BIOCHEMICAL AND PHYSICAL CHANGES IN A SELECTED FRUIT AND VEGETABLE DURING STORAGE AND RIPENING AT AMBIENT TEMPERATURE

Thesis submitted to
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for the degree of
DOCTOR OF PHILOSOPHY
in
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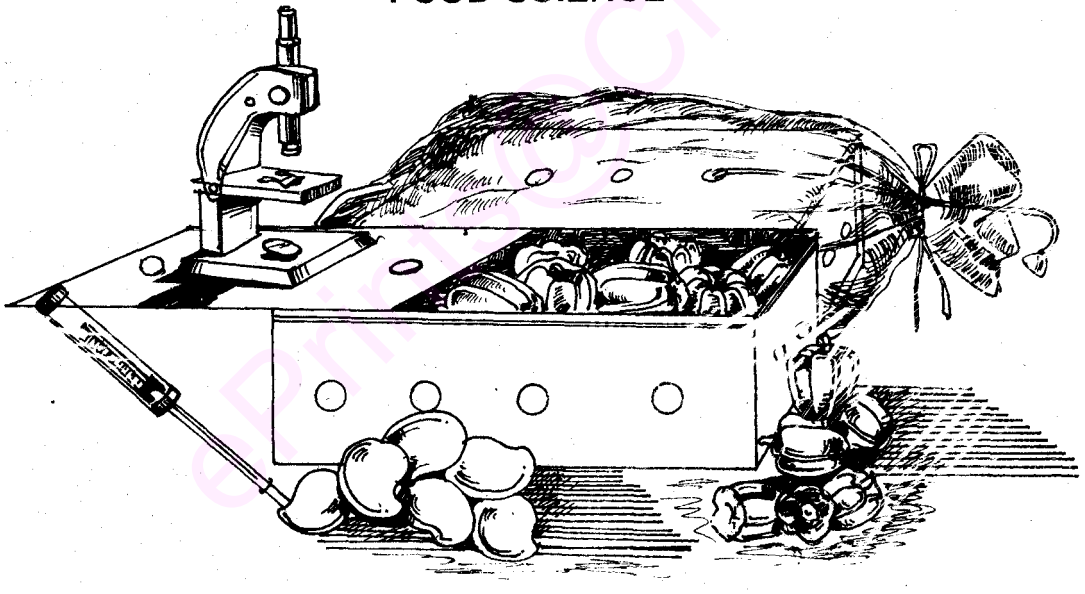

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DECLARATION

I hereby declare that the thesis entitled "Biochemical and Physical changes of selected fruit and vegetable during ripening and storage at ambient temperature" submitted to the ***University of Mysore*** *for the award of Degree of* ***Doctor of Philosophy*** *in* ***Food Science*** *is the result of work carried out by me under the guidance of Dr. M.V. Patwardhan, Ex-area Co-ordinator, fruit, vegetable and plantation crops discipline, Central Food Technological Research Institute, Mysore - 570 013, India, during the period 1982-1986. I further declare that these results are not submitted for the award of any other degree or fellowship.*

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CERTIFICATE

I hereby certify that the thesis entitled "Biochemical and Physical changes of selected fruit and vegetable during ripening and storage at ambient temperature" submitted by Miss Padmini Nagaraj, for the degree of the **Doctor of Philosophy** of the University of Mysore is the result of research work carried out by her in the Fruit, vegetable and plantation crops discipline, CFTRI Mysore - 570 013, India, under my guidance during the period 1982-1986.

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Supervisor of Research.

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CONTENTS

CHAPTER 1

REVIEW OF LITERATURE

Ripening and/or senescence are essential, inevitable processes in fruits and vegetables and begin soon after harvest. The perception that guarantee quality with or without delay in ripening and senescence are the concern of horticultural research. In this context various post harvest treatments have been employed to regulate ripening and senescence depending on the suitability of the method & intended use of the commodity. The ripening changes along with various physico-chemical treatments employed to regulate ripening and senescence are described in this chapter. Emphasis is given to treatments relevent to this study. Although an encyclopedic documentation has not been provided for some aspects, key journal articles and reviews that should provide appropriate entry to literature in this field have been cited.

