugars from starch or other reserve carbohydrates and the interconversion of the released sugars results in sweetness. As mentioned earlier, the unripe banana fruit contains roughly 20 to 25 % starch by weight and starch degradation, results in sugar enrichment of up to 20 % of fruit weight (PALMER, 1971). In fruit with substantial carbohydrate reserves, for example apples, postharvest ripening, which occurs naturally or is stimulated by applied ethylene, is usually accompanied by an increase in sweetness and the attainment of edible quality (LESHEM, HALEVY and FRENKEL, 1986). SCRIVEN, GEK and WILLS (1989) stated that naturally ripened banana fruits were more fruity, less green, and softer than ethylene–treated fruit. They found that ethylene caused the skin and flesh to ripen out of phase, with ripening in the flesh occurring more slowly. This suggestion is endorsed by two previously reported biochemical studies. RIPPON and TROCHOULIAS (1976) found that when the rate of ripening (example skin colour change) is accelerated by

increasing storage temperature, flesh softening is inhibited. VENDRELL and McGLASSON (1971) also suggested that when no exogenous ethylene was administered peel ripening depends on ethylene produced by the flesh. SCRIVEN, GEK and WILLS (1989) concluded that consumers complaints that the taste of fruit is not as good as they are used to probably basing the time of optimum eating quality on skin colour when fruit is ripened without ethylene. Ethylene–ripened bananas do have a different taste since they have a more advanced skin colour than naturally ripened fruits although the pulp may be at a lesser degree of ripening.

Organic acids which are stored principally in the vacuoles of fruits are responsible for their acidity. The bulk of acids are represented by malic and citric acid (ULRICH, 1970). Acid accumulation may be due to the sequestration of the tricarboxylic acid cycle intermediates, the dark CO_2 fixation process, deamination of amino acids, and by acid mobilisation from other plant parts (ULRICH, 1970). The acid content, for example in lemons and other acidic fruit, usually decreases during ripening, probably as it is used as substrate in respiration (SOLOMOS, 1983). However, in the ripening banana, the production of organic acids leads to an increase in acidity (WYMAN and PALMER, 1964). Nevertheless, the organic acid levels at any stage of banana development are less when compared with other produce (WILLS, McGLASSON, GRAHAM, LEE and HALL, 1989). The sugar–acid ratio and sugar content are often used as a parameter of fruit sensory quality (KADER, MORRIS, STEVENS and ALBRIGHT–HOLTON, 1978).

An important component of taste is astringency which is apparently a function of

the tanning action of condensed tannins on proteins and enzymes. As fruit ripen astringency disappears and this action is attributed to a further condensation of the active tannins, a process which may render them less soluble or reactive (VAN BUREN, 1970).

Aromatic compounds contribute to a great extent toward the overall fruit sensory quality. Fruit aroma is a composite of a large number of compounds, consisting of alcohols, aldehydes, esters, phenolics, heterocyclic compounds, terpenes, hydrocarbons and sulphur–containing substances (NURSTEN, 1970). The composition of the aroma compounds is specific to species and often variety. In the banana the typical green, ripe and overripe aromas can be ascribed to α–hexenal, eugenol and isopentanol respectively (WILLS, McGLASSON, GRAHAM, LEE and HALL, 1989). KADER, MORRIS, STEVENS and ALBRIGHT–HOLTON (1978) stated that a deficient complement of volatiles results in lesser quality of fruits, whereas in sufficient complement contribute to the desirability of the fruit. GALLIARD and MATTHEW (1977) described some pathways for formation of fruit volatiles from amino acids and from oxidative degradation of lipids.

Change in fruit colour is one of the most familiar and readily perceived processes. Fruit ripening in most fruit is accompanied by the disappearance of the green background colour and the development of yellow, orange, red, blue or other colours (LESHEM, HALEVEY and FRENKEL, 1986). The fruit interior often undergoes changes in colour which may be externally visible, as in ripening tomatoes, and may be used to evaluate the degree of ripeness. The banana is also an example of the latter phenomenon (SCRIVEN, GEK and WILLS, 1989).

Fruits are modified leaves and contain chlorophyll (ESAU, 1965). The green background colour is derived mainly from the chlorophyll content and composition of the tissues in developing fruits. However, the importance of chlorophyll is unclear since it may contribute only marginally to fruit growth and development or to the photosynthetic and photorespiratory activity of the fruit (CLIJSTERS, 1969; CLIJSTERS and PERRIN, 1974). Chlorophyll disappearance begins at the early stages of ripening. In the banana and pear, the disappearance of chlorophyll is associated with the breakdown of the chloroplast. In the tomato and orange, however, the chloroplasts undergo ulstrastructural and functional transformations into chromoplasts (GOLDSCHMIDT, 1980). Chromoplasts specialise in the production and accumulation of carotenoids. The carotenoids, normally present in the chloroplast become unmasked and dominate the background colour as the chlorophylls disappear for example in ripening banana or pear (LESHEM, HALEVY and FRENKEL, 1986). The carotenoids consists of the carotenes, a family of hydrocarbons with a basic carbon skeleton comprised of eight isoprenoid units, and of xanthophylls – the oxygenated derivatives of the carotenes (SPURGEON and PORTER, 1980). Pigment production in fruit, for example in tomato (LESHEM, HALEVY and FRENKEL, 1986), and degreening in banana (SEYMOUR, JOHN and THOMPSON, 1987) declines precipitously at elevated temperatures (>25 °C). The production of full colour in some warm regions may therefore be restricted. Ethylene or ethylene–releasing compounds are known to enhance colour production in citrus, banana and other fruit whereas other growth regulating substances, notably gibberellins are inhibitory to the process (GOLDSCHMIDT, 1980). Pigment changes occur somewhat independently of other ripening processes (GOLDSCHMIDT, 1980; JEFFEREY, SMITH, GOODENOUGH, PROSSER and GRIERSON, 1984). These authors showed that chlorophyll loss occurs

slowly when tomato fruits are removed from the plant, however, its loss is to a large extent enhanced by ethylene. In spite of the diversity in composition and fruit tissue origin, there is great similarity in the control of colour production in fruit by plant growth regulators.

Radiation

The discovery of X–rays in 1895 by Von Roentgen was followed by the discovery of radioactivity by Becquerel in 1896. Seven years later Madame Curie determined the atomic mass of radium. She also described for the first time the existence of alpha, beta and gamma rays (GOLDBLITH, 1966). Shortly thereafter the preservation properties of irradiation with respect to foodstuffs was realised.

The source of gamma rays used for food preservation are ^{60}Co or ^{137}Ce. Gamma rays have the singular property of deep penetration into material with negligible heating effect. The energy–rich photons of gamma rays, cause ionizations in the medium through which it travels (i.e., they are capable of removing electrons from atoms or molecules).

A further property of ionizing radiation, problematic to cellular radiology, is their extraordinary effectiveness in disturbing cellular growth and development. A certain number of cells exposed to ionizing radiation eventually die or lose the ability to divide. Some cells contain abnormal chromosomes or transmit their chromosomes abnormally, while others exhibit heritable changes (gene mutations). The proportion of affected cells usually rises with increasing exposure to radiation. The changes occurring in cells are due to absorbtion of

some of the radiant energy. The important differences between light and gamma rays are the following (BECKING, 1979):

i The energy of gamma rays is of the order of 10^4 higher than that of light. There is therefore little similarity between the interaction processes of gamma rays with matter and those of visible light.

ii The absorption of light depends on the molecular structure of the absorbent and to a small extent on its atomic composition. The spectrophotometric determination of various chemical compounds is based on this principle as each substance has its own characteristic absorption peaks.

iii When gamma rays are passed through an absorbent, equal amounts of energy of the gamma rays are absorbed. The gamma rays are not selective for a particular compound. The absorption is primarily related to the atomic structure of the irradiated compound and not the way in which these atoms are arranged in molecules.

iv When a quantum of visible or ultraviolet light is absorbed by a chemical compound, the energy absorbed is stored in the molecule. The molecule can then undergo one or more reactions, some of which will lead to chemical changes (such as molecular dissociation) and others to physical effects (such as fluorescence, heating, etc.). If, however, an atom absorbs a quantum of gamma rays, it loses an electron. The energy taken up by the atom mostly exceeds the quantity necessary to produce this ionization (i.e. to eject an electron from the atom) and the surplus energy appears as kinetic energy of the ejected electron. In this case two events may occur. First, the ejected electron will obtain all the energy of the gamma ray. This effect is called the Photo–effect. Or the electron will obtain only part of the total energy of the gamma ray, which is the so–called Compton

effect. In the latter case a secondary gamma ray will appear containing the remaining energy. In other words, it will continue its existence as an electromagnetic wave with a longer wavelength.

v The ejected electron in being itself the product of an ionization, is sufficiently energetic to produce ionization in the atoms through which it passes. In fact almost all ionizations are produced by ejected electrons and the effect of the initial absorption of the quantum gamma rays is usually negligible. Owing to this phenomenon, the ions produced are not distributed at random in the solutions as in the case of light absorption, but they are concentrated along the track of the ejected electrons. This represents another fundamental difference between ultraviolet or visible light absorption and ionizing radiation.

vi Radiation damage in living organisms can be divided into two categories, which cannot be separated in the living cell. Firstly, there is the direct action, for example the molecular damage in an essential molecule, which absorbs the radiation energy. Secondly, there is the indirect action, for example the formation of highly reactive free radicals in cells. The formation of free radicals from water and oxygen is far more destructive to the cell than changes in macromolecules [like DNA (deoxyribonucleic acid) and enzymes] which can lead to mutations and other molecules (like ATP or co–enzymes) without in most cases affecting the survival of the cell.

During irradiation the metabolism of cells becomes increasingly disturbed and the molecular damage is 'amplified' to give rise to easily–discernible changes in cells. Three main stages can be recognized (Figure 6). The physical stage is concerned with energy absorption processes, which occur extremely quickly (10^{-13} seconds)

and over dimensions measured in atomic diameters. The chemical stage covers the period in which activated molecules react with one another and with the normal cell constituents, and ends when chemical stability is restored. This usually takes place within a millionth of a second and involves distances in the order of a nanometre or less. Important problems at this stage concern the identity of the targets, the kinds of damage which inactivate them, and the ways in which this damage is produced. Finally the physiological stage is concerned with the metabolic consequences of the radiation–induced biochemical change, and may take more than 10^6 seconds to complete. Important problems at this stage are the ways in which biochemical damage is translated into metabolic disturbance, and the mechanisms by which cells can repair or bypass damage (LAWRENCE, 1971).

Gamma rays used in food preservation with energies from 1.17 to 1.33 MeV (1.6×10^3 Joule) can not create any radioactivity. The energy content of electromagnetic rays is determined by the following relationship:

$$E = \frac{h.c}{\lambda}$$

E = energy level (eV)

h = Planck's constant (6.6256×10^{-34} J s)

c = speed of light (3×10^{17} nm s^{-1})

λ = wavelength (nm)

The most useful unit for food technologist is the rad (radiation absorbed dose), which is defind as follows:

1 rad = 100 ergs g^{-1} of material irradiated

1 krad = 1000 rad

= .01 kGy

The great advantage of using the rad is that it refers specifically to the amount of energy absorbed by the material.

The unit of activity is the curie which is defind as:

1 curie = 3.67 x 10^{10} disintegrations s^{-1}

or alternatively, 1 curie will give a dose of approximately 1 rad over a 1 metre distance in material of unit density in 1 hour.

In plants, exposure to ultraviolet light results in a slowing and deterioration of many metabolic mechanisms and in certain instances in the acceleration of senescence (LESHEM, HALEVY and FRENKEL, 1986). The effect of gamma rays (more potent than ultraviolet light due to electrons expelled from compounds, which in turn leads to the generation of the superoxide anion radical (O_2^-) and its toxic derivatives [hydroperoxy radical ($HOO^{\cdot}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$)] on the parameters of ripening as discussed in the literature survey was examined in an attempt to determine the maximum tolerable radiation dose required to slow down the rate of banana fruit ripening.

CHAPTER 3

FRUIT SENESCENCE

INTRODUCTION

After harvest bananas are often stored at 12 to 13 °C, conditions under which respiration is low, to delay the onset of ripening (SAMSON, 1980). Under these storage conditions the fruits remain metabolically active and will ripen. Certain physiological processes in bananas are related to temperature, and storage below 12 °C may result in chilling injury which may manifest itself in various ways, including failure to ripen, poor colour and green and hard ripeness (SALUNKHE and DESAI, 1984). Fungal activity may occur during prolonged storage below 12 °C, resulting in extensive tissue damage (WARDLAW, 1961).

Bananas are climacteric fruits (BIALE, 1960a, 1960b; BURG, 1962) and exhibit a rise in ethylene production just prior to the onset of the respiratory climacteric (BURG and BURG, 1962). This phenomenon is known as autocatalytic ethylene production (ABELES, 1973). A sensitivity to ethylene gradually develops during maturation, particularly after harvesting (BURG and BURG, 1962; SAWAMURA, KNEGT and BRUINSMA, 1978). The control of ethylene synthesis and sensitivity is regarded as the most important means of regulating the shelf life of harvested fruits (BRUINSMA, 1983).

Treatment of bananas in the preclimacteric phase with ethylene will accelerate their ripening. Since the fruits are physiologically active when stored at 13 °C, they are still sensitive to their own endogenous ethylene, depending on their stage of development. MAXIE, AMEZQUITA, HASSAN and JOHNSON (1968) stressed the fact that because of the small difference between minimum storage temperatures (11 to 13 °C) and ripening temperatures (18 °C), bananas in long distance transit often ripen during transport. The fruit is also susceptible to browning caused by handling after it has reached the all–yellow colour. If not marketed immediately the product will be unattractive to the consumer, often resulting in serious losses or price reductions. Secondly, ethylene contamination from adjacent ripening rooms may trigger autocatalytic ethylene production in bananas. Various techniques have been investigated to eliminate ethylene from the storage atmosphere or to counteract the effect of ethylene on the fruit (SCOTT, McGLASSON and ROBERTS, 1970; NDUBIZU, 1976; SALTVEIT, BRADFORD and DILLEY, 1978).

Any preservation method that can slow down ripening will be of economic advantage to the banana industry. An extended period of ripening in bananas after irradiation with gamma rays has been reported by various research workers (AMEZQUITA, 1965; FERGUSON, YATES, MacQUEEN and ROBB, 1966; MAXIE, AMEZQUITA, HASSAN and JOHNSONE, 1968; THOMAS, DHARKAR and SREENIVASAN, 1971; AKAMINE and MOY, 1983; BRODRICK, THORD–GRAY and STRYDOM, 1984). It is clear from the results of these studies that the physiological status of the fruit at the time of irradiation is of importance to obtain maximum benefit from this technique. For gamma irradiation

treatment to prolong ripening of bananas effectively, the fruit must be treated prior to the onset of the climacteric rise in respiration (MAXIE, AMEZQUITA, HASSAN and JOHNSONE, 1968). Nevertheless the literature is contradictory regarding the maximum tolerable exposure. This can probably be attributed firstly, to a failure to realize that climacteric and non–climacteric fruit respond differently to the same gamma irradiation exposure. In climacteric fruit, gamma irradiation can be applied to delay ripening and fruit senescence. Most of the non–climacteric fruits are already in a state of ripening when harvested and are therefore more tolerant to higher gamma irradiation exposures (transcription, translation and activation of the ripening enzymes have been completed). Secondly, the differences between the maximum tolerable irradiation exposure for climacteric and non–climacteric fruit can perhaps be attributed to differences in climatic conditions, soil characteristics, variation between cultivars, and storage conditions after irradiation. A fact that should also be borne in mind is that irradiation at the maximum tolerable exposures can only be rationalised by means of the principles of radiation biology as described by CASARETT (1968) and LAWRENCE (1971). LAWRENCE (1971) described three distinct stages of development in cells exposed to ionizing radiation, namely a physical stage (ionized macromolecules, ionized molecules), a chemical stage (macromolecular radicals, free radicals) and a metabolic stage (undamaged macromolecules, damaged macromolecules) (Figure 6). Therefore, after irradiation the banana fruit will metabolically function abnormally or normally after 11 days depending on the radiation exposure it received.

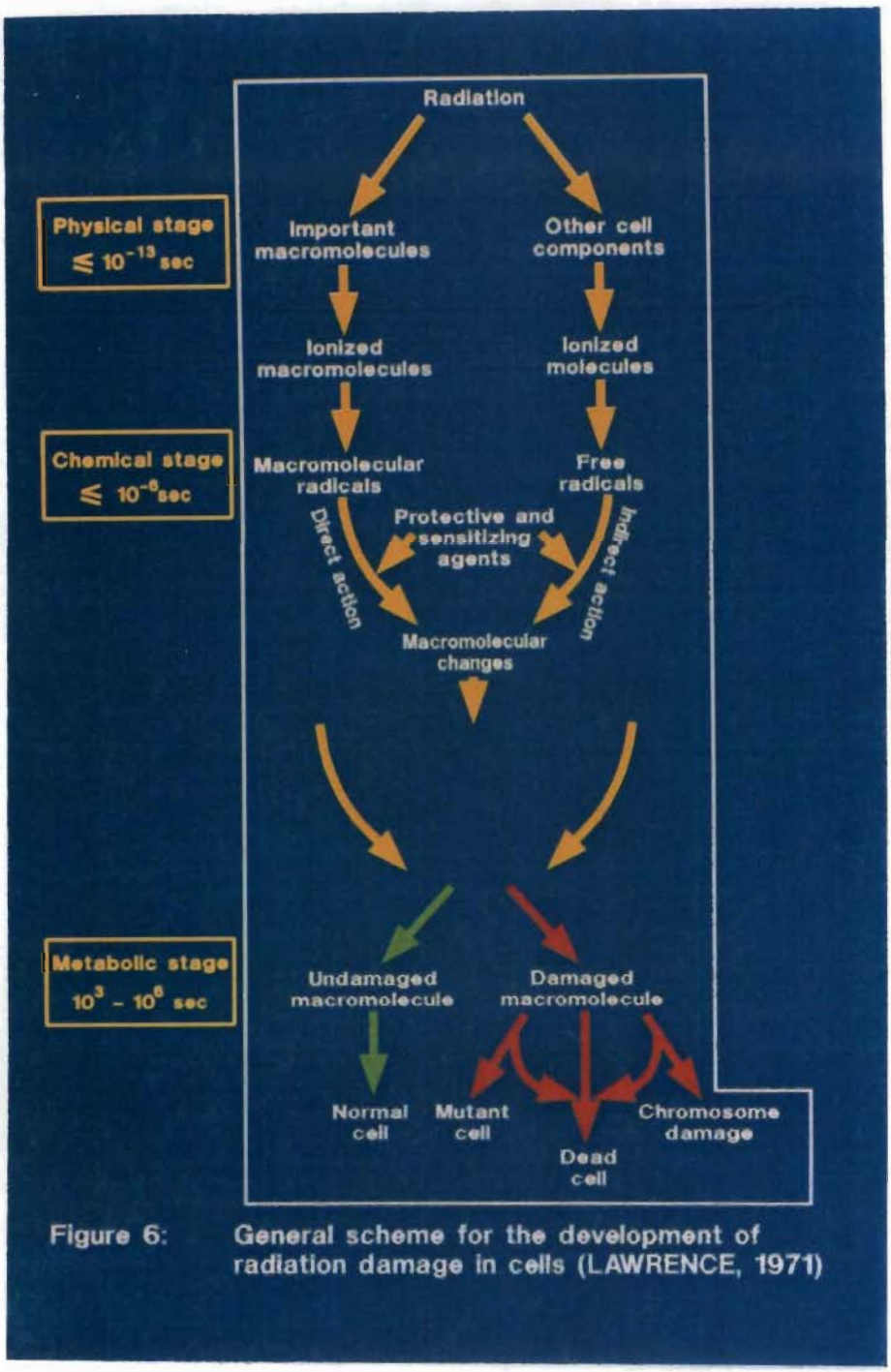


Figure 6: General scheme for the development of radiation damage in cells (LAWRENCE, 1971)

MATERIALS AND METHODS

Plant material

Bananas (*Musa acuminata* Collar cv. Dwarf Cavendish) from the Northern Transvaal were harvested at regular intervals. Fruits, the third row from each bunch at colour index no 1 (VON LOESECKE, 1950) were treated with thiabendazole [2–(4–Thiazolyl)benzimidazole] in the packhouse and packed as hands (cluster of fruit) into cartons. Maturity of the fruit at harvest was three quarters full, as judged by their angularities. They were without bruises and abrasions. The fruit was cooled to 15 °C and transported to the laboratory by insulated road vehicles one day after picking. The rate of ethylene production upon arrival in the laboratory was less than 0.1 nℓ g^{-1} $hour^{-1}$. This confirmed that the fruits were still in the preclimacteric stage.

Gamma irradiation

Detached hands of bananas were exposed to gamma irradiation from a cobalt source in a Gamma Beam 650 installation (Atomic Energy of Canada Ltd., 30 000 curies) to average doses of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 kGy, at a dose rate of 7.35 kGy $hour^{-1}$ (1 kGy = 100 krad). The ratio of maximum to minimum exposure received within the container was 1.3. Irradiation was performed in air at 20 to 25 °C. After irradiation the fruits were immediately transferred to incubators preset at 21±2 °C

and 75±2 % relative humidity, to simulate conditions in a commercial ripening chamber (WILLS, McGLASSON, GRAHAM, LEE and HALL, 1989).

Ethylene determination

For the measurement of ethylene production single bananas were enclosed in 1 dm^3 flasks filled with locking inlets and outlets. These were then flushed with ethylene and CO_2–free air and placed in incubators at 21 °C and 75 % relative humidity. At the end of 1.5 hours 1 mℓ air samples were withdrawn from each flask with an airtight syringe, and the ethylene concentration in each sample determined using a Varian 3700 gas chromatograph equipped with a flame ionization detector and a 1 m column of 80 to 100 mesh alumina (Supelco). Helium was used as carrier gas with a column pressure of 20 pSi. The gas chromatrograph conditions were: column temperature 100 °C; injector temperature 140 °C; flame ionization detector temperature 140 °C. The ethylene output was expressed as nℓ ethylene g^{-1} fresh weight (HALEVY, WHITEHEAD and KOFRANEK, 1984). The gas chromatograph was calibrated before each measurement with 1 mℓ standardized ethylene (3 $\mu\ell$ ℓ^{-1}) in nitrogen.

Respiration measurements

Single bananas were sealed in 1 dm^3 air–tight flasks fitted with locking inlets and outlets and kept in incubators at 21 °C and 75 % relative humidity for 1.5 hours. At the end of the incubation period air samples were withdrawn from the flasks and O_2 and CO_2 levels were determined using a gas chromatograph equipped with a thermal conductivity detector (bridge current 134 mA) and a 75 cm stainless steel column packed with a 40 to 60 mesh 5A Molecular Sieve (Supelco). The

column oven was kept at ambient temperature and helium was used as carrier gas with a flow rate of 24 mℓ min^{-1}. The increase in CO_2 levels and decrease in O_2 levels were calculated to determine the respiration rate of the banana fruits.

Ethylene sensitivity

To induce ripening banana fruits were treated 14 days after irradiation with a gas mixture of 1 000 $\mu\ell$ ethylene ℓ^{-1} air for 24 hours at 18 °C and 75 % relative humidity. Thereafter, all the fruits were kept in incubators at 21 °C and 75 % relative humidity, and evaluated 7 days later. Ethylene sensitivity was quantified by observing the rate of colour and textural changes as described below.

Fruit softening measurements

The texture of the pulp (meso–endocarp) of banana fruit was measured with a firmometer (Figure 7) as described by SWARTS (1980).

Colour evaluation

Colour changes were scored as described by VON LOESECKE (1950). A numerical colour value was assigned to each individual fruit corresponding to: 1 – green; 2 – green with yellow tracks; 3 – more green than yellow; 4 – more yellow than green; 5 – yellow with green tips; 6 – all yellow; 7 – yellow flecked with black spots (Figure 8). A mean colour score was calculated for each sample of fruit.

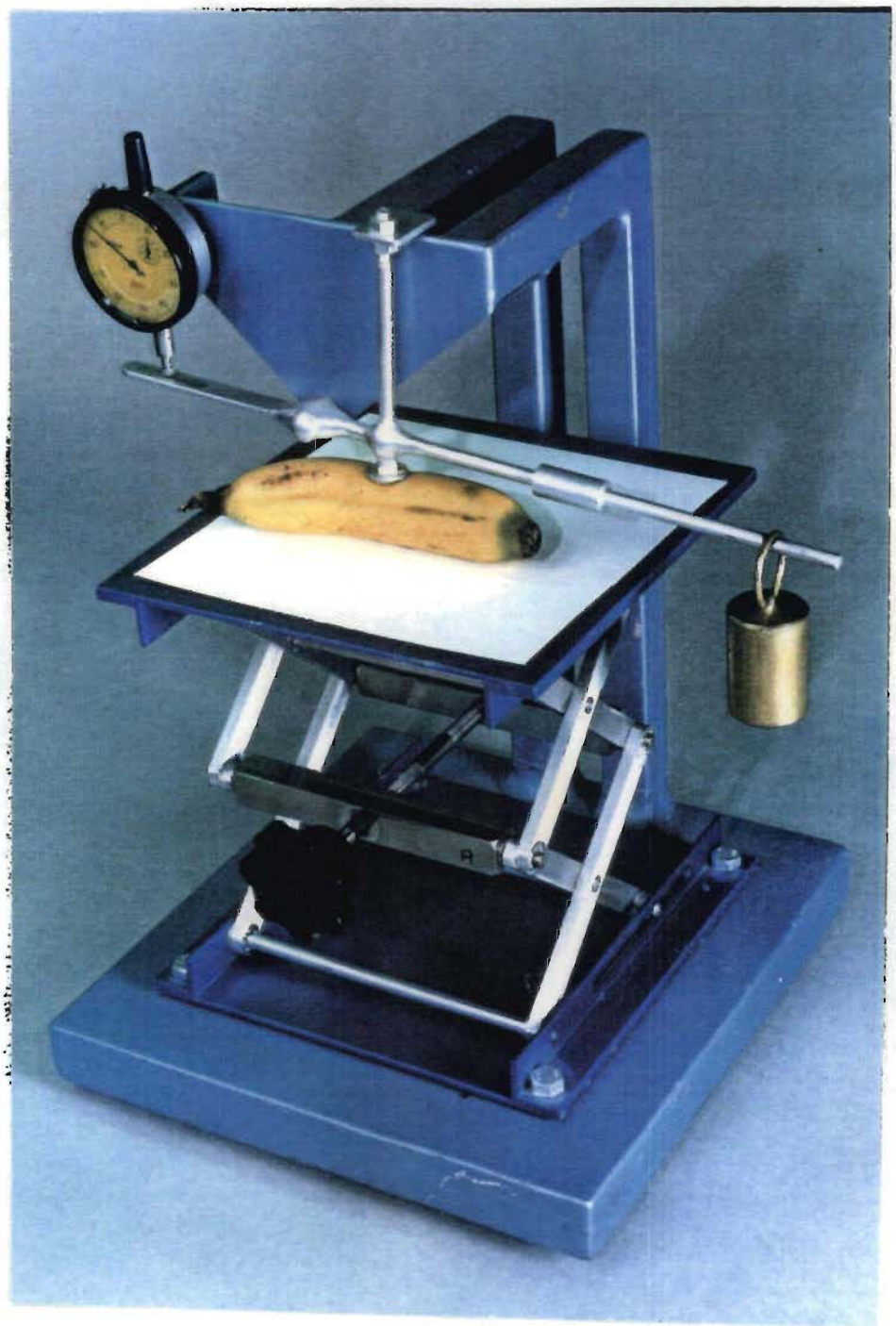


Figure 7: Device (firmometer) for measuring fruit firmness

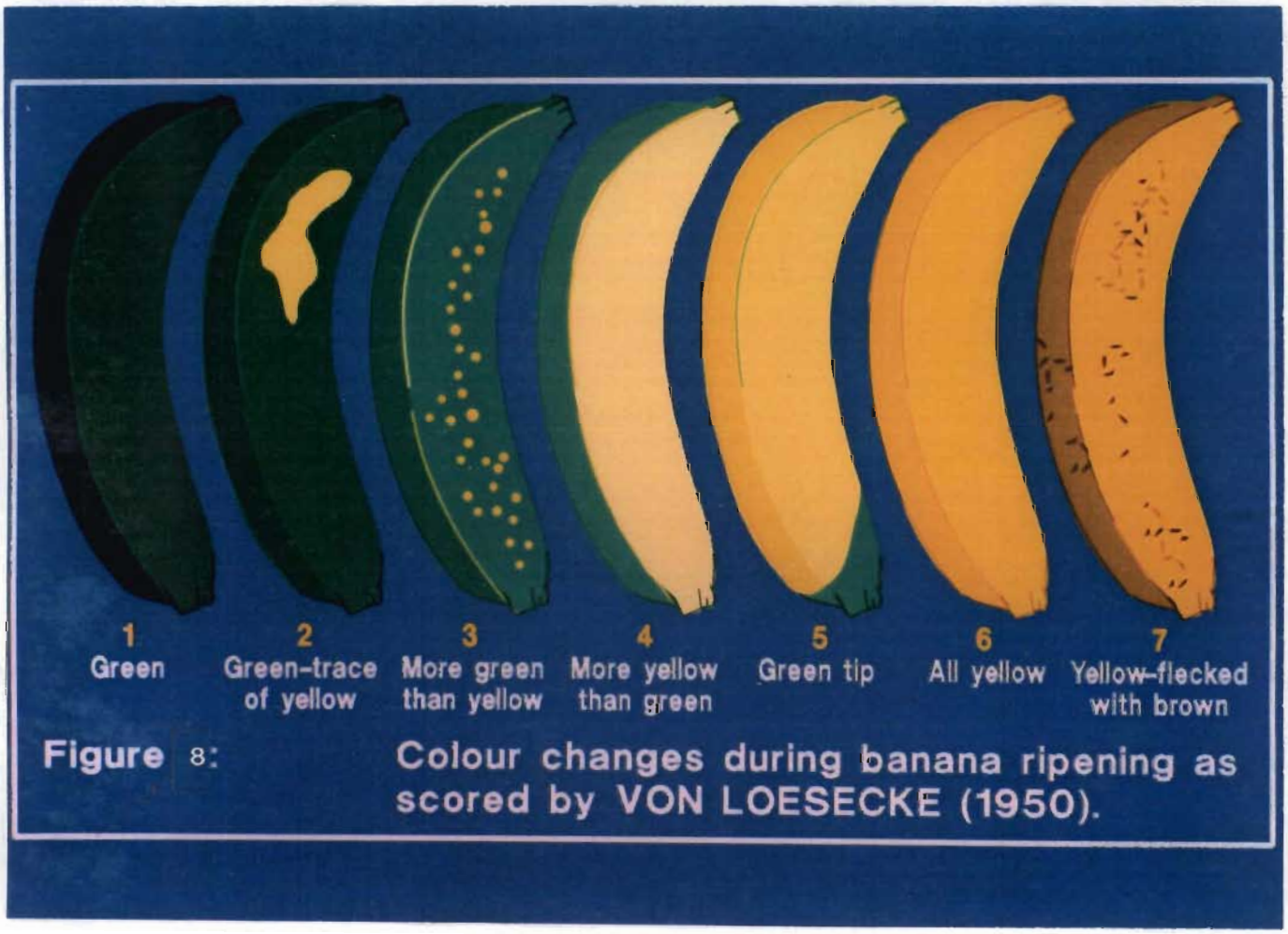


Figure 8: Colour changes during banana ripening as scored by VON LOESECKE (1950).

Chlorophyll determination

Exocarp samples were taken from bananas at different stages of ripening. Chlorophyll was extracted from each sample by homogenisation in 40 mℓ 80 % acetone. After centrifugation at 4 000 rpm for 5 min in a BECKMAN benchtop centrifuge, the supernatant liquid was decanted into a 100 mℓ flask and the residue was re–extracted with a fresh 30 mℓ aliquot of 80 % acetone. The homogenate was again centrifuged for 5 min and the supernatant was decanted into the flask. The final volume was then adjusted to 100 mℓ with 80 % acetone and total chlorophyll content was determined spectrophotometrically (BRUINSMA, 1961).

Sensory evaluation

Fourteen days after irradiation freshly harvested green bananas were treated with a gas mixture of a 1 000 $\mu\ell$ ethylene ℓ^{-1} air for 24 hours at 18 °C and 75 % relative humidity to induce ripening. Thereafter, fruits irradiated at different doses were kept in separate incubators until ripe. Fruits at colour stage 6 (all yellow) were selected for sensory evaluation by a panel of 12 judges. Each fruit was peeled, divided into portions and placed in paper cups coded with random three digit numbers. A set of three samples was presented to each judge. Each set contained two identical and one odd sample. In addition to choosing the odd sample, the judge stated whether he or she preferred the odd or duplicating sample. Three of these triangle tests were evaluated by each judge on three different days. The results of the triangle tests were analysed by chi–square using P–1/3 to establish significant differences and P–1/2 to establish preferences from correctly identified samples.

Statistical analysis

The statistical layout for all experiments was according to a randomized block design with 6 treatments and 3 replicates consisting of 10 fruits per replicate.

Where statistical analysis was undertaken on data (Chapters 3, 4 and 5) the data were subjected to the standard error test. In most cases the analyses were used to support the often marked differences between the treatments, therefore the method of analysis used was considered to be adequate for the purposes of the present study.

RESULTS

Figure 9 indicates that 0.2 kGy is the optimum radiation dose for Dwarf Cavendish bananas kept at 21 °C and 75 % relative humidity for 28 days. No differences could be observed in the texture and quality of these bananas when compared to the control non–irradiated fruit stored under similar conditions. However, radiation damage did occur at exposures higher than 0.2 kGy. This damage was manifested as undesirable uneven colour development, the development of nodular structures and black necrotic spots on the exocarp and, in the case of 1.0 kGy irradiated fruit, lengthwise necrotic splits in the exo– and meso–endocarp. The exocarp was brown and brittle, whereas the meso–endocarp of the banana fruits were mealy and elastic, with crumbly zones. The appearance of these fruit 28 days after gamma irradiation is shown in Figure 10.

The ripening of non–irradiated control fruit was accompanied by a climacteric rise in ethylene production in the postharvest ripening period (Figure 11). However, the amount of ethylene produced by irradiated fruit during the climacteric maximum differed from that of the control. The extent to which ethylene production was affected depended on the radiation exposure. The twofold increase in the climacteric ethylene production at 0.2 kGy could possibly be ascribed to stress–induced ethylene (LEVITT, 1980). The same could apply for the 0.4 and 0.6 kGy irradiated fruit (ABELES, 1973; LEVITT, 1980; BEYER, MORGAN and YANG, 1984). Bananas irradiated at 0.8 and 1.0 kGy however, showed a decrease in ethylene production during the same period. The ethylene climacteric for all treatments began on about day 16.

Figure 9: The effect of different doses of gamma irradiation on the physical appearance of ripening banana fruits 28 days after irradiation. Arrow indicates maximum tolerable radiation exposure

Figure 10: The effect of gamma irradiation (1.0 kGy) on the appearance of the peel and pulp of bananas 28 days after treatment.

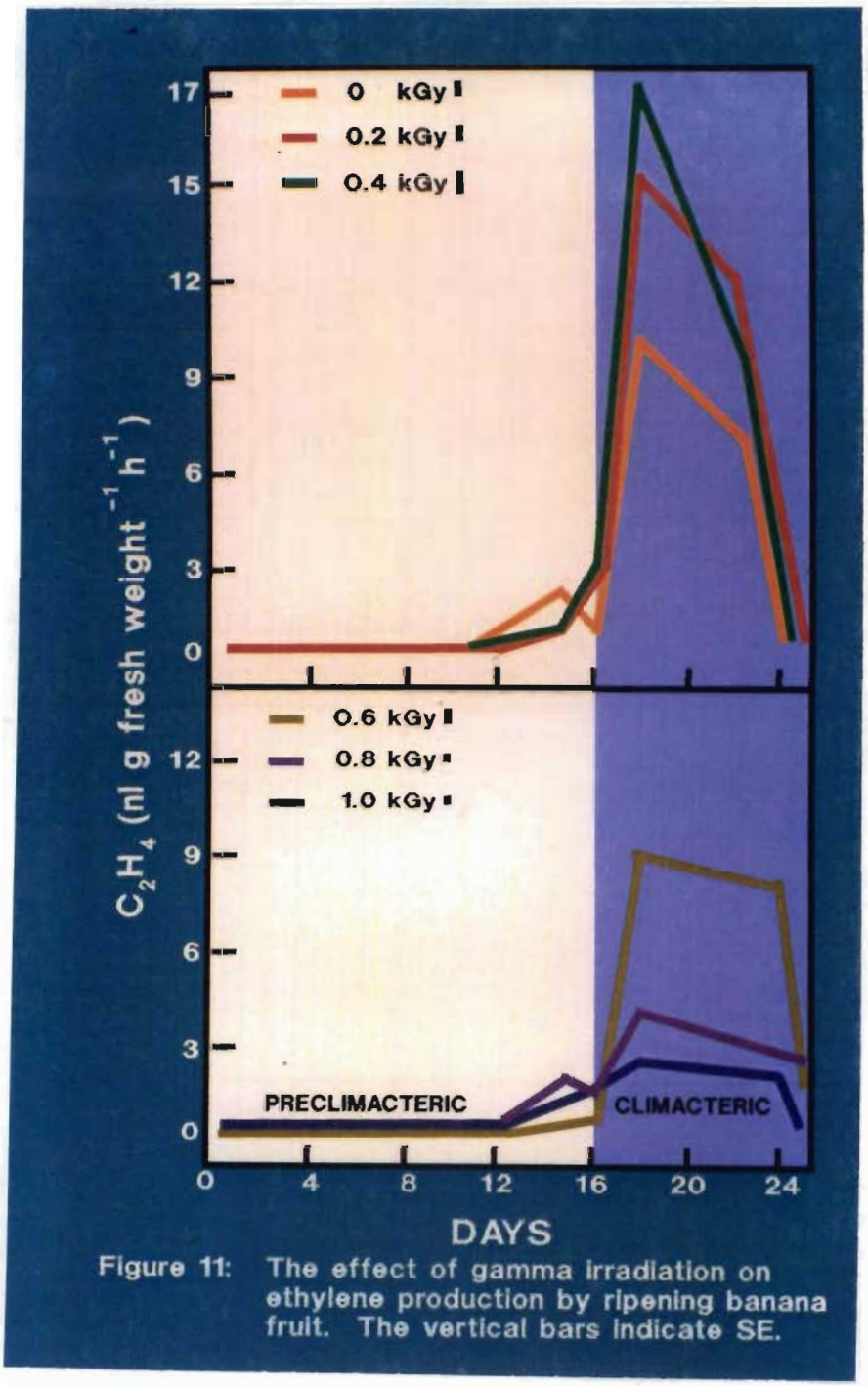


Figure 11: The effect of gamma irradiation on ethylene production by ripening banana fruit. The vertical bars indicate SE.