INTRODUCTION

Fruits and vegetables produced need to be protected from post harvest losses to safe guard growers investment and also to ensure the demands of consumers and processors. Vast food resources (25 to 50%) are wasted simply because of improper handling and inadequate storage facilities. In recent years with revolutionery advances in horticulture technology, the production of fruits and vegetables has increased substantially. Although in India there is considerable increase in the production of fruits and vegetables, it has not been accompanied with proportional increase in consumption. The average per capita consumption being as low as 80g per day as against 100g in other tropical countries and world's average of 277gms. The recommended level is 355g person per day. The main reasons being improper ripening, heavy spoilage after harvest in fruits and weight loss, shrivelling and early senescence in vegetables. While economic factors also play a significant role is shaping the fruits and vegetable choice. The problems have been multiplied due to their seasonal availability and localised production. Thus in India for majority of the population fruit is a luxury rather than necessity and vegetable choice depends on availability rather than acceptability. This obviously necessitates proper post harvest protective measures from harvest to consumption. Various post harvest treatments have been evolved depending upon the ultimate use of the produce either to hasten or delay the ripening, alleviating spoilage, weight loss etc. Common among these treatments are low temperature, controlled atmosphere hypobaric or high humidity storage or treatments with plant growth hormones like ethylene or heat treatments, etc. The success of these treatments depends on their suitability simplicity and economic feasibility.

Thus, ripening and senescence seem to be the crucial changes that may be monitered for specific quality. These two distinct features of post harvest biology of fruits and vegetables are studied in the present investigation with respect to :

- *Accelerated ripening changes in 'Alphonso' Mangoes induced by Acetylene.*
- *Delayed senescence in Capsicum stored under commodity Modified Atmosphere.*

In this connection literature regarding various physico-biochemical changes during ripening and senescence in fruits and vegetables has been reviewed in detail. In addition, literature regarding accelerated ripening in Mangoes and MA storage of fruits and vegetables along with various other post harvest treatments is also presented.

REVIEW OF LITERATURE

RIPENING CHANGES

Ripening involves a series of changes occurring during the early stages of senescence of fruits in which the structure and composition of the unripe fruit is altered so that it becomes edible (217). Ripening is a desired change in fruits while in most vegetables it renders them unacceptable and hence not desired. The major ripening changes that occur in fruits are: changes in respiration rate, colour, softening, ethylene production, production of flavour volatiles, carbohydrate composition, increase in membrane permeability, etc. (206). Rhodes (217) has reviewed the ripening changes in fruits.

RESPIRATION

Respiration is an essentially irreversible active metabolic process of fruits and vegetables. This is associated with cataclysmic physiological, histochemical and biochemical changes (167,214,222). Respiration is considered as programmed continuation of both catabolic and anabolic processes. The various physico-biochemical changes involved in respiration are well reviewed (29,30,114,181,217,244).

Two major types of respiration were observed in fruits and vegetables - climacteric and non-climateric. Kidd and West (143,145) observed that the rate of respiration in apples decreased initially

and there was a sudden upsurge and then declined at the end of ripening. This sudden rise in respiration was termed climacteric or respiratory climacteric, the initial decrease or pre-climacteric minimum and the last phase, the post-climacteric (181). Subsequently this phenomenon was observed in various fruits and vegetables with equal exceptions. Biale (27) grouped fruits into climacteric (e.g. apple, avocado, banana, mango, etc.) and non-climacteric (e.g. pinapple, citrus, grape, melon, etc.)

Climacteric manifests the climax of all metabolic activities ultimately heralding the onset of senescence. The various metabolic activities interrelated with respiration are dealt below:

Endogenous ethylene evolution

Ethylene is produced endogenously in all climacteric fruits (89). Its biosynthesis and influence on various ripening changes were worked out by several workers (1,167,291).