The respiratory climacteric of irradiated fruits are shown in Figure 12. There was no meaningful difference in the overall carbon dioxide (CO_2) production tendency of the control and 0.2 kGy irradiated fruits during the preclimacteric phase. The non–irradiated fruit showed the typical respiratory climacteric maximum. The climacteric maximum was still present but greatly reduced in the 0.2 kGy irradiated fruit. The 0.4 to 1.0 kGy irradiated bananas showed an immediate increase in CO_2 production directly after irradiation in the preclimacteric period. This was followed by a climacteric maximum intermediate between that of control and 0.2 kGy treated fruits. The total amount of CO_2 produced by 0.4 to 1.0 kGy irradiated fruit over the period observed was substantially higher than that of the control and 0.2 kGy irradiated bananas. It appears that the pattern of oxygen (O_2) consumption was similar to that of CO_2 production. However, irradiation had a marked effect on O_2 consumption during the ripening stage. This effect was visible even at doses of 0.2 kGy, where the maximum O_2 consumption peaked broadly at day 11 some 6 days before the control maximum (Figure 13). Higher doses of irradiation resulted in an increase in O_2 consumption during both the preclimacteric and climacteric phases. The respiratory rate reached a maximum for all treatments, with the exception of the 0.4 kGy irradiated fruit, on day 17. The control and 0.2 kGy irradiated bananas had the same respiratory quotient pattern (increase in respiration) during ripening. The respiratory quotient of the control and 0.2 kGy irradiated bananas was very close to unity at the climacteric (Figure 14). The 0.4 to 1.0 kGy irradiated fruit, however, started with a relatively high respiratory quotient and ended with a low

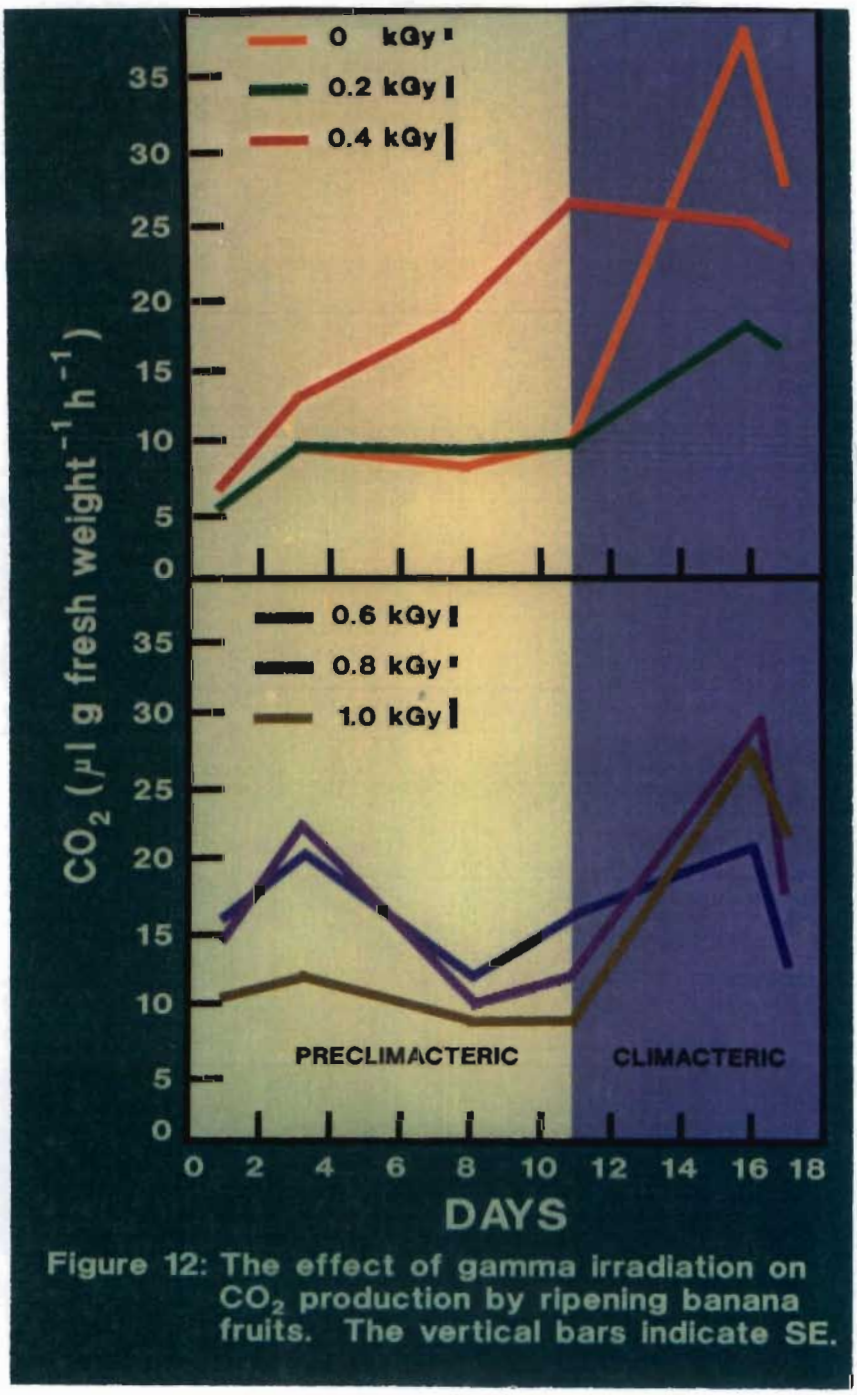


Figure 12: The effect of gamma irradiation on CO_2 production by ripening banana fruits. The vertical bars indicate SE.

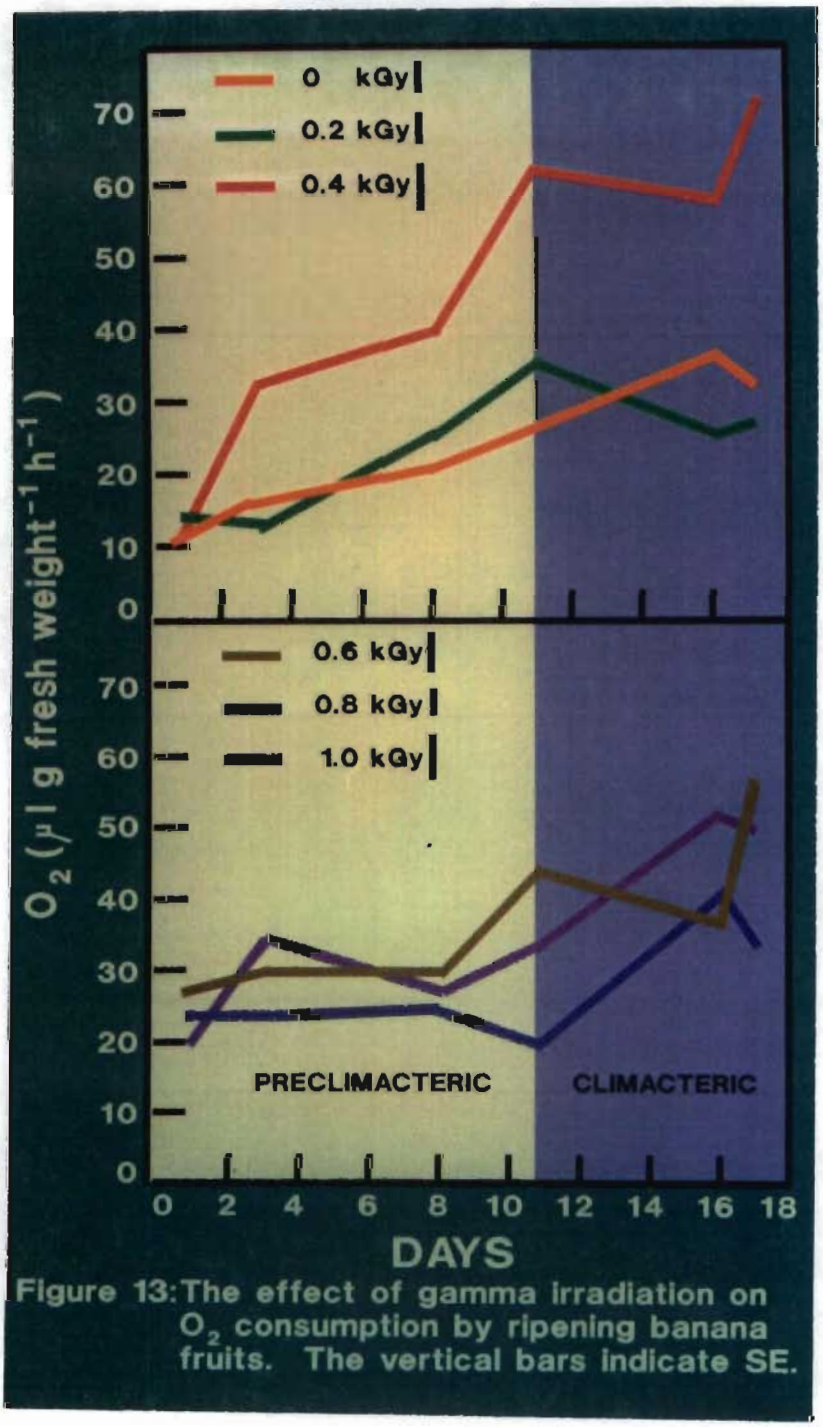


Figure 13: The effect of gamma irradiation on O_2 consumption by ripening banana fruits. The vertical bars indicate SE.

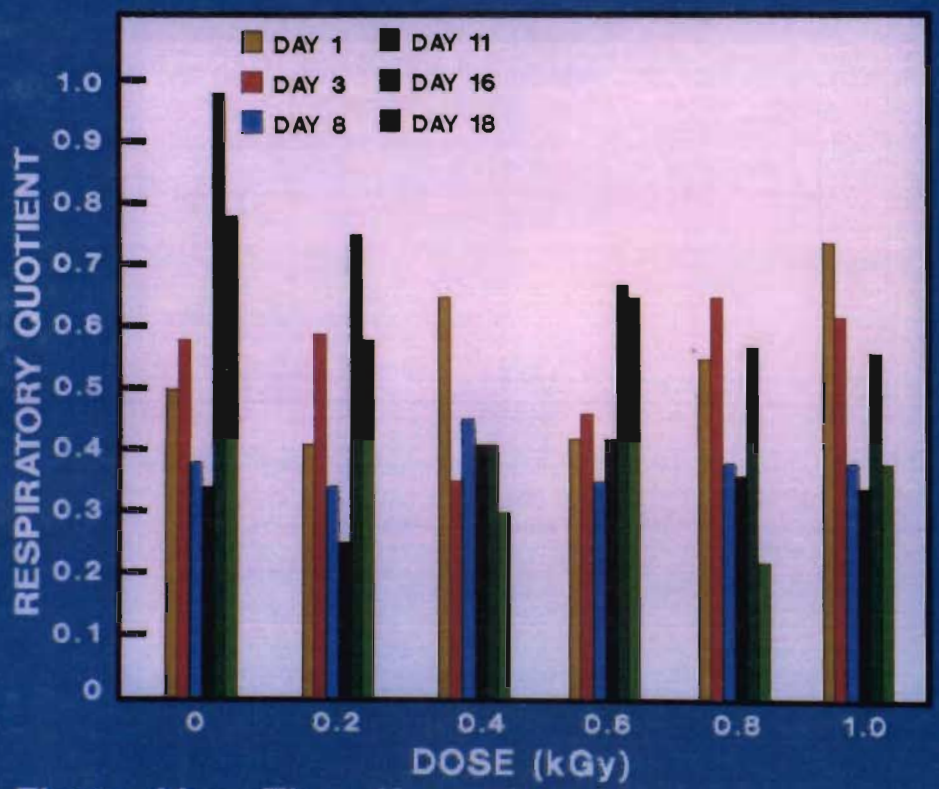


Figure 14: The effect of gamma radiation on the respiratory quotient of ripening banana fruits.

respiratory quotient during the respiratory climacteric. The respiratory quotient gives an indication of the type of substrate that is being metabolised during respiration. At a low respiratory quotient lipid metabolism is possible. A respiratory quotient of 1 represents the complete oxidation of glucose, whereas a respiratory quotient of higher than 1 represents organic acid metabolism (WILLS, McGLASSON, GRAHAM, LEE and HALL, 1989).

Treatment of the fruit with ethylene for 24 hours, 14 days after irradiation, only slightly affected the rate of colour change and firmness in bananas irradiated at doses ranging from 0.2 to 0.4 kGy. Bananas irradiated with 0.6 kGy showed a 50 % reduction in ethylene sensitivity, whereas at 0.8 and 1 kGy the loss of sensitivity was even more pronounced (Figures 15 and 16).

The banana fruit firmness (meso–endocarp) of the 0.2 kGy irradiated fruit over the period of observation was generally higher than that of the other treatments up to day 16 (Figure 17). After 14 days the control fruits were yellow (colour score of 6.25), with a firmometer reading of 7.92. The colour of the 0.2 kGy irradiated bananas was more green than yellow (colour score of 3.32), with a firmometer reading of 4.62. The 0.4 kGy irradiated fruit were yellow with green tips (colour score of 3.95), with a firmometer reading of 5.66. Fruits irradiated at 0.6 kGy were more yellow than green (colour score of 4.65), with a firmometer reading of 4.30. The 0.8 to 1.0 kGy irradiated fruit were bronze–brown–black (more bronze–brown at 1.0 kGy), with a firmometer reading of 9.52 and 8.15 (colour score 6.10 and 6.20) respectively.

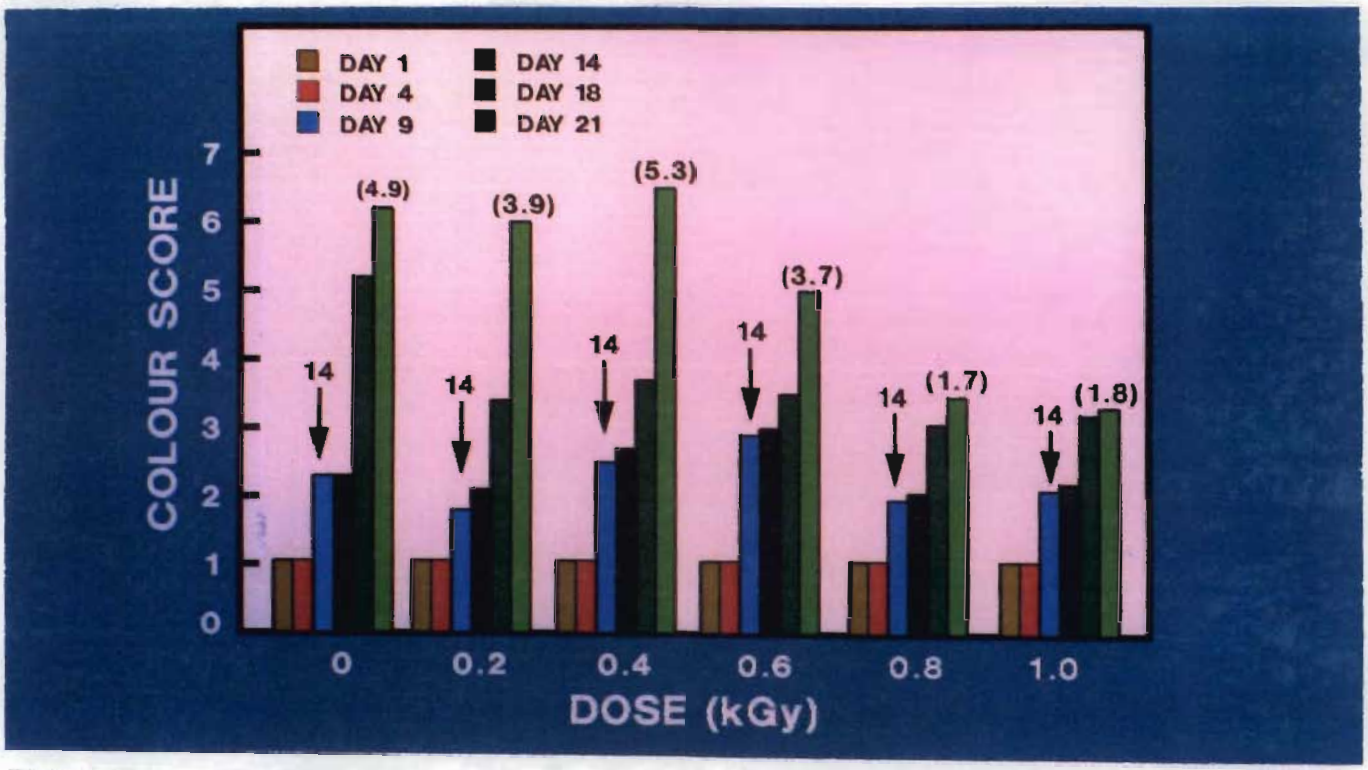


Figure 15: The effect of ethylene on colour and firmness in irradiated bananas. Arrows indicate the time of ethylene treatment. The firmometer scores are indicated in brackets.