Ethylene triggers respiratory CO_2 evolution and ripening in fruits (207). Ethylene production preceeds the onset of climacteric; its production was proportional to peak rise in respiration (7,136,215, 216,218).

Traces of ethylene trapped in the intercellular spaces of fruit tissues during early stages of development were detected without any rise in respiration (6,180). Later it was found that a minimum induction level of ethylene is necessary to initiate rise in respiration and to stimulate the coordinated ripening process. This level varies for different fruits and cultivars.

Table 1. Classification of fruits according to their maximum ethylene production rate*

Ethylene production rate (µl/kg/hr at 20°C)	Fruits
Very low (0.01-0.1)	Cherry, citrus, grape, pomegranate strawberry
Low (0.1-1.0)	Blue berry, kiwifruit, peppers, persimmon, pineapple, raspberry
Moderate (1.0-10.0)	Banana, fig, honey dew melons, mango, tomato
High (10.0-100.0)	Apple, apricot, avocado, cantalope nectarine, papaya, peach, pear and plum
Very high (> 100.0)	Cherrimoya, mamey apple, passion fruit, sapota

(* From Kader [130])

Carbohydrate metabolism

The carbohydrates are the main respiratory substrates and principal source of energy (28). A marked reduction in starch content with increase in sugars with respiratory rise was noted in fruits (117). These changes were well reflected by increased activity of glycolytic enzymes; phosphofructokinase (PFK) and pyruvatekinase (PK) which promote glycolysis during climacteric (242,267). In addition activation or **de novo** synthesis of hydrolytic enzymes like α-amylases, β-amylases was suggested (293).

Degradation of cell wall carbohydrates (pectin and cellulose) by their respective degradative enzymes in relation to the trends in respiration in avocadoes was studied by Awad and Young (14).

They have found that cellulase activity is more pronounced than PG and PME at respiratory peak unlike in the case of tomatoes (113).

Effects on TCA

No major changes were observed in the functional capacity of mitochondria at various stages of climacteric. Pyruvate levels as substrate for oxidative activities however increased with ripening (162). In banana TCA cycle acids increased with ripening resulting in decreased pH (198). In apple decrease in malic acid was observed (118).

Malic enzyme which catalyses the oxidative decarboxylation of malic acid, is synthesised during ripening in apples and pears. The activity of enzyme pyruvate decarboxylase, which decarboxylates pyruvate to acetaldehyde and CO_2 is increased in bananas during ripening (117).

Protein and nucleic acid metabolism

Utilization of generated ATP during respiration for protein synthesis is less elucidated. Data indicate that there was sharp rise in protein synthesis during pre-climacteric and drastic decrease at the peak of respiration in avacadoes (219), pears (155) and banana (39).

Cellular organization

Increase in membrane permeability during senescence is a common phenomenon. Increase in permeability was recorded just prior to climacteric rise in many fruits (106). Subsequently enhanced potassium ion leakages in cytosolic concentration which is a requirement for PK and a positive modulator of PFK, is expected to enhance respiration (213,271).

In non-climacteric fruits, the ripening changes are the result of biochemical aging process unlike perfective changes during climacteric fruit ripening (90).

CHANGES IN COLOUR DURING RIPENING

Colour change in fruits and vegetables during ripening often serves as visual maturity index. The main pigments in fruits and vegetables are chlorophylls, carotenoids and anthocyanins. Transition from chloroplasts rich in chlorophyll to those rich in red or yellow carotenoid pigments occurs during ripening.

Chlorophyll dominates in unripe stages of fruits and vegetables. Chlorophylls a and b are most common in all photosynthetic and storage tissues. The surface pigment changes in ripening/senescing fruits and vegetables are recorded (97), but, the **in vivo** degradation of chlorophyll and its derivatives is least understood (72). Chlorophyllase activity is correlated with chlorophyll degradation (21,236), and also with its synthesis (10). Enzymic cleavage of chlorophyll into small colourless fragments and distribution of chlorophyll a and b in various chlorophyll protein complexes has been suggested (260).

Carotenoids are a group of yellow pigments present in green fruit tissues prior to maturation and dominant in ripe fruits. Maturation does not always involve accumulation of carotenoids but, is common among various fruits and vegetables. The process of chloroplast to chromoplast transformation and its associated biochemical and ultrastructural changes along with the role of genetic aspects are well studied in tomatoes (42,43), capsicums (52,147,168) and citrus fruits (74).

Carotenoids differ in their composition from one species to another. They are relatively simple in tomato with lycopene and β-carotene (22) but, highly complex with 115 different carotenoids

in mature citrus fruits (249) and with less degree of complexity in capsicum (53) and mango (78).

Anthocyanins are water soluble, phenolic pigments known to be stored in vacuoles. Six principal anthocyanidins occur in fruits in 3 glycosides that impart red colour to apple varieties, strawberries, grapes, etc. These pigments may interact with other phenols, such as tannins and some flavanoids resulting in copigmentation or discolouration (240).

Factors affecting pigment change

These pigment changes are brought about by an array of complex biochemical reactions (60,117,238) which are influenced by several internal and external physico-chemical factors (94).

(a) Light

Light delays loss of chlorophyll (165). Loss of chlorophyll was enhanced by red light in harvested tomatoes wherein lycopene and β-carotene formation was induced (124). The effect can be nullified by far red and dark treatments (142,257).

(b) Temperature

Lower temperatures favour pigment change. Low temperature (15°C) can be effectively utilized in degreening citrus fruits without ethylene (281). Temperature of 25°C is optimum for biosynthesis of lycopene. Above 30°C it is suppressed while β-carotene synthesis remains unaffected (62). Contrary to this, in grapes and citrus, temperatures of 30-35°C favoured accumulation of lycopene but not of carotenes (250).

(c) Endogenous plant hormones

Auxins, cytokinins and gibberellins occur in low quantities in maturing fruits (83,196). Auxins are known to delay fruit maturation and associated colour change. The oxidative turnover of auxins has been related to the onset of ripening in pears (87).

Gibberellins and cytokinins are known to delay senescence in many fruits and vegetables. They interfere with chlorophyll degradation and biosynthesis of carotenoids and anthocyanins (96,221). Decreased levels of gibberellin was observed in ripening orange fruit treated with ethylene (95). This quantitative antagonism between exogenous ethylene and gibberellin or cytokinins in the control of citrus degreening has been demonstrated (237).

Abscisic acid and succinic acid 2,2-dimethyl hydrazide (SADH) enhanced the colouration of various fruits and vegetables (63). Their action is similar to auxins.

Pigment changes appear to be controlled separately from other processes of ripening such as softening, sugar accumulation, respiration, etc. However there is striking similarity in the hormonal control of pigment changes occurring within plastids (chlorophylls, carotenoids) and in the vacuole (anthocyanins). Also, pigment changes in maturing fruits are similar to patterns of leaf senescence (94).

TEXTURE AND ITS ASSOCIATED CHANGES

Texture is an attribute, difficult to define and measure. Fruits and vegetables are characterized by different textural types that alter with advance of ripening and senescence. The texture profile varies according to maturity and is greatly influenced by structure and composition of the cell walls (38). In large parenchymatous tissues of fruit cells, the degree of contact between cells is also an added factor. The chemical composition and nature of cell walls

has been studied and established by several workers (4,5,150,152).