Figure 16 The effect of ethylene on colour changes in irradiated banana fruit. Ethylene was administered 14 days after irradiation. Arrow indicates the maximum acceptable dose. Fruits were exposed to a 1,000 $\mu\ell$ of C_2H_4 per litre of air for

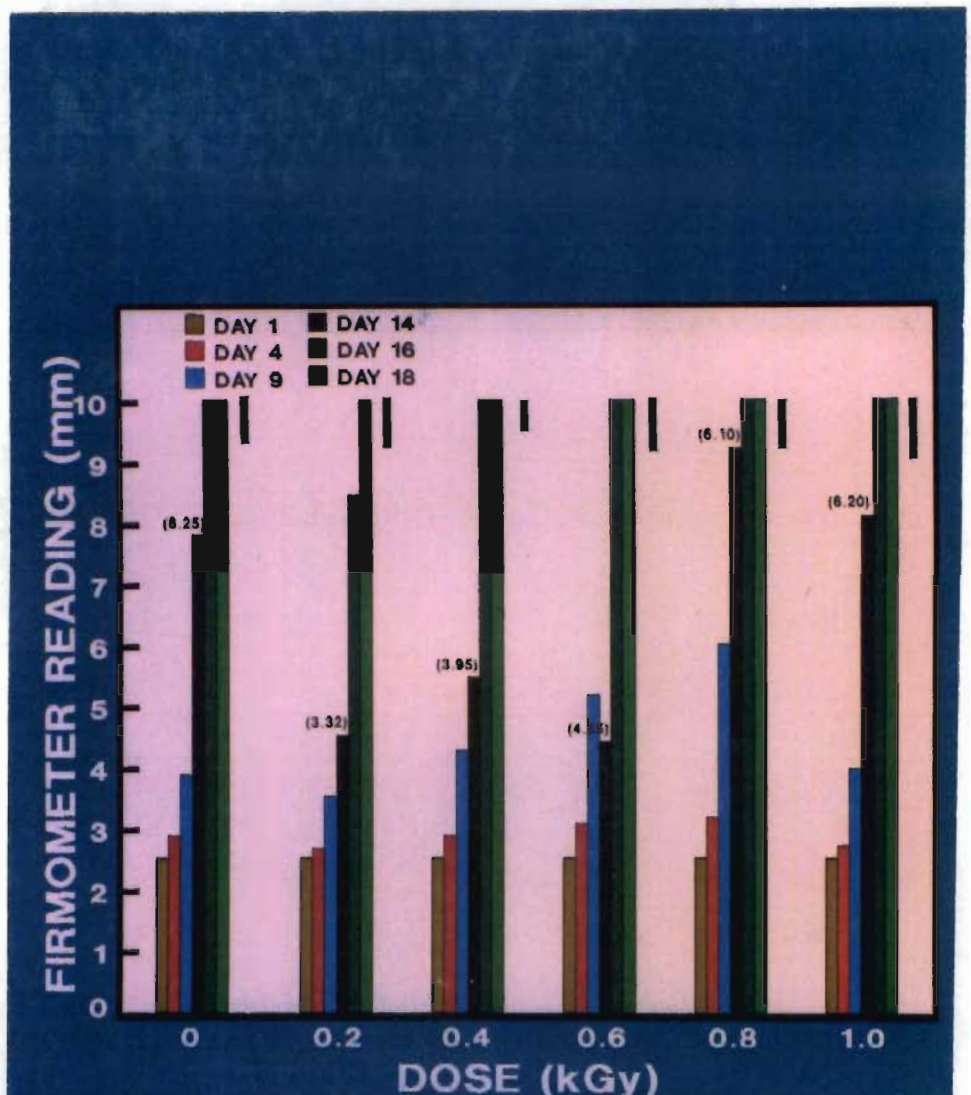


Figure 17: The effect of gamma irradiation on structural changes of banana during ripening. The vertical bars indicate SE. Colour score in brackets.

Irradiation of bananas at doses up to 0.6 kGy resulted in a slower change in the colour of the exocarp from green to yellow (Figure 18). Although the rate of colour change at 1.0 kGy irradiated bananas were indistinguishable from those of the control, irradiation at this high exposure resulted in the formation of an undesirable yellow–bronze–black colour. Degreening of the exocarp was generally accompanied by a rapid loss in chlorophyll content (Figure 19). However, in the 0.2 kGy irradiated fruit very little change in chlorophyll content could be observed during the first 20 days after irradiation.

No significant differences could be detected between the non–irradiated and irradiated bananas, with respect to texture, flavour and aroma (quality attributes) at doses up to 0.6 kGy (Table 2).

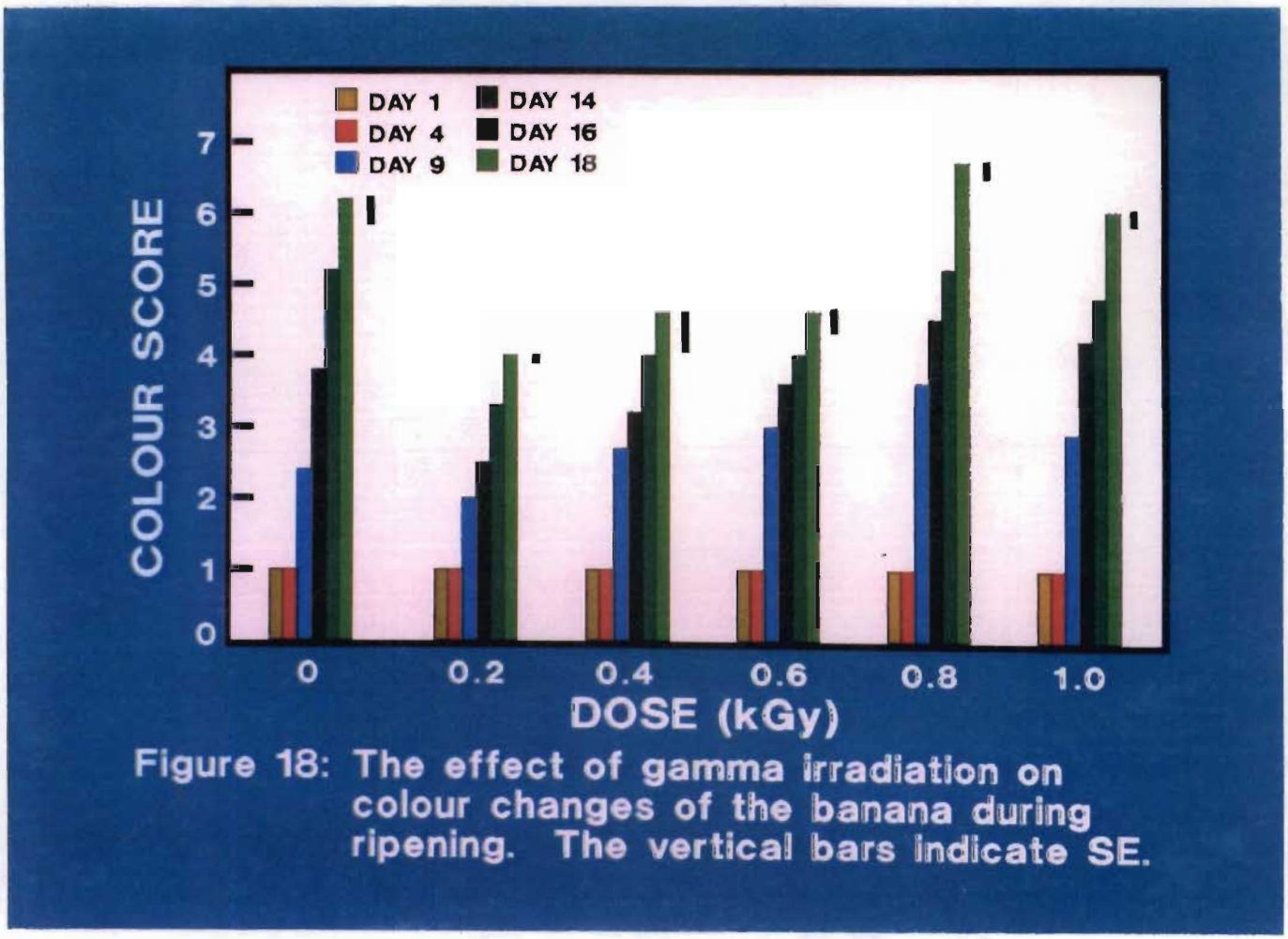


Figure 18: The effect of gamma irradiation on colour changes of the banana during ripening. The vertical bars indicate SE.

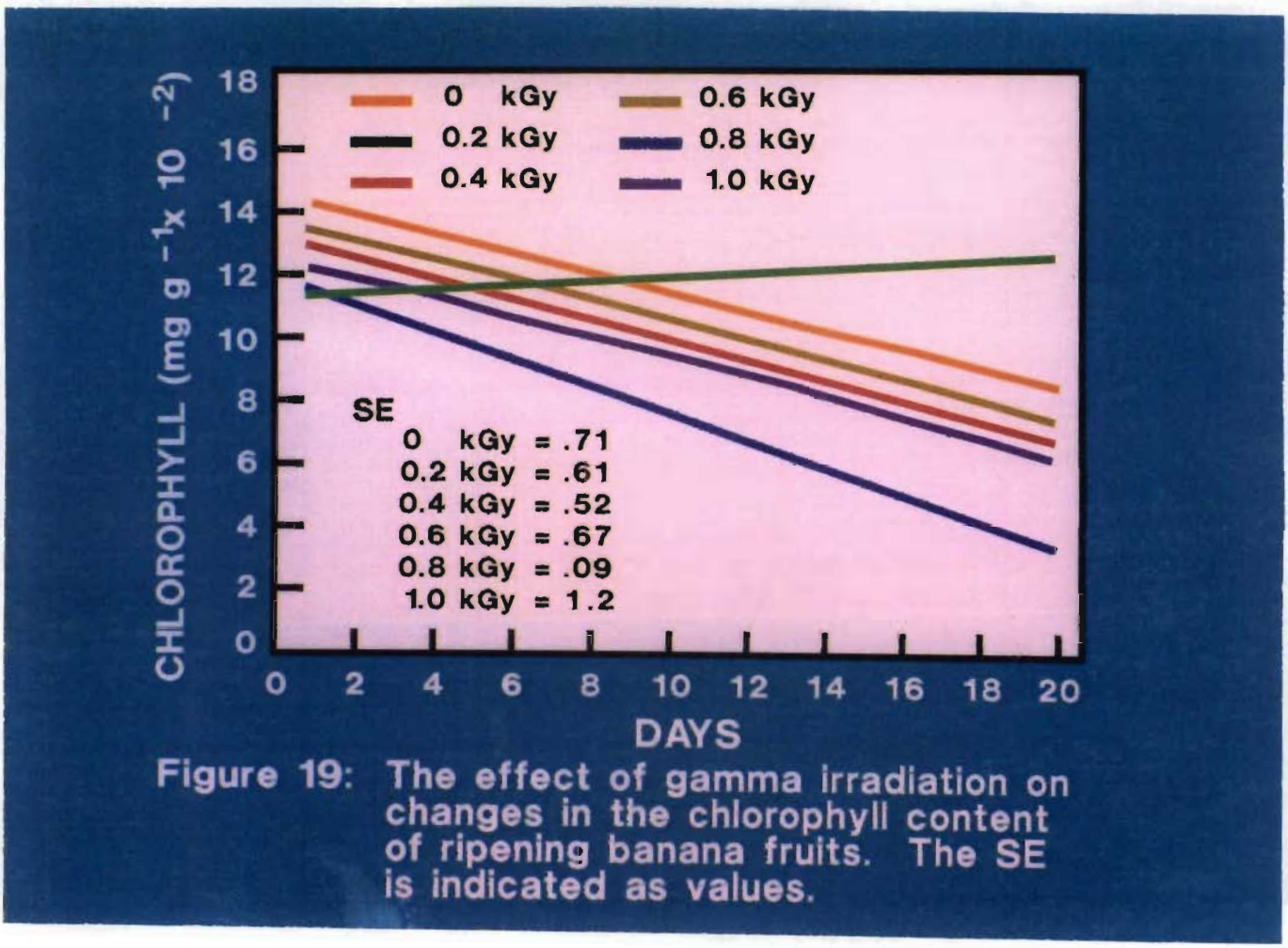


Figure 19: The effect of gamma irradiation on changes in the chlorophyll content of ripening banana fruits. The SE is indicated as values.

Table 2: The effect of gamma irradiation on the sensory quality of bananas.

COMPARISON	CORRECT IDENTIFICATION OF ODD SAMPLE	PREFERENCE
0 kGy vs. 0.2 kGy	2 of 8 judgments	0 kGy = 5; 0.2 kGy = 3
0 kGy vs. 0.4 kGy	2 of 8 judgments	0 kGy = 5; 0.4 kGy = 3
0 kGy vs. 0.6 kGy	1 of 8 judgments	0 kGy = 4.5; 0.6 kGy = 3.5
0 kGy vs. 0 kGy (C_2H_4)	4 of 8 judgments	0 kGy = 6; kGy (C_2H_4) = 3
0.2 kGy vs. 0.4 kGy	2 of 8 judgments	0.2 kGy = 4; 0.4 kGy = 4
0.2 kGy vs. 0.6 kGy	3 of 8 judgments	0.2 kGy = 5; 0.6 kGy = 3

Based on the chi-square using P-1/3 as described in Materials and Methods. Values reported are the average of three independent estimations. No significant difference was found between the treatments.

DISCUSSION

Gamma rays cause ionizations when they collide with molecules (CASARETT, 1968), and three distinct stages of progression have been identified based on reaction times (LAWRENCE, 1971). The first stage represents the actual physical collision and is very short ($\leq 10^{-13}$ sec). The variety of reactions which follow in the second stage are essentially rapid chemical reactions ($\leq 10^{-6}$ sec). It is during this second stage that macromolecular radicals and free radicals such as O_2^- and its toxic derivatives ($HOO^{\cdot}$, H_2O_2 and $OH^{\cdot}$) may be formed (FRIMER, 1986). This second stage leads to the third or metabolic stage of reactions with reaction times of between $10^3 - 10^6$ seconds. During this time there is the greatest danger of the superoxide anion radical products and the potential to attack DNA, membrane lipids and other cell components (FRIDOVICH, 1978). In multicellular tissues such as fruits, the results are seldom so clear cut as to result in death (AKAMINE and MOY, 1983; POLLARD, 1983). In many fruits the cells comprising tissues discontinue cell division before harvest so that the consequences of DNA damage may be less severe than in tissues with rapidly dividing cells. Furthermore, during the metabolic stage the maximum irradiation exposure will do little harm to the cell as chemical changes will be minimal. Most types of molecules are replicated many times within each cell, so that the loss of function in a few of them will be unimportant, while in other cases their function will be unimpaired by the chemical alteration, or the non–essential damage can be bypassed or even repaired. In a few instances, however, irreparable damage will occur within certain critical structures such as cell membranes, the integrity of which is essential to the cell. Serious metabolic disability may also result. When considering the above rationale the maximum radiation exposure for the Dwarf Cavendish banana appears to be 0.2 kGy. This treatment does not appear to damage the physiological

status and nor does it affect the quality of the fruit. If the radiation exposure of 0.2 kGy is exceeded, colour change are seen with eventual cell death in the exo– and meso–endocarp of the banana. The higher the dose the more severe the damage. When the maximum dose is exceeded peel browning occurs in the banana. This is ascribed to an increase in polyphenoloxidase (E.C. 1.3c.3.1) activity as a result of cell membrane damage with consequent contact between enzymes and substrates (THOMAS and NAIR, 1971). Polyphenoloxidase reacts with dopamine (the primary substrate for browning reactions in the peel of the banana) in the exocarp (epidermis cells) of the fruit (PALMER, 1971), with the eventual formation of melanin pigments (GRIFFITHS, 1959; KARLSON, 1970). The radiation–induced reaction is known as phytotoxicity, and may be used as an indicator of overdose. It takes 7 days for the above–mentioned radiation damage to manifest itself on the quality attributes of the banana. This time falls within the limits (10^3–10^6 sec) for the development of radiation induced damage (or no damage as is the case at 0.2 kGy) as described by LAWRENCE (1971). The principles of radiation biology are thus relevant with respect to all the parameters described and outlined for senescence, ultrastructural changes, sugar metabolism and enzymic studies of the banana fruit.

The climacteric pattern of ethylene production and respiration rate can be used as a measure of physiological status or degree of ripening during the postharvest life of bananas (MAXIE, AMEZQUITA, HASSAN and JOHNSON, 1968). During the normal development of harvested bananas the initiation of the autocatalytic climacteric rise in ethylene production may be taken as the first indication of ripening. Although this is true for non–irradiated bananas the same may not necessarily apply to irradiated fruit. When the rate of ripening in the present study is examined in relation to changes in other processes, it became evident that

the timing of the climacteric rise in ethylene production was not related to the onset of the other ripening parameters under ethylene control, particularly in fruit exposed to radiation doses of 0.2 to 0.6 kGy. Irradiation clearly did not affect the timing of the climacteric maximum in ethylene production during the postharvest period (STRYDOM and WHITEHEAD, 1990). This can be due to the fact that bananas were of the same physiological status (fully mature) when harvested for experimentation. However, the respiratory (THOMAS, DHARKAR and SREENIVASAN, 1971) and ethylene climacteric (AMEZQUITA, 1965; MAXIE, AMEZQUITA, HASSAN, JOHNSON, 1968) of 70 to 80 % mature irradiated and non–irradiated banana fruits occurred later in the climacteric phase than in fully mature irradiated and non–irradiated fruit (Figure 20). The delay of the ethylene and respiratory climacterics is therefore possibly the result of prolonging the maturity phase of the banana fruit and not the result of lowering ethylene sensitivity by means of gamma irradiation. BRUINSMA (1983) stated that the control of ethylene biosynthesis and sensitivity is the ultimate way in regulating the shelf life of climacteric fruit. It would also be more feasible for marketing reasons to irradiate fully mature fruit. Fruit does not "mature" after harvest; an immature fruit may ripen but will be of low quality (MAXIE and ABDEL–KADER, 1967).