The main components of cell wall carbohydrates are pectins, cellulose and other polygalacturones (8,210). Pectins make up one third of the dry matter of primary CW of fruits and vegetables and are mainly present in the middle lamella. The chemistry of these substances has been extensively studied (205). Alongwith pectins, hemicelluloses contribute to the bulk of non-cellulosic dry material of primary cell wall and are considered to exhibit microdispersity (197). Cellulose (β, 1→4 glucon) is present in primary cell wall in linear association of the polymer molecules called fibrils. They greatly influence the cell wall properties (274). These fibrils usually form a loose network (123) but parallel arrangement is not uncommon (197).

In addition to these complex carbohydrates, 0.5-2% of the dry weight of fruits and vegetable cell walls is constituted by proteins (151,163).

Lignin is absent or present in very low concentrations in the cells of fruits and vegetables at their edible stages. It appears in senescent vegetables in their secondary cell walls (194). It is a complex polymer derived from phenolic compounds.

Ripening changes

Fruits soften, while vegetables toughen during later stages of storage. Degradation of pectic and cellulytic material results in textural weakening of senescent tissues (140), and damage to the semipermeability of cell membranes leading to loss of turgor and softening (178). A reduction in cell wall pectin due to increased solubility has been reported in a whole range of ripening fruits (205). Changes in poly uronides have been intensively investigated by Labavitch (157). In addition a net loss of non-cellulosic neutral sugar residues has been recorded (4,102,153).

These changes are brought about by an array of carbohydrate degrading enzymes of which polygalacturonase (PG), pectin methyl esterase (PME) and cellulase are the most important (208). Their activity is low or absent in unripe fruit and increases during ripening. PG is cell wall bound and brings about solubilization of pectin. The qualitative and quantitative difference of this enzyme among varieties of peaches (209) and tomatoes (266) has been studied. The enzyme PME is also wall bound and its role is to modify pectin structure (to decrease pectin methylation) prior to PG action. It promotes PG activity and softening. Its activity has been established in tomatoes (112), peaches (25) and avacadoes (14). The turnover of cellulase, however, has not been demonstrated adequately. Its activity preceeding PG activity has been demonstrated in avacadoes (14). Its activity has been established in tomatoes (46), avacadoes (13,204) and capsicum (26). β -1-4 glucanase activity is demonstrated is involved in the decrease in cell wall galactose content in apples (22).

Measurement of texture

Texture is influenced by varieties, maturity stages and several pre- and post-harvest factors. Various methods are used to measure these properties. They are based on the principles of puncture, deformation and extrusion (36). Comprehensive reviews are available on the principles, methods and instrumentation of textural evaluation of horticultural crops (36,38,202,272).

Magnus Taylor pressure tester is widely used to test the maturity of fruits and vegetables. This determines firmness by force necessary for the plunger to penetrate the sample to a given distance. Fruit pressure tester and its practical application has been reviewed (104).

Shear device instruments are used to measure tenderness in fruits and vegetables. Relative tenderness is measured by the force required to shear them through standard grid.

In the compression test, force is applied to a commodity until it is disrupted and extruded. The maximum force required is an index of textural quality. The behaviour of various fruits and vegetables to compression test has been reviewed.

The main shortcoming in the above mentioned tests is that they measure only a small phase of mechanical properties rather than a complete description of all the parameters of physical characteristics. This has been overcome by the development of a set of instruments, viz. Instron Universal Testing Machine, Maturo Meter, Nametre Acoustic Spectrometer, Ottawa Texture Measuring System, MIT Texturometer, etc. These instruments quantify as many textural parameters as possible from the force/distance provided by texture testing instruments. In addition, they have the ability to perform texture profile analysis (37,40). The use and application of these multipurpose, multidimensional texture instruments are detailed by Bourne (38).

Sensory measurement of texture

The common sensory texture testing methods are squeezing in the hand, bending, biting between the teeth, etc. The subjective sensory methods for measuring textural properties of food with relation to human perception is well reviewed by Christensen (61).

CHANGES IN AROMA AND FLAVOUR

Fruits and vegetables are not only important sources of vitamins, fibres, minerals, etc. but equally valued for their aesthetic qualities such as aroma and colour. Flavour of fruits and vegetables is a complex mixture of several high molecular weight volatile compounds. Earlier, sensory evaluation was the only method of evaluating quality of aroma or odour. The advent of GC and various sophisticated instruments led to characterization and evaluation of various

chemical compounds. In 1963, a few hundred compounds were listed (280), and since then the list has grown to 4000 compounds in 1983. The methodology of extraction and determination of various volatile compounds has been reviewed by many workers (17,119,276). A comprehensive review of tropical fruit flavours has been reported (212). and in particular on tomato (146), capsicum (51,222), apples (282), kiwi fruit (292). Several other reviews on fruits and vegetables flavours are also reported (71,81). The role of amino acids, peptides and proteins in food flavour is reviewed by Compos and coworkers (62).

Since a review on all the fruit and vegetable flavours is beyond the scope of this dissertation only mango and capsicum have been dealt with.

Mango

Mango flavour is constituted by more than 150 compounds comprising of hydrocarbons, esters, alcohols and lactones. Bandyopadhya and Gholap (18,19) studied the flavour volatiles of Alphonso, Langra, Totapuri, Raspuri and Neelum varieties and observed an influence of ratio of palmitic to palmitoleic acids on the flavour characteristics among these varieties.

Engel and Tressl (80) identified 114 compounds in the volatiles of Alphonso and Baladi var. of which 81 were new by liquid solid chromatography, GC and GC-MS. Alphonso var. contained large amounts of C_6-aldehydes (hexanal, E-2 hexanal) and C_6-alcohols(1-hexanal, Z-3, E-3 and E-2 hexanal) which were absent or in traces in Baladi variety. They concluded that no single component is responsible for characteristic mango aroma.

Studies on the Venezuelan var. (183) revealed 3 major compounds viz. terpene hydrocarbons (68%), monoterpenes (54%) and 4 sesquiterpenes (14%) of total. Important constituents were α-pinene, car-3-ene,

limonene, γ-terpenes, α-hemulene, β-selinene, acetophenone, benzaledehyde and dimethylstyrene. New compounds identified were β-selinene, car-3-ene and dimethyl styrene considered to contribute to characteristic mango flavour.

In a recent study by Sakho and coworkers (224) on African var. by analytical GC and GC-MS, 72 components were recorded of which comphene, ethylstyrene, isolongifolene, α-bergesmotene, aromadendrene, α- and δ-guaiene eremophiline, alloaroma dendrene, α-murolene, butyric and hexanoic acids, benzyl furfuryl alcohols, 2-acetyl pyrrole and dihydro-actinidiolide were newly reported.

The contribution of lipid metabolism in development of flavour (acids, esters and lactones) of mango fruit during ripening has been studied (91,92). In Alphonso mangoes, C_6 aldehydres and alcohols are formed during crushing depending upon the enzyme status of the fruit and other storge conditions (81,138).

Capsicum

Presence of Capsaicin, N-(4-hydroxy-3-methoxy-benzyl)-8-methyl-non-trans-6-enamide, a non-volatile compound responsible for pungency is widely known (129,174,227,251,262).

Isolation identification and sensory characterization of some major volatile components of bell peppers were carried out by GC-MS. IR & proton magnetic resonance (PMR) spectra (51). Totally 63 components have been identified of which major components were limonene, trans-β-ocinene, 2-methyoxy 3-isobutyl pyrazine and methyl salicylate. 2-methoxy, 3-isobutyl pyrazine imparts a characteristic bell pepper aroma with an absolute threshold of 2 parts/10^{12} in water. Haymon and Aurand (109) recorded 125 components as a neutral volatile fraction from ***C. frutescence*** *(tabasco peppers) of which, only 24 were identified by infrared MS. The major components were Isohexyl-isocaproate*

(28%), 4-methyl-1-pentyl-2-methyl butyrate and 3-methyl-1-pentyl-3-methyl butyrate. The isohexyl isocaproate along with C_6 acids and alcohols contributes to overall green aroma of tobasco pepper.