The rate of ethylene production at the climacteric maximum differs widely with the different irradiation treatments used in this study. Irradiation at 0.2 and 0.4 kGy resulted in a two–fold increase in the rate of ethylene production at the climacteric which could possibly be due to stress–induced ethylene. However, irradiation at exposures in excess of 0.6 kGy resulted in a suppression of ethylene production during this phase (STRYDOM and WHITEHEAD, 1990). Many other aspects have been found to promote ethylene synthesis. These include gamma irradiation,

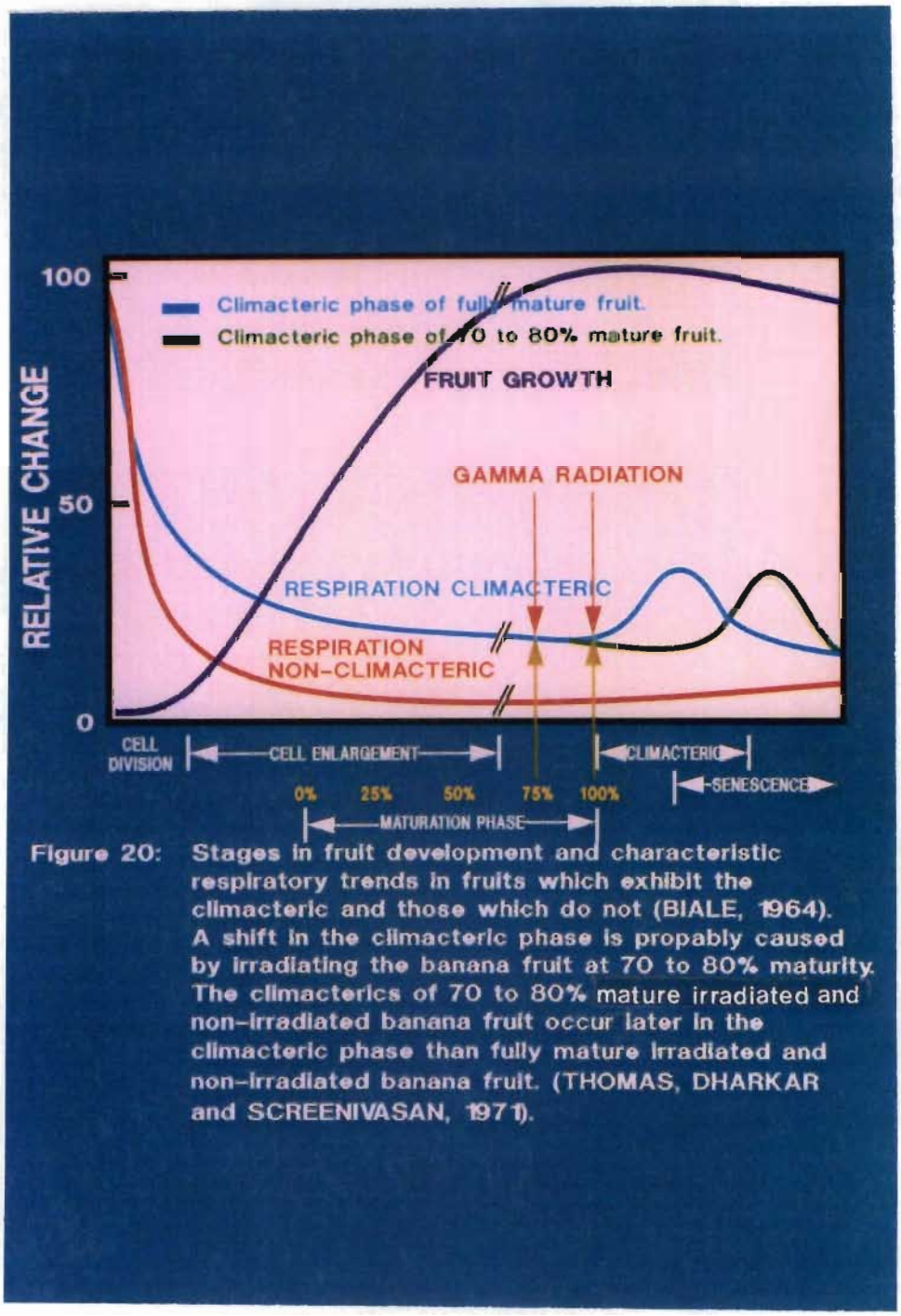


Figure 20: Stages in fruit development and characteristic respiratory trends in fruits which exhibit the climacteric and those which do not (BIALE, 1964). A shift in the climacteric phase is propably caused by irradiating the banana fruit at 70 to 80% maturity. The climacterics of 70 to 80% mature irradiated and non-irradiated banana fruit occur later in the climacteric phase than fully mature irradiated and non-irradiated banana fruit. (THOMAS, DHARKAR and SCREENIVASAN, 1971).

cytokinins, abscisic acid and numerous stress phenomena (ABELES, 1973). Stress–ethylene appears to act as a "second messenger" to communicate the effect of stress to the plant in a way that allows a response. Stress–ethylene may also be involved in the production of phytoalexins, wound healing and increased disease resistance. Stress as well as the natural ripening stimuli induces the synthesis of ACC synthase which in turn causes the accumulation of ACC from SAM in the cell cytoplasm and the onset of stress–ethylene production (BEYER, MORGAN and YANG, 1984). Stress–induced ethylene by gamma irradiation could therefore be part of the mechanisms by which cells can repair or bypass damage. The stereospecific conversion of ACC (the immediate precursor of ethylene) is a membrane–related process (IMASEKI, 1986). In over–ripe fruit, considerable ACC accumulation can occur, possibly because of the inactivation of the membrane–bound ethylene forming enzyme during the gradual loss of membrane integrity, which may contribute to the decrease and eventual termination of ethylene production (HOFFMAN and YANG, 1980). The results of studies on the effect of ionizing radiation on membrane permeability indicated that high doses of radiation may result in a loss of membrane semi–permeability in various plant tissues (STADELMANN, 1968; WATTENDORF, 1970). Irradiation at high doses (> 0.4 kGy) resulted in extensive damage as indicated by the development of necrotic areas on the exocarp of bananas. It has been proposed that this may account for the observed decrease in the rate of ethylene production during the climacteric phase (STRYDOM and WHITEHEAD, 1990).

The preclimacteric rise in CO_2 production following irradiation at the higher dosages (>0.2 kGy) was immediate and persisted for some days. The corresponding stimulation of CO_2 production following irradiation with the processes involved in

the climacteric phase needs further elucidation. It could be expected that an acceleration of the climacteric will be correlated with the acceleration of the ripening process which occurs, as for example, in peaches and nectarines (MAXIE, JOHNSON, BOYD, RAE and SOMMER, 1966). Furthermore as the rate of CO_2 production at these higher dosages was increased during the preclimacteric in the bananas (a stage when the ripening processes can be inhibited), it cannot be assumed that the immediate stimulation of respiratory activity is due to processes which control the climacteric phase. A hypothesis was proposed that could link the stimulated CO_2 production at these higher exposures with the onset of senescence and accelerated ripening, by analogy with the effect of NAD, which induces loss of phosphorylative control by the electron chain (CLARKE and HAYES, 1965). Evidence against this hypothesis was, however, obtained with pear mitochondria (ROMANI, YU, KU, FISCHER and DEHGAN, 1968). This clearly demonstrated the resistance of the phosphorylative and oxidative processes in fruit mitochondria to radiation (ROMANI, YU, KU, FISCHER and DEHGAN, 1968). A second hypothesis postulated that the stimulated respiratory exchange is related to repair of damage induced by these higher dosages. In support of this hypothesis, an increase in protein synthesis was observed in isolated organelles from irradiated pre–climacteric tissues (ROMANI and FISHER, 1966). Earlier work had shown that the protein content of irradiated pears increased during storage (CLARKE and FERNANDEZ, 1961). There is however, no direct evidence that this excess protein may be involved in repair processes. In post–climacteric pears the ability to repair radiation–damaged mitochondria was lost (ROMANI, YU, KU, FISCHER and DEHGAN, 1968). Neither of these two hypotheses explains the inhibition at the succinic dehydrogenase step of the tricarboxylic acid cycle (Figure 21). Evidence for the sensitivity of this particular step to radiation has been obtained

for carrot and apple (MASSEY and BOURKE, 1967). They reinvestigated the findings of ROMANI and BOWERS (1963) and ROMANI (1964) which indicated a general stimulation of both O_2 consumption and CO_2 production during the irradiation period (0.5 kGy), subsiding to near–normal rates shortly following the cessation of treatment. These studies with carrot tissues revealed a general reduction in all metabolic activity of the irradiated system and accumulation of the substrates glucose and pyruvate. This however, could not explain the observed increase in the respiratory rate of the tissue following irradiation. Further experimentation indicated that irradiation stimulated the catabolism of acetate to CO_2 and that the complete tricarboxylic acid cycle was operative. The increase in respiratory CO_2 was shown to be proportional to the irradiation dosage. It can therefore be deduced that the effect of radiation upon acetate metabolism explains the increased CO_2 production. It can be postulated that the normal energy source of the tissue stems from tricarboxylic acid activity and that the radiation exposures applied stimulated this pathway, resulting in an increased rate of O_2 consumption and CO_2 production (Figure 21). All reactions therefore associated with glycolysis are decreased by the exposure to radiation, while all associated with the tricarboxylic acid cycle and acetate catabolism are increased. Anabolic reactions were also reduced by irradiation. The change in the respiratory processes during the preclimacteric ripening of banana irradiated at higher exposures (> 0.2 kGy) could therefore possibly be due to similar causes as suggested for carrot and apple tissue.

The suppression of CO_2 production during the climacteric as well as the changes

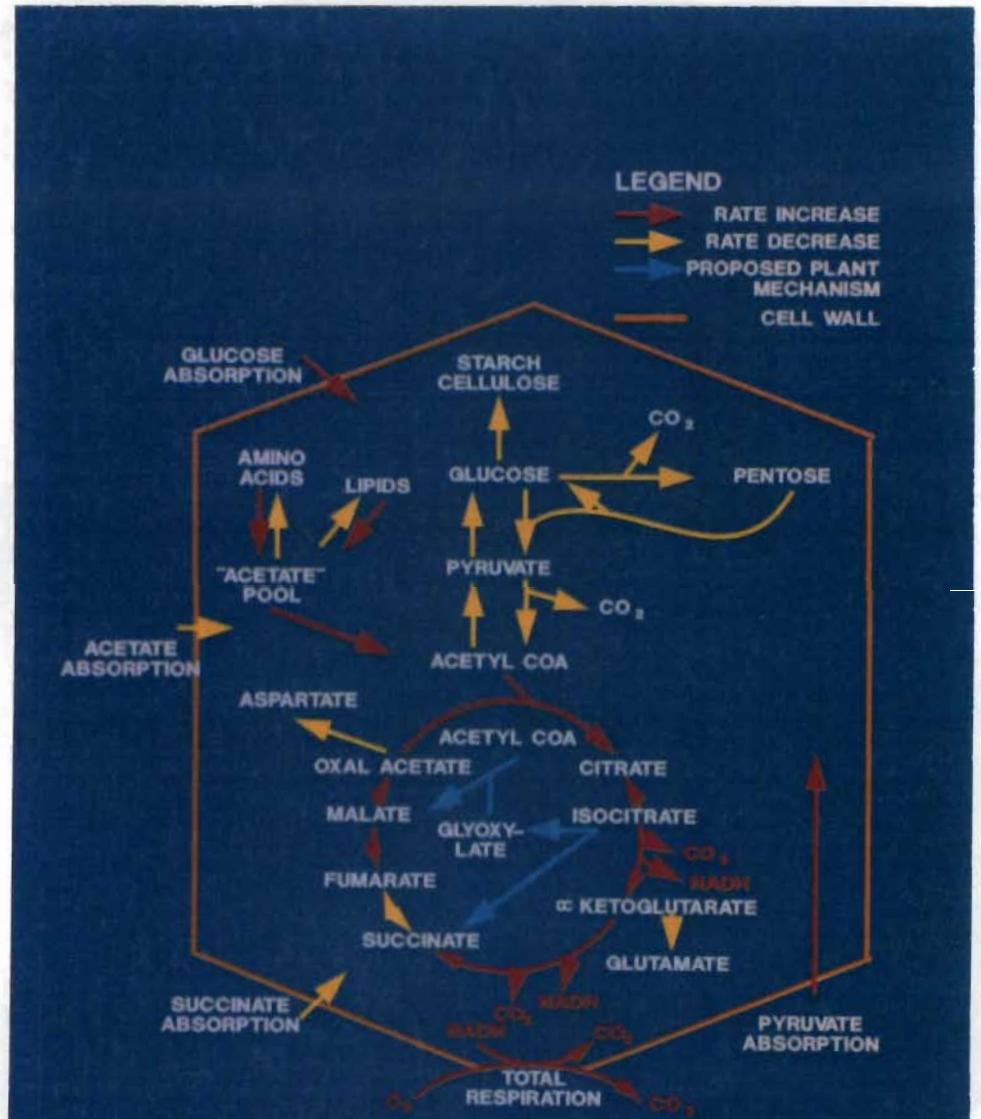


Figure 21: The effect of gamma irradiation on the intermediary metabolism of carrot slices (MASSEY and BOURKE, 1967).

observed in the levels of O_2 consumption during the post–irradiation period suggest that irradiation resulted in considerable changes in the metabolic processes normally used as indicators of ripening (STRYDOM and WHITEHEAD, 1990). It is well–known that suppression of the respiratory climacteric is associated with an increase in the longevity of various climacteric plant tissues (SOLOMOS, 1983). Since irradiation with doses up to 1.0 kGy resulted in a marked decrease in CO_2 production during the climacteric, it may be concluded that irradiation may result in an increase in the postharvest life of bananas. However, when CO_2 production by irradiated banana tissues is compared with that of the control in relation to O_2 consumption, it becomes evident that irradiation resulted in considerable changes in the respiratory metabolism of the tissues that cannot be accounted for by a simple suppression of respiratory processes. The suppression of the climacteric in CO_2 production in irradiated fruit was not accompanied by a similar suppression in O_2 consumption. Irradiation at 0.2 kGy resulted in a climacteric pattern in O_2 consumption during the post–irradiation period similar to that observed in non–irradiated fruit. However, the climacteric maximum in O_2 consumption took place approximately 6 days earlier than the control. Higher levels of irradiation resulted in an increase in O_2 consumption during both preclimacteric and climacteric stages, indicating an early increase in catabolic activity during the preclimacteric stage and a change in the respiratory processes operating during the postharvest period (STRYDOM and WHITEHEAD, 1990). The respiratory quotient studies indicate the possibility of lipids being respired, possibly as a result of membrane degradation. The respiratory quotients support the idea that the preclimacteric and climacteric changes in respiration and metabolism caused by exposures exceeding the maximum tolerable dose of 0.2 kGy are due to damage. It has been suggested that the rise in respiration during fruit ripening is the result of ethylene action (BIALE,

1960a,b; RHODES, 1970; McMURCHIE, McGLASSON and EAKS, 1972). However, in the case of irradiated fruit no clear correlation could be obtained between respiration and the rate of ethylene production, indicating that irradiation resulted in changes in the normal mechanisms of ethylene action in banana fruit tissues (STRYDOM and WHITEHEAD, 1990).

BOROCHOV and FARAGHER (1983) concluded that both ethylene and ultraviolet–irradiation promote a sequence of reactions in flower tissues similar to those of natural senescence. These are changes in membrane properties, loss of membrane integrity, membrane leakage, cell water loss, and petal inrolling. However, ultraviolet–irradiation initiates reactions which are independent of those which ethylene initiates, in as much as blocking the primary site of ethylene action with silver thiosulfate did not inhibit senescence caused by ultraviolet–irradiation. The site of action of ultraviolet–irradiation is possibly lipids or protein components of membranes. Ultraviolet–induced lipoxidation (WRIGHT, MURPHY and TRAVIS, 1981) and protein cross–linkage (BRAUN and BUCKNER, 1981) has been noted in membranes of wheat roots and flower tissues. These changes may also be induced by gamma irradiation. It has furthermore been demonstrated that there is a substantial increase in the level of peroxide and presumably of other active O_2 species during natural fruit ripening and in senescing leaves. This change in peroxide levels is not one which is a result of the ageing process but rather one which induces it (LESHEM, HALEVY and FRENKEL, 1986). Therefore the rate at which senescence proceeds may be influenced by varying the level of tissue peroxides. In ripening fruits the rise in peroxide level is accompanied by a parallel rise in ethylene concentration. Furthermore, an application of ethylene stimulates peroxide

formation in both fruit and potato tubers (LESHEM, HALEVY and FRENKEL, 1986). This increase is coincident with the respiratory rise, which leads one to conclude that the role of ethylene is to stimulate those systems which utilize O_2. From the results of this study it appeared that gamma–irradiation and not ethylene, stimulate O_2 utilization at the higher doses applied (0.4 to 1 kGy). Therefore it is possible that senescence is not initiated by ethylene in the 0.4 to 1.0 kGy irradiation fruit but possibly by elevated levels of toxic active oxygen species presumably as a result of enzymic activity (HALLIWELL, 1982) and gamma irradiation. This is in agreement with the conclusion of COOK and VAN STADEN (1988) that ethylene does not initiate the senescence process in carnation flowers but accelerates the irreversibility thereof. It is thus possible that ethylene action developed through evolution and cultivation to assist and accelerate ripening of climacteric fruits.

Up to 0.6 kGy there was little effect on the pulp (meso–endocarp) of the bananas. In the range of 0.8 to 1.0 kGy–treated fruits, there was a marked loss in texture. THOMAS, DHARKAR and SREENIVASAN (1971) reported that Dwarf Cavendish bananas irradiated to doses of 0.3 kGy to 2 kGy failed to reveal any increases in reducing sugars, suggesting that in bananas it is unlikely that starch degradation by radiation contributes much to a loss in texture. However, the possibility of random hydrolytic cleavages of starch molecules to fragments of lower molecular weight like dextrins and oligosaccharides cannot be ruled out (THOMAS, DHARKAR and SREENIVASAN, 1971). The data from previous studies with other fruit suggest that most of the textural changes at higher radiation exposures ($\geq$ 0.5 kGy) may be

attributed to depolymerization and conversion of protopectin into soluble pectic substances (KERTESZ, GLEGG, BOYLE, PARSONS and MASSEY, 1964; SOMOGYI and ROMANI, 1964). Of further interest is that the textural and colour changes in the 0.2 to 0.6 kGy–treated banana fruit showed a clear temporal separation from each other.

Colour changes during ripening of the exocarp from green to yellow is the result of degreening of the tissue, caused mainly by a loss in chlorophyll content during the ripening process. The levels of carotenoids in the exocarp do not change significantly during the postharvest period (VON LOESECKE, 1950; PALMER, 1971). However, SEYMOUR, JOHN and THOMPSON (1987) reported that ripening of bananas at 35 °C resulted in increased levels of carotenoids in the exocarp of the fruit. It was concluded that "the effect of elevated temperature on peel colour development is mediated through a suppression of chlorophyll breakdown" (SEYMOUR, JOHN and THOMPSON, 1987). Irradiation of bananas at exposures ranging from 0.4 to 1.0 kGy resulted in a loss of chlorophyll similar to that found in control fruit. However, in 0.2 kGy–irradiated fruit very little change in chlorophyll content was observed during the first three weeks following irradiation. The change in colour to yellow–green could be the result of a stimulation of carotenoid synthesis rather than chlorophyll loss, as was the case in bananas ripened at elevated temperatures, and may not be a definitive indicator of ripening (SEYMOUR, JOHN and THOMPSON, 1987).

Ripening of banana fruit is associated with a sharp rise in respiration and ethylene evolution, massive breakdown of starch, concomitant with a rise in free sugars, total degradation of chlorophyll, and softening (PALMER, 1971). None of the above

ripening indicators, except ethylene production, respiration and free sugar accumulation occurred in the 0.2 kGy irradiated fruit, 28 days after irradiation. However, when bananas were exposed to exogenous ethylene 14 days after irradiation fruits treated with doses of 0.2 and 0.4 kGy slowed the overall rate of colour change and firmness (whole fruit). Irradiation thus increased the concentration of ethylene required to elicit a response (fruit ripening), indicating that gamma irradiation at 0.2 kGy possibly may just damage binding sites or induce a competitive inhibitor of ethylene action. It is thought that in climacteric fruits and flowers the autocatalytic effect is irreversible once the climacteric has been initiated (YANG and HOFFMAN, 1984). The present results show that this is not the case for bananas, and that inhibition of ethylene action after the banana fruits have entered the climacteric stage results in reversal of ripening symptoms and extends the life of the 0.2 kGy–treated banana fruit. This result is in contrast to the widely held belief that the rate of banana fruit ripening is fixed and irreversible once the fruits enters the ethylene climacteric. The ethylene sensitivity of bananas irradiated at higher doses (0.6 to 1.0 kGy) was drastically reduced in comparison with non–irradiated fruit. Even exposures to very high levels of ethylene could not counteract the effect of irradiation (STRYDOM and WHITEHEAD, 1990). The extensive tissue damage caused by irradiation at higher exposures could result in changes in membrane properties that would account for the observed loss in sensitivity to ethylene, since ethylene will partition mainly in the lipid regions of the cell membrane (BURG and BURG, 1967; GOODWIN and MERCER, 1983).

The result of the sensory evaluation indicated that no significant differences

as regards taste, aroma and texture were recorded between the control and 0.2 to 0.6 kGy–treated fruit. However, SCRIVEN, GEK and WILLS (1989) reported that fruit ripened naturally were considered more fruity, less green, and softer than ethylene–treated fruit. Exogenous ethylene probably causes the skin and flesh to ripen out of phase, with ripening in the flesh occurring more slowly. RIPPON and TROCHOULIAS (1976) found that when the rate or ripening (yellowing of fruit) is accelerated by increasing storage temperature, flesh softening is inhibited. VENDRELL and McGLASSON (1971) also suggested that in the absence of exogenous ethylene, peel ripening depends on ethylene produced by the flesh. It might be expected that irradiated fruit and non–irradiated fruit ripened with ethylene would, at a more advanced skin colour, attain an eating quality similar to that of fruit allowed to ripen naturally.

The results show that irradiation of 'Dwarf Cavendish' bananas at 0.2 kGy and a dose rate of 7.35 kGy $hour^{-1}$ will delay ripening and allow storage of the fruit for up to 28 days at room temperature, provided that the relative humidity is kept at a level of 75 % to prevent desiccation. Since the sensitivity of the fruit to exogenous ethylene is not affected at this radiation dose, irradiated fruit can still be ripened with ethylene after storage and this has potential benefits for marketing nationally as well as internationally.

CHAPTER 4

ULTRASTRUCTURAL STUDIES

INTRODUCTION

Bananas occupy an important place amongst fruits in South Africa. The postharvest quality of fruits and consumer acceptance ultimately depends on the appearance of the outer tissues. Pectins and their bonds with calcium play an important role in plant tissue during ripening. During senescence the middle lamella disappears from the cell walls, the fruits become soft and cell wall reticulation can be seen. Within the cell the plasmalemma separates from the wall, the tonoplast from the cytoplasm, and plastids, mitochondria and nuclei become disorganised (KOVACS, KERESZTES and KOVACS, 1988). Many investigations have been undertaken on the potential use of ionizing radiation in reducing market losses and extending the shelf–life of fruits and vegetables (FOA, JONA and VELLANIA, 1980). There exists an extensive literature on the postharvest physiology and biochemistry of bananas (MARRIOTT, 1980). Doubts have however, been raised about the prospects for ionizing radiation exposures as a post–harvest treatment for fresh produce (MAXIE, SOMMER and MITCHELL, 1971). Exposures of 0.3 to 0.35 kGy were identified as the minimum required to inhibit ripening in bananas (AMEZQUITA, 1965). Irradiation of preclimacteric bananas with doses above 0.5 kGy resulted in severe

peel discoloration and fruit splitting, irrespective of the variety tested (THOMAS, DHARKAR and SREENIVASAN, 1971). Reports on the effect of gamma irradiation on the ultrastructure of cells of soft fruits are almost non–existent.

It has previously been shown that irradiation with 0.2 kGy delayed the ripening of bananas for up to 28 days (STRYDOM and WHITEHEAD, 1990). Since the ripening process involves, **inter alia,** changes to the plastids, the present study was undertaken with specific emphasis on the effect of gamma radiation on the ultrastructure of these organelles in the banana peel and the ultrastructure of the banana peel with respect to the quality attributes of interest to the consumer.

MATERIALS AND METHODS

Plant material

Green bananas of *Musa acuminata* Collar cv. Dwarf Cavendish were harvested for study and were of colour index no. 1 (VON LOESECKE, 1950). Fruits, the third row from each bunch, were treated with thiabendazole [2–(4–Thiazolyl) benzimidazole] in the warehouse and packed as "hands" (clusters of fruit). At harvest the preclimacteric fruit was fully mature as judged by their angularities and ethylene production of < 0.1 nℓ g^{-1} hour^{-1} (STRYDOM and WHITEHEAD, 1990). Fruits were cooled to 15 ^{0}C and transported to the laboratory by insulated road vehicles 1 day after picking.

Gamma irradiation

The bananas were irradiated as "hands" with average exposures of 0, 0.2, 0.4, 0.6 and 1.0 kGy at an exposure rate of 1.38 kGy hour^{-1} (1 kGy = 100 krad) in a Gamma Beam 650 (Atomic Energy of Canada, Ltd.), in air at ambient temperatures (20 to 25 ^{o}C). The ratio of maximum to minimum dose received within the container was 1.1. Dosimetry was confirmed using a Fricke (ferrous ammonium sulphate) dosimeter. Immediately after irradition the bananas were placed at 21 ^{o}C and a relative humidity of 75 % to simulate conditions considered to be the optimum for the ripening of most fruits (WILLS, McGLASSON, GRAHAM, LEE and HALL, 1981).

Ultrastructure

For transmission electron microscopy pieces of tissue (1 mm^3) were removed from the banana peel in the centre of the fruit (Figure 22) and fixed at room temperature in 0.03 M PIPES (1,4 – piperazinediethanesulfonic acid) buffer, pH 7.2, containing 2 % formaldehyde and 2.5 % glutaraldehyde. The specimens were rinsed in PIPES buffer after 4 hours in the fixative. The tissue was post–fixed in 1 % osmium tetroxide, similarly buffered. After washing in distilled water tissue dehydration was by a graded alcohol series before infiltration in Spurr's resin. Thin sections were cut with a diamond knife on a LKB ultramicrotome, stained with uranyl acetate and lead citrate (REYNOLDS, 1963), and examined with a Jeol CX 100 electron microscope at 80 kV.

For scanning electron microscopy samples of banana peel, from the centre (Figure 22) of the fruit, were placed in 3 % glutaraldehyde in 0.5 M sodium cacodylate buffer at pH 7.2 for 1 hour. This was followed by 2 buffer washes and subsequent post–fixation in 2 % osmium textroxide for 1 hour. After two further washes in cacodylate buffer, tissue samples were dehydrated through a graded ethanol series. Critical–point drying of tissues was accomplished in a Hitachi HCP 2 instrument. Samples were gold–coated in a vacuum evaporator and examined in a Hitachi S–570 scanning electron microscope operated at 4 kV. All studies were done on five replicates and each ultrastructural study was repeated three times.

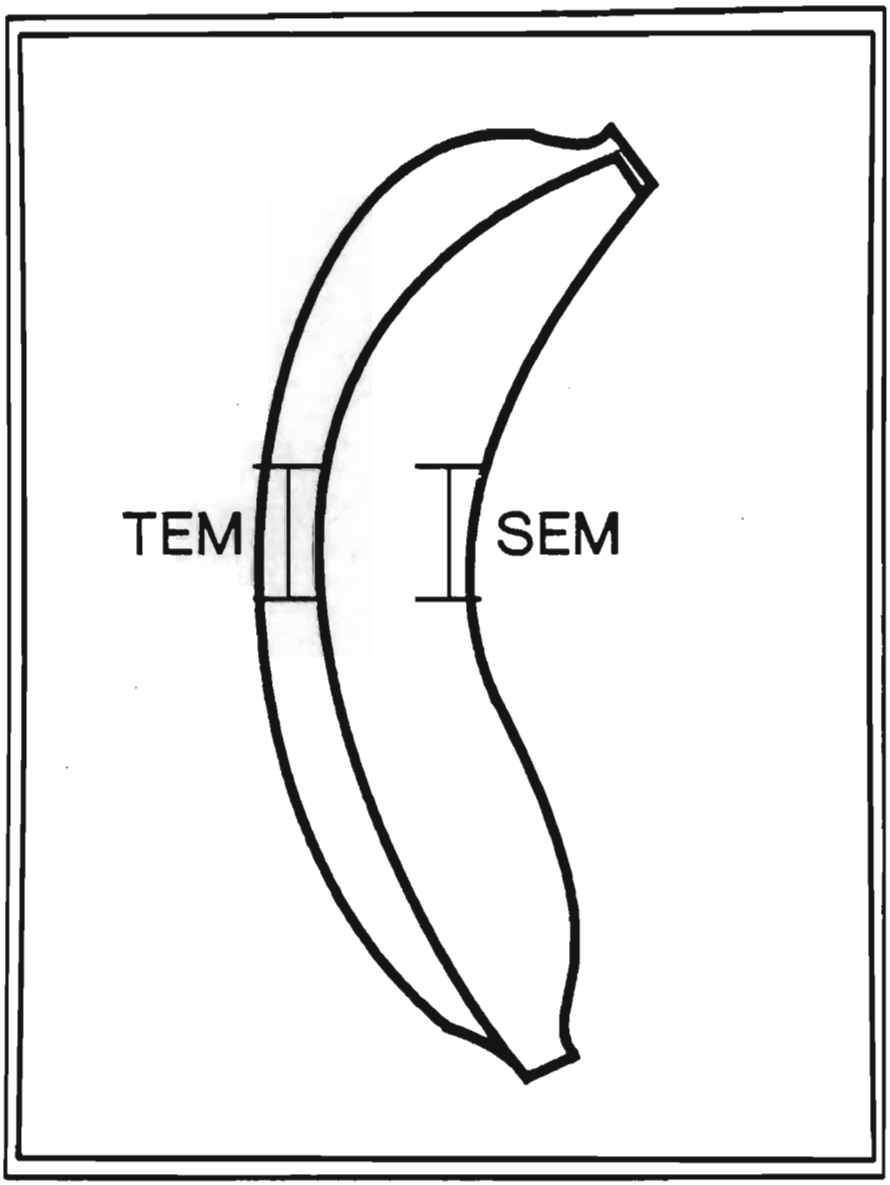


Figure 22: Regions of the banana peel sampled for electron microscopy

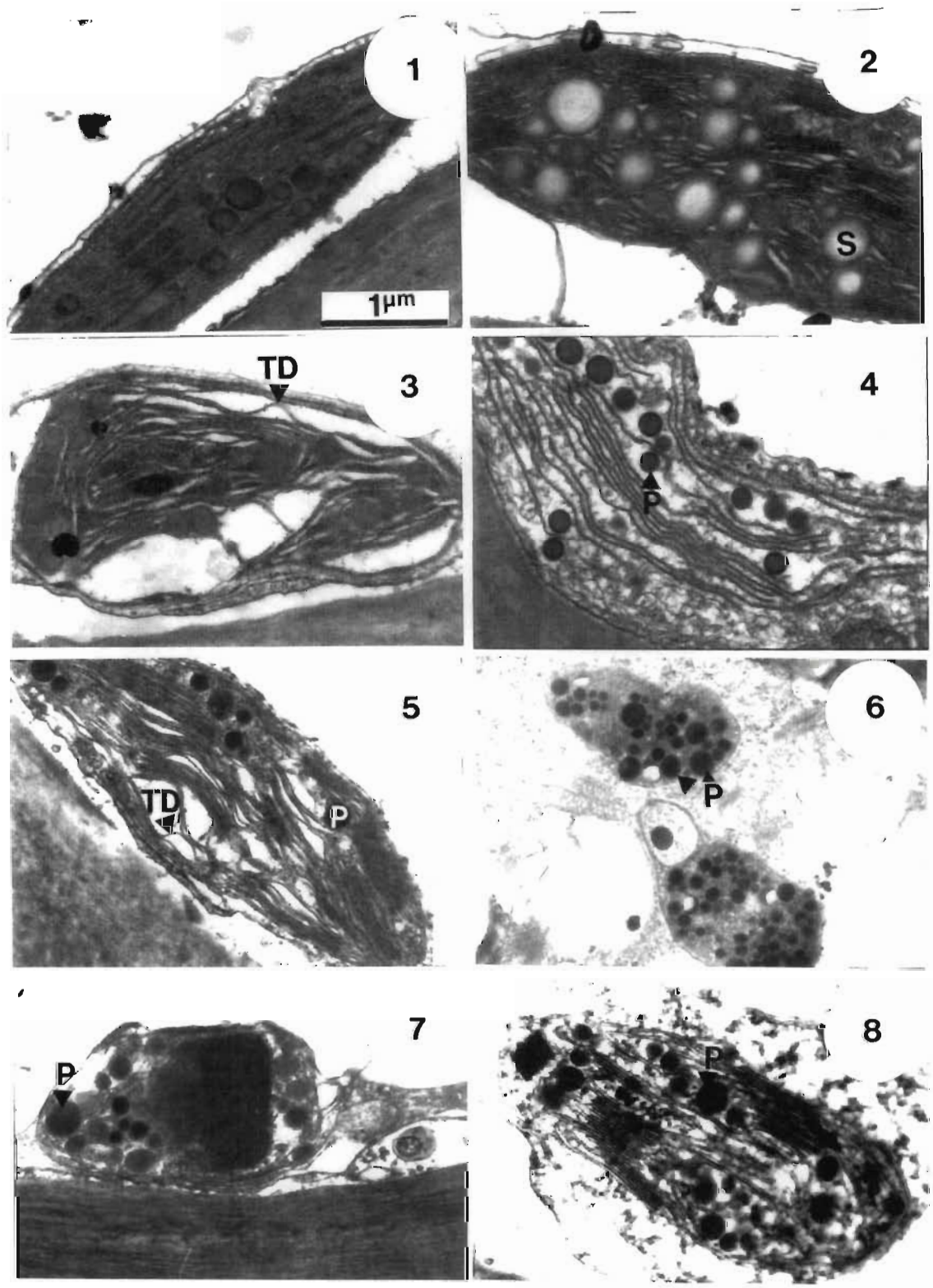
RESULTS

One day after irradiation no differences were evident between the chloroplasts of control, preclimacteric fruits which were not irradiated (Figure 23.1) and those receiving irradiation exposures of 0.2 kGy. At a radiation exposure of 0.4 kGy there was extensive formation of starch grains which were interspersed between the thykaloids (Figure 23.2). Loss of granal structure and dilations between thylakoid lamellae were evident in chloroplasts 1 day after irradiation with doses of 1 kGy (Figure 23.3) and 14 days following radiation exposures of 0.2 kGy (Figure 23.5). This pattern of degeneration differed from that associated with chloroplasts of ripened, non–irradiated fruits where the loss of granal stacking was complete (Figure 23.4).

Osmiophilic, spherical bodies which were considered to be plastoglobuli, were present in chloroplasts of banana fruits during after 14 days of natural senescence (Figure 23.4). Plastoglobuli were also observed in the chloroplasts of fruits 14 days after irradiation following exposures of 0.2, 0.4, 0.6 and 1 kGy (Figures 23.5 to 23.8). The chloroplasts of bananas 14 days after irradiation with 0.4 kGy contained numerous plastoglobuli (Figure 23.6) and an apparent absence of a thylakoid system. A dominant component of some chloroplasts irradiated with 0.6 kGy after 14 days was a large, uniformly staining osmiophilic, crystaline body, and some remnants of a thylakoid system (Figure 23.7). This contrasted with chloroplasts from fruits irradiated with 1 kGy where a persistence of thylakoid stacking and a distension of the chloroplast envelope were evident (Figure 23.8).

At exposures of up to 0.4 kGy the walls of the hypodermal parenchyma appeared isodiametric and regular when viewed by scanning electron microscopy (Figures 23.9 and 23.10). Following irradiation with 0.6 kGy and 1 kGy wall profiles were irregular, suggestive of wall collapse (Figures 23.11 and 23.12). These visual observations were supported by physical measurements with the firmometer which indicated that fruits subjected to a 0.2 kGy irradiation treatment were firmer than all other treatments. Fruit softening only became evident 4 days after the start of the experiment, and marked differences were only evident between 2 and 3 weeks after irradiation treatments (Figure 17). Loss of structural strength of the whole fruit increased progressively with exposure between 0.4 and 1 kGy.

A marked delay in colour change was noted in bananas irradiated with 0.2 kGy (Figure 18), while an intermediate retardation of yellowing was seen at doses of 0.4 and 0.6 kGy. Colour changes at 1 kGy were indistinguishable from those of control, non–irradiated fruit. The sensitivity of fruits to applied ethylene, as evaluated by colour change, further confirmed chloroplast membrane stabilization of 0.2 kGy irradiated fruits (Figure 16).


1
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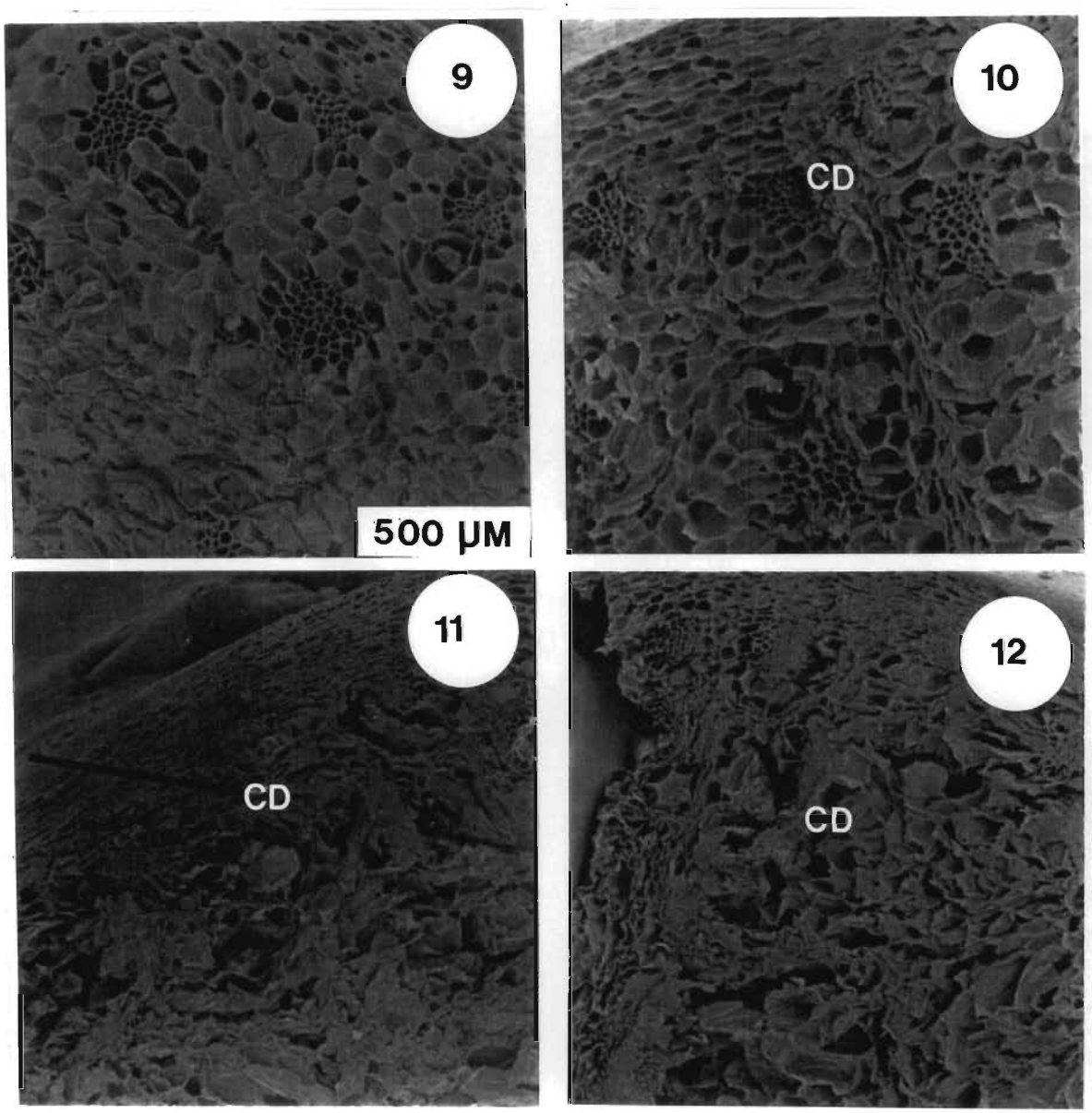
9
500 µM
10
CD
11
CD
12
CD

DISCUSSION

It is well–known that elevated levels of toxic active O_2 species are symptomatic of senescence processes in plant tissue, presumably as a result of enzymatic activity determined and controlled by the genetic code (LESHEM, HALEVY and FRENKEL, 1986). Oxygen toxicity in senescence (ageing) is therefore the result of an imbalance in a fine and sensitive equilibrium (HALLIWELL, 1982; RABINOWITCH and FRIDOVICH, 1983). Moreover the fact that oxidation conditions stimulates senescence makes it plausible that oxidation processes have a general effect on the transition from the reduced state of non–senescing cells to the highly, oxidized state of ageing cells (LESHEM, HALEVY and FRENKEL, 1986). Nevertheless this too leaves the fundamental question with regard to the mechanism of action and control unanswered. Of particular relevance to the present study would therefore be the damaging effects of $O_2^{\cdot -}$ and its derivatives to the chloroplast membranes as part of natural senescence and those generated by gamma–irradiation. Usually intrinsic defence mechanisms such as β–carotene, superoxide dismutase, catalase and natural antioxidants including vitamins C and E protect the chloroplast from free radicals produced during normal photosynthesis (HALLIWELL, 1982; RABINOWITCH and FRIDOVICH, 1983; FRIMER, 1986).

In spite of the visible macroscopic changes of peel browning and the development of necrotic spots, which can be attributed to increased mixing of polyphenol oxidase with cellular substrates following a loss of compartmentation (SKOU, 1963; THOMAS and NAIR, 1971), as well as obvious ultrastructural damage to the chloroplasts, irradiated bananas exhibited a climacteric pattern of ethylene production in the exposure range 0.2 to 0.6 kGy (STRYDOM and

WHITEHEAD, 1990), and were able to respond to exogenous ethylene in a similar manner to control, non–irradiated fruits. It has been suggested that the inability of fruits to respond to exogenous ethylene at higher radiation doses may point to altered metabolism or changes in membrane properties (STRYDOM and WHITEHEAD, 1990). The latter suggestion may be significant in view of the fact that ethylene production and action is possibly a membrane bound process (BURG and BURG, 1967; HOFFMAN and YANG, 1980). Furthermore, it would seem reasonable to assume that up to 0.2 kGy the natural defence mechanisms were able to quench any free radical species and return cells to a metabolic status quo, without the above mentioned cell damage.

Doses above 0.2 kGy appeared to alter the normal pattern of starch to sucrose interconversions in some way, as evidenced by increasing levels of chloroplastic starch. Retention of plastid starch has also been observed in apples and pears irradiated with 1 kGy (KOVACS, KERESZTES and KOVACS, 1988). Further investigations are needed to determine why the starch content is higher in the irradiated bananas than in the controls.

Chlorophyll appears to be organised into complexes with specific thylakoid membrane proteins (HILLER and GOODCHILD, 1981). Chlorophyll degradation during fruit ripening or leaf senescence is accompanied by breakdown of the internal membrane systems of the chloroplast (THOMAS and STODDARD, 1980; THOMSON and WHATLEY, 1980; BURTON, 1982). It has been proposed that the plastoglobuli are involved with the process of thylakoid breakdown (HARRIS and ARNOTT, 1973; HURKMAN, 1979). Plastoglobuli

isolated from the chloroplasts contain mainly triacylglycerols, plastohydroquinone and α–tocopherol (STEINMüLLER and TEVINI, 1985). Typical symptoms of chloroplast senescence in control tissue as well as in fruit irradiated with 0.4 to 1 kGy and left for 14 days were a loss of thylakoids, ribosomes and the appearance of numerous plastoglobuli. Fruits irradiated with 0.2 kGy appeared to show a delay in the normal pattern of chloroplast senescence. SEYMOUR, JOHN and THOMPSON (1987) have suggested that pigment stability in the non–yellowing mutant of *Festuca* may be due to an alteration in the chloroplast lipid turnover. This may be the means whereby irradiation at 0.2 kGy kept the chloroplast thylakoids intact and thus retarded ripening. At the present time it is not possible to conclude whether this response is a result of changes to the chloroplastic or extrachloroplastic genome or at some other level. The chloroplasts of stressed or infected plants frequently contain protein bodies and these are thought to be centres of crystallized ribulose bisphosphate carboxylase (WELLBURN, 1987). The electron–dense structures in the chloroplasts of fruits exposed to doses of 0.6 kGy (Figure 23.7) may be such crystals.

No evidence of irregular wall profiles or tissue softening was observed at radiation doses of 0.2 kGy. This is in keeping with the observations of other workers (AMEZQUITA, 1965; THOMAS, DHARKAR AND SREENIVASAN, 1971). Radiation doses from 0.4 to 1 kGy caused an increase in tissue softening probably as a result of the conversion of insoluble protopectin to soluble pectin, loss of cellular turgor as a result of membrane damage, and possible degradation of cell wall polysaccharides (SOMOGYI and ROMANI, 1964; SRB and

HLUCHOVSKY, 1963; FOA, JONA and VELLANIA, 1980).

The interpretation of responses in irradiated tissue is complicated by repair or turnover of organelles and damage to the chloroplastic genome, or both. It was clear that irradiation with a dose of 0.2 kGy resulted in retardation of ripening and a banana fruit of excellent postharvest quality as judged by ultrastructure.

CHAPTER 5

SUGAR AND ENZYMIC STUDIES

INTRODUCTION

Banana ripening is associated with respiratory and ethylene climacteric, a massive breakdown of starch concomitant with a rise in simple sugars, total degradation of chlorophyll, and softening (PALMER, 1971).

During ripening, starch, which accounts for 22 % of the pulp of the green banana, is hydrolysed to sugars (VON LOESECKE, 1950; PALMER, 1971) which concurrently increase from 1 % in green fruit to almost 20 % of the pulp of ripe fruit (PALMER, 1971). Total carbohydrates decrease from 2 to 5 % during ripening, presumable as sugars are utilized in respiration.

The green peel of the banana contains pectin, cellulose and hemicellulose (PALMER, 1971). In the banana pulp insoluble protopectin decreases from 0.5 to 0.3 %. Soluble pectin shows a corresponding increase during ripening (VON LOESECKE, 1950). Cellulose levels (2 to 3 %) decrease only slightly during ripening. Hemicellulose appears to constitute the largest fraction of cell wall materials (8–10 %); hemicellulose decreases to about 1 % in ripe fruits (PALMER, 1971; BRADY, 1986). Pectinic material is demethoxylated, presumably by the action of pectin methyl esterase. PRESSEY (1977) stated that the enzyme is present in sufficient quantities throughout the development of the banana fruit. It is however, not clear why the pectinic material becomes a

preferred pectin methyl esterase substrate during ripening.

It is possible that specific pectin methyl esterase isozymes catalyze the demetoxylation in defined pectin domains so that the compounds may be rendered susceptable to the action of polygalacturonase. Pectin methyl esterase may actually catalyze the esterification of free carboxyl groups in the pectic fraction of the middle lamella as a mechanism for disrupting the calcium cross–linkages and subsequent cell separation (KNEE and BARTLEY, 1981).

Chlorophyllase is a ubiquitous plant enzyme which hydrolyzes chlorophyll and the related pheophytin into their component moieties chlorophyllide and pheophorbide respectively, plus the isoprenoid alcohol phytol (HOLDEN, 1967). Its role **in vivo** is not clear. Evidence suggest that chlorophyllase is involved in chlorophyll biosynthesis and no consistant relationship between chlorophyllase activity and senescence had been found (THOMAS and STODDART, 1980). HOLDEN (1967) described an enzyme system in legume seeds where long chain fatty acids are peroxidised by lipoxygenase and other enzymes. The hydroperoxides are then broken down by a lipoperoxidase leading to free–radical formation and chlorophyll oxidation. There is no reason to believe that this is the only way or even the usual way that chlorophyll is broken down **in vivo** (HOLDEN, 1967). MARTINOIA, DALLING and MATILE (1982) and LüTHY, MARTINOIA, MATILE and THOMAS (1984) found that in senescing leaves an 'oxidase' catalysed chlorophyll degradation. The above enzyme was localised in the chloroplast lamellae and was thought to be activated by linolenic acid.

The objective of this part of the study was to determine the effects of low dose irradiation (0.2 to 1 kGy) on sugar accumulation and the activities of certain enzymes in the banana fruit during the preclimacteric, climacteric and post–climacteric stages of the fruit in an effort to throw more light on the effect of gamma–irradiation on the biochemistry of the fruit.

MATERIALS AND METHODS

Plant material

Bananas (*Musa acuminata* Collar cv. Dwarf Cavendish) from the Northern Transvaal were harvested at regular intervals for study and were of colour index no 1 (VON LOESECKE, 1950) treated with thiabendazole [2–(4–Thiazolyl) benzimidazole] in the packhouse and packed as hands (clusters of fruit) into cartons. The harvested fruits were fully mature and preclimacteric as judged by their angularities and ethylene production. Fruits were cooled to 15 °C and transported to the laboratory by insulated road vehicles 1 day after picking. The rate of ethylene production upon arrival in the laboratory was less than 0.1 nl g^{-1} $hour^{-1}$. This confirmed that the fruits were still in the preclimacteric stage (STRYDOM and WHITEHEAD, 1990).

Irradiation of bananas

The bananas were irradiated as "hands" with average exposures of 0, 0.2 and 1.0 kGy at an exposure rate of 1.25 kGy $hour^{-1}$ (1 kGy = 100 krad) in a Gamma Beam 650 (Atomic Energy of Canada Ltd., 30 000 curies) in air at ambient temperatures (20 to 25 °C). The ratio of maximum to minimum dose received within the container was 1.3. Dosimetry was recorded using a Fricke (ferrous ammonium sulphate) dosimeter. After irradiation the fruit were immediately transferred to incubators at 21 °C ± 2 °C and 75 ± 2 % relative humidity, to simulate conditions in a commercial ripening chamber.

Sampling method

Mesocarp tissue of bananas used for pectin methyl esterase and sugar analyses were ground in liquid nitrogen using a mortar and pestle and the fine powder stored at –70 °C until required. All studies were done with 3 treatments and 3 replicates consisting of 10 fruits per replicate.

Extraction and assay of chlorophyllase

Crude chlorophyllase was prepared from banana peel according to the method of TANAKA, KAKUNO, YAMASHITA and HORIO (1982) with some modifications. Banana peel tissue (100 gram) was homogenized in 180 mℓ of 0.35 M NaCl for 2 minutes at 4 °C using a Waring Blender (model 33 BL 12, Dynamic Corp. of America, New Hatford, Connecticut) at top speed. The homogenate was filtered through two layers of cheesecloth to remove the coarse debris. Chloroplasts were sedimented by centrifugation of the filtrate at 20 000 x g for 20 min, washed three times by suspending in 100 mℓ 80 % (v/v) acetone (–20 °C), followed by a final washing in 100 mℓ hexane (–20 °C). The washings removed pigments that could interfere with the chlorophyllase assay. The chloroplasts were recovered by centrifugation (5 000 x g, 4 °C), and the final pellet was freeze dried and ground to a fine powder. The chloroplast preparation was stored at –20 °C. A crude chlorophyll sample was prepared from the above pooled 80 % acetone extracts by dioxane precipitation according to the method of IRIYAMA, OGURA and TAKAMIYA (1974). The recovery of chlorophyll at the "dioxane precipitation" step was about 95 % (IRIYAMA, OGURA and TAKAMIYA, 1974).

The assay for chlorophyllase activity (TANAKA, KAKUNO, YAMASHITA and HORIO, 1982) consisted of 10 $\mu\ell$ of enzyme sample, 0.2 mℓ of 0.2 M sodium phosphate buffer (pH 6.5), 0.6 mℓ of acetone (30 % in final concentration) 10 $\mu\ell$ chlorophyll in acetone, 20 $\mu\ell$ of 0.1 M ascorbate and water to make the volume to 2.0 mℓ. The reactions were carried out at 30 °C for 30 minutes in the dark in 10 mℓ graduated centrifuge tubes with stoppers. The absorbance of chlorophyllide was measured at 645 nm, 663 nm and 652 nm respectively (BRUINSMA, 1961). One unit of chlorophyllase is defined as that amount which hydrolyses 1 μ mol of chlorophyll per min.

Sugar determination

The assay for sugar determinations (TANOWITZ and SMITH, 1984) consisted of twenty grammes of frozen fine mesocarp powder. The mesocarp powder was boiled in 80 % ethanol for 15 min and left at room temperature until cooled. After removal of the ethanol under vacuum at 30 °C two grammes of polyvinylpyrrolidone (Merck) was added to the aqueous solution. The mixture was centrifuged at 3 800 x g for 15 min. Glass columns containing 2 grammes of resin (Dowex 50 W–X 8 H^+ 100–200 mesh) were washed with distilled water. The aqueous extracts were passed through the column slowly (3 mℓ min^{-1}). The effluent contained the sugars. A 0.5 mℓ aliquot of aqueous sugar solution containing sucrose, glucose and fructose was placed in a test tube and the solution reduced to dryness at 40 °C under a constant air stream. A 0.5 mℓ aliquot of a solution containing 25 mg mℓ^{-1} of hydroxylamine hydrochloride (Mallinkrodt) and 6 mg mℓ^{-1} of phenyl–β–D–glucoside (Internal standard) dissolved in dry silylation grade pyridine (Pierce Chemicals) was added to the test tube. The tube

was stoppered and heated in a water bath at 40 °C for 20 minutes. After allowing the solution to cool to room temperature, a 100 $\mu\ell$ aliquot was removed and transferred to a 3 mℓ reactivial (Pierce Chemicals). A 200 $\mu\ell$ sample of trisilylimidazole (Pierce Chemicals) was added and allowed to react at room temperature for 15 minutes. A 1.0 $\mu\ell$ sample was injected into a Varian 3 700 gas chromatograph equipped with a 2 meter x 2 mm (i.d.) glass column packed with 3 per cent OV–17 on Chromosorb W 100 to 120 mesh and a flame ionization detector. Chromatographic conditions were as follows: injector temperature 280 °C; detector temperature 300 °C and initial oven temperature 140 °C. Programme conditions were 1.0 minute hold, 8 °C min^{-1} rate for 9 minutes (to 212 °C), then 30 °C min^{-1} rate to a final temperature of 270 °C and a final hold of 5.5 minutes. Integrator parameters were at attenuation 10^{-11}, slope sensitivity 1.0. Nitrogen carrier gas flow rate was 27 mℓ min^{-1}. A standard curve was used for the quantifaction of sugars. The data was expressed as μ mol g^{-1} (fresh weight).

Pectin methyl esterase determination

Pectin methyl esterase was determined according to the method of CHERRY (1973). Twenty grammes of frozen fine mesocarp powder was well homogenized in a precooled blender with 20 mℓ cold 1 M K_2HPO_4 [di–Potassium hydrogen phosphate, (pH7)]. Methanol, which is generated by the enzyme pectin methyl esterase from a pectin solution (Sigma), released from tissue pectin by alkali, or exists free in the tissue as the result of prior enzyme activity, is converted into methyl nitrate by reacting the sample with a nitriting reagent.

Methyl nitrite is assayed by gas liquid–chromatography. One mℓ air samples were withdrawn from each nitrating tube with an airtight syringe, and the pectin methyl esterase concentration in each sample determined using a Varian 3700 gas chromatograph equipped with a flame ionization detector and a 2 m x 2 mm (i.d.) glass column of 80 to a 100 mesh Chromosorb 102. Nitrogen was used as carrier gas with a column pressure of 20 pSi. The gas chromatograph conditions were: column temperature 140 ^{o}C; injector temperature 200 ^{o}C; detector temperature 200 ^{o}C. The pectin methyl esterase activity was expressed as μ mol g^{-1} min^{-1}.

RESULTS

There was no meaningful increase in the chlorophyllase activity (degreening) of the 0.2 kGy irradiated fruit during subsequent ripening, in comparison with the increasing chlorophyllase activity levels of the 1.0 and 0 kGy irradiated fruit (Figures 6 and 24). The control fruit showed the highest level of chlorophyllase activity.

The levels of glucose and fructose in the 0, 0.2 and 1.0 kGy irradiated banana fruit showed a rapid increase after day 10 until fully ripe (Figure 25). These increases parallelled the increase in respiration observed earlier (Chapter 3, Figure 12). In comparison the sucrose levels of the 0 and 0.2 kGy irradiated fruit showed a slight increase during ripening and overall sucrose levels were much lower. There was, however, an immediate two–fold increase in the sucrose level of the 1.0 kGy irradiated fruit after 10 days which decreased rapidly.

Pectin methyl esterase activity for the 0 and 0.2 kGy irradiated banana fruit showed a slight increase in activity during the ripening phase, although there was a marked increase in controls at day 20 when marked fruit softening is seen (Figure 17). In contrast the 1.0 kGy irradiated fruit showed a transitory increase in pectin methyl esterase which peaked at day 12 but declined to low levels at day 18 (Figure 26).

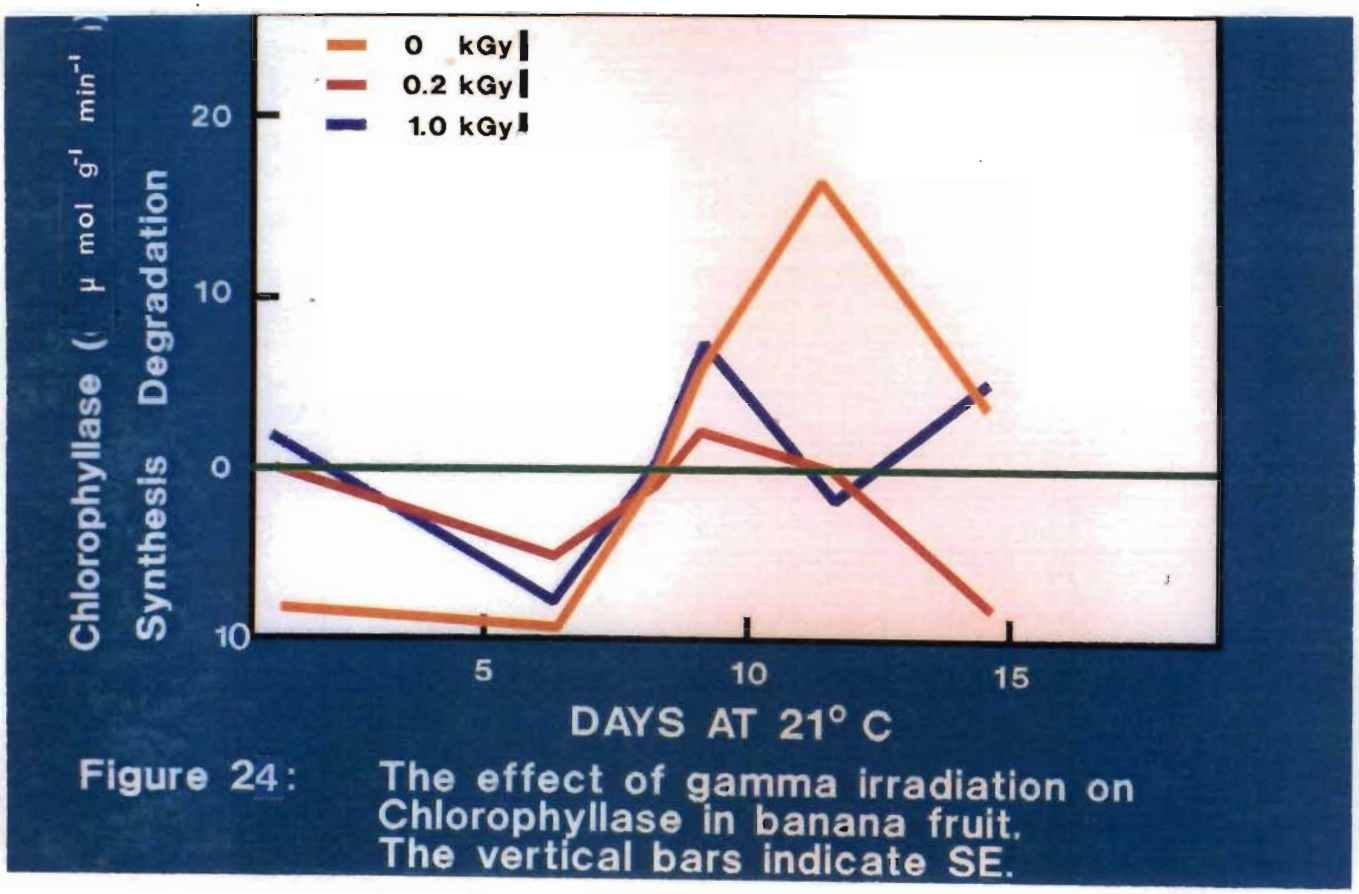

Figure 24: The effect of gamma irradiation on Chlorophyllase in banana fruit. The vertical bars indicate SE.

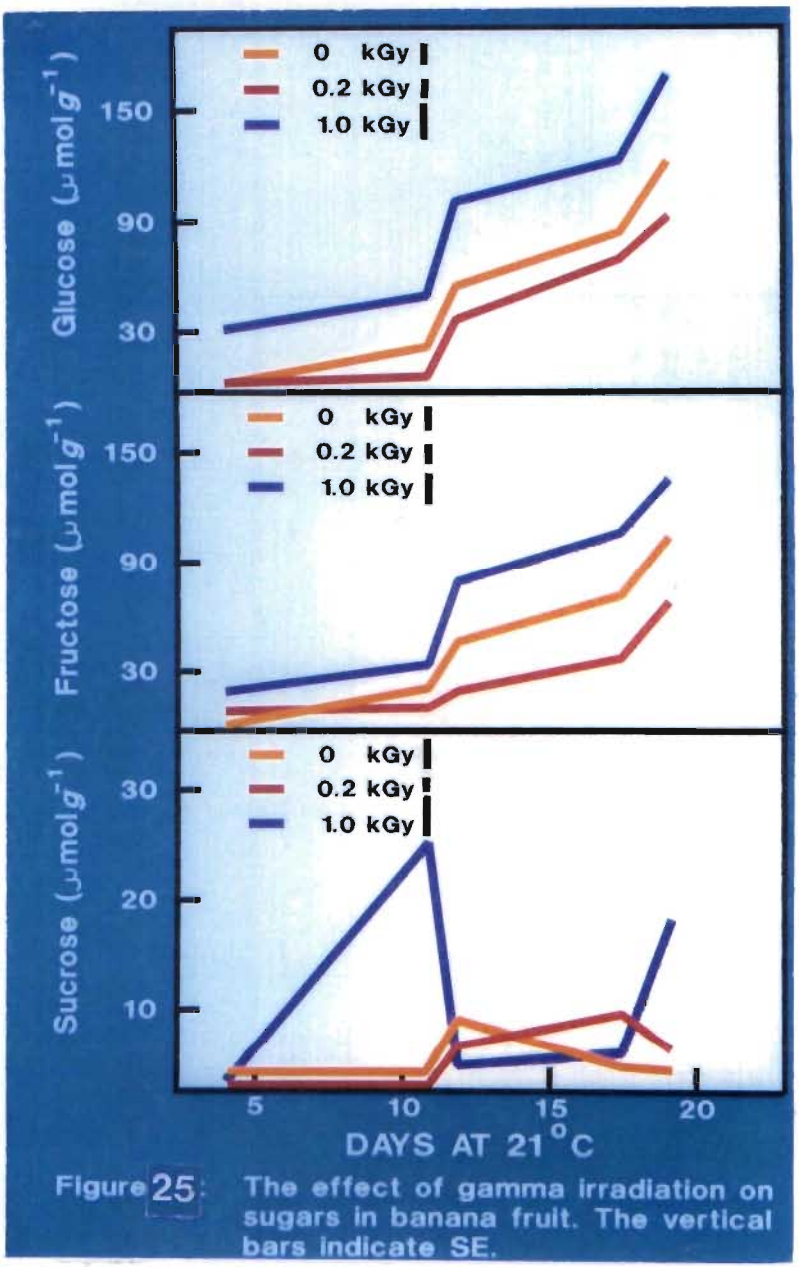

Figure 25: The effect of gamma irradiation on sugars in banana fruit. The vertical bars indicate SE.

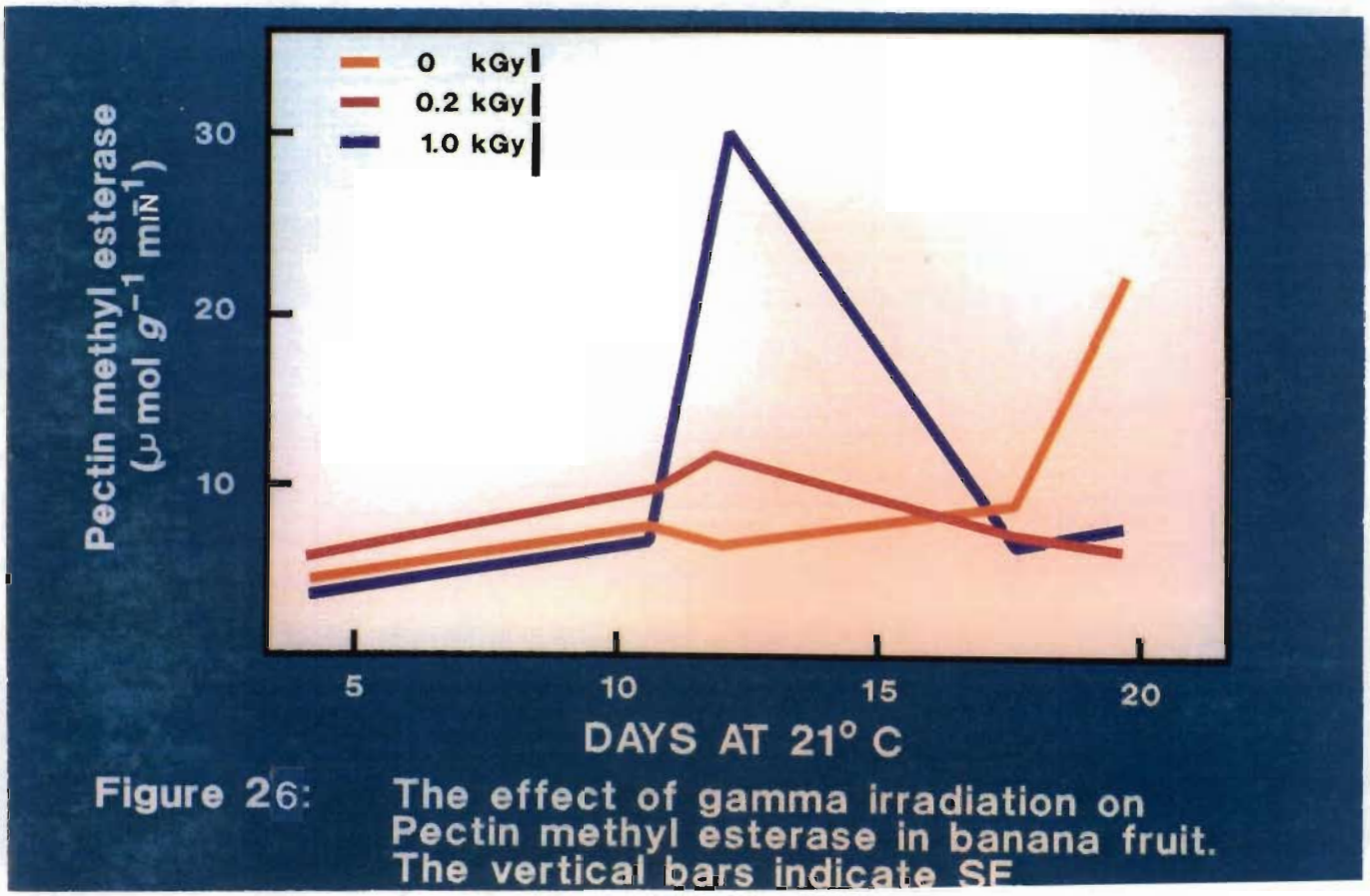

Figure 26: The effect of gamma irradiation on Pectin methyl esterase in banana fruit. The vertical bars indicate SE

DISCUSSION

The degradation of chlorophyll is usually symptomatic of leaf senescence and the maturation of many fruits and vegetables (MARTINOIA, DALLING and MATILE, 1982). Chlorophyllase (E.C. 3.1.1.14) which cleaves the phytol side chain with the production of free phytol and chlorophyllide has been found in citrus peel and tea leaves. However, there is evidence that chlorophyllase is involved in chlorophyll biosynthesis but no consistent relationship between chlorophyllase activity and senescence could be found (THOMAS and STODDARD, 1980). The results obtained during this study indicated that chlorophyllase catalysed the synthesis of chlorophyll during the climacteric and post–climacteric phase of 0.2 kGy irradiated fruit. The chlorophyll content in the 0.2 kGy irradiated fruit showed a slight increase during the first three weeks after radiation (STRYDOM and WHITEHEAD, 1990).

The average starch content of the preclimacteric banana reaches 26 % on a wet weight basis (80 to 95 % of the dry weight matter) and declines within a few days of the climacteric peak to less than 1 % (TERRA, GARCIA and LAJOLO, 1983). The glucose (from starch hydrolysis as well as from sucrose) and fructose (from sucrose) content of the 0, 0.2 kGy and 1.0 kGy increased and 'preceeded' the formation of sucrose during banana fruit ripening. The result is in accord with BIALE's (1960b) conclusion that almost complete hydrolysis of starch occurs in the banana during ripening giving rise to approximately equal concentrations of glucose and fructose, together with a little sucrose. Glucose and fructose in the 0, 0.2 and 1.0 kGy irradiated fruit are therefore probably produced directly from sucrose that may not accumulate because it is transient. Accumulation of glucose

and fructose occurred immediately after the banana fruit are removed from the banana plant. The accumulation of glucose, fructose and sucrose were not stimulated by ethylene. These results indicate a clear temporal separation between those biochemical processes that appear to be stimulated by ethylene (respiration, fruit softening and colour change) as discussed in chapter 3 from those that are not(sugars). The sucrose content of the 1.0 kGy irradiated banana fruit was enhanced by gamma–irradiation. Sucrose also accumulated in sweet potato tubers and roots upon irradiation doses of 1 to 3 kGy (HAYASHI and AOKI, 1985). It was further established that the activities of phosphorylase, sucrose synthase and sucrose phosphate synthase were significantly enhanced by irradiation (1 to 3 kGy) and remained at a high level, while sucrose accumulated apparently because sucrose breakdown was restricted in irradiated tubers (HAYASHI and AOKI, 1985). The increase in sucrose content in banana fruits irradiated at a dose of 1 kGy may also be enhanced due to enzyme activities.

Pectin methyl esterase activity in relation to ripening of various fruits has been reported to remain constant, increase or to decrease. The information concerning pectin methyl esterase activity in the banana is however conflicting. PALMER, (1971) and BRADY (1976) found that the activity of pectin methyl esterase remains constant during banana ripening. HULTIN and LEVINE (1981) reported a large increase in the activity of pecin methyl esterase during banana ripening. During this study a slight increase in pectin methyl esterase activity at the 0 and 0.2 kGy irradiated fruit was observed [supporting the results of HULTIN and LEVINE (1981)] compared to a very large increase in pectin methyl esterase activity in the 1.0 kGy irradiated fruit. SOMOGYI and ROMANI (1964) concluded that most of the textural changes (softening) at higher irradiation

exposures (> 0.5 kGy) could be due to depolymerization and conversion of protopectin into soluble pectic substances. Therefore it could be concluded that 1.0 kGy irradiation rendered more pectic substances available (to a lesser degree in the 0 kGy and 0.2 kGy irradiated fruit) through depolymerization per unit time during banana ripening to be demethoxylated by pectin methyl esterase.

GENERAL DISCUSSION

The delay in ripening of the 0.2 kGy irradiated fruit was the result of a reduced ethylene sensitivity. The maximum dose of 0.2 kGy retards the ripening and senesence process of preclimacteric Dwarf Cavendish banans in an indirect manner without affecting the quality of the fruit. It appeared as if the biochemical changes in the 0.2 kGy irradiated bananas take place more slowly in the pulp than in the peel (yellow colour of the 0.2 kGy irradiated fruit after application of exogenous ethylene, versus firmness). This is an indication that the reduced ethylene sensitivity is restricted primarily to the meso–endocarp of the banana fruit.

At irradiation doses of 0.2 to 1.0 kGy tissue damage increased until cell death eventually occurs. The various biochemical processes during the climacteric still occurred during ripening and senesence in the 0.4 to 1.0 kGy irradiated bananas but in a modified form. In this study it seemed as if gamma irradiation and not ethylene stimulate O_2 activation at 0.4 to 1.0 kGy. It can be concluded that senescence was not initiated by ethylene but possibly by elevated levels of toxic active O_2 species. It is thus possible that ethylene action developed through evolution and cultivation to assist and accelerate ripening of climacteric fruits.

There has been a slowdown in the rate of agricultural innovation due to biological limitations and the increased loss of arable land through erosion and desertification. Combined with the steady increase in world population, it is apparent that food shortages will continue to grow unless new approaches are developed to assure food security.

While parts of the world can be somewhat secure, the developing countries face the greatest challenges in meeting basic foods needs. Losses are especially high in developing countries where transport and storage systems are often old or inadequate, surrounding temperatures and humidity are high, and the need to improve food supply is greatest.

Concerns are mounting over increasing food–borne diseases; persistently high food losses from infestation, contamination and spoilage; as well as growing international trade in food products that must meet stiff import standards of quality and quarantine. In Africa, postharvest food losses are a fundamental cause of food shortages.

For thousands of years, preservation methods have evolved from the earliest days of sun drying to salting, cooking, smoking, canning, freezing and chemical preservation. To this list the most recent addition is irradiation, the exposure of foods to carefully measured amounts of ionizing radiation as was the case with the banana fruit.

When compared with no irradiation, it is evident that the 0.2 kGy radiation dose yields superior results in all respects with regard to ripening and senesence mechanisms during the climacteric. This alone should establish gamma irradiation of banana fruit in the food industry. It can furthermore be employed synergistically with other techniques like hypobaric pressure and cooling if necessary.

When compared with conventional methods of food preservation, irradiation has proved to be energy–conserving and can replace or drastically reduce the use of certain food additives and fumigants which pose hazards for consumers as well as for workers in farming communities.

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