Capsicum has widely varying aromas and flavours among its varieties. Of these, pyrazines have been most studied. 2-sec-butyl-3-methoxy-pyrazine and 2-isobutyl-3-methoxy pyrazine have been described as green bell pepper like in character (51,199,253).

Factors responsible for volatile production

The primary aroma and flavour development occurs during ripening while the secondary aroma components are formed during chewing, crushing or processing. Various pre- and post-harvest factors responsible for the manifestation of aroma in fruits and vegetables are well reported in tomato (294), apples (286), banana (179) and peaches (23). The aroma development is generally very low in unripe and pre-climacteric fruit and starts with the onset of climacteric and develops fully after the climacteric rise (99,264). However, 2 range of "critical temperature" were observed at 10-12°C and 27-30°C in bananas (179).

Volatiles dissolved in non-cellular cuticular wax is recorded in many fruits due to their solubility in polar solvents (122,139).

Biosynthesis of aroma

Volatiles of fruits originate from several metabolic pools - fatty acids, amino acids or metabolites, mevalon yl CoA, shikimic acid (70,82,126,195,290). However, two major groups of precursors are: long chain amino acids (leucine, isoleucine and valine) and unsaturated fatty acids (linoleic and linolenic acids).

The biosynthesis and formation of pungent principles of capsicum was studied by Fujiwaka ***et al.****, (88). The ratio between 16:0 to 16:1 was correlated with the differences in aroma among mango varieties by Gholap and Bandhyopadhyay (18).*

STRUCTURAL CHANGES DURING RIPENING

The visible ripening changes are the result of histological and histochemical changes in the underlying tissues. Although all other facets of ripening have been extensively investigated, the structural/histological changes have received very little attention. A comprehensive account of anatomical structures of plant tissues including fruits and vegetables have been published by Esau (84). The material preparation techniques for light microscopy have been described by Johansen (127) and Lacquni and Langeron (158). The advent of electron microscopy is an impetus for the study of the ultrastructural changes. The microstructure of fruits and vegetables with particular reference to cell wall components, changes occurring during maturation ripening and processing have been detailed by Sebek and Schlotter (231). The preparatiǫn procedure for ultramicroscopy depends on the morphology, structure and composition of the material. Techniques of preparing biological materials for SEM are dealt in detail by Hayat (108) and Juniper and Jeffrey (128).

Surface changes

SEM provides a characteristic three dimensional view of the material and hence widely used to study the surfaces of fruits and vegetables.

Faust and Shear (85) studied the fine structure of cuticle on fruit surface of different apple cultivars. In ripe apples the structure of cuticle is homogenous and evenly spread on the surface, often interspersed with pores and transcuticular canals (185,186). But in ***Vaccinium elliotti*** *the structure of surface waxes in raw immature*

fruits varied from flat to upright platelets either horizontally or vertically oriented. These variations disappeared with advance of maturity and ripening. At edible ripe stage only a limited amount of platelets or waxes were retained with complete absence of rodlets (9). Glenn and coworkers (93) showed changes in cuticular permeability in relation to calcium penetration in apples at various developmental stages.

The change in the surface waxes of cherries at various stages of development were observed both by LM and SEM. Cracking of cuticle at ripe stages of berry was correlated with water absorption. This could be overcome by ethyl oleate treatment which helps in redistribution of waxes (93). The total epicuticular waxes increased continuously throughout fruit development in Washington navel oranges (79).

Anatomical changes

Inter- and intra-cellular structural changes of fruits and vegetables during ripening have been studied by SEM and TEM. The softening of fruits during ripening was evident by the changes in the degree of cell separation, cell wall thickness and starch hydrolysis in some cases. Earlier microscopic studies on different fruits and vegetables revealed that the parenchymatous cell wall became extremely thin and tenuous during ripening (247). But in avacadoes, cell walls lost their shape and structure without any alteration in thickness (204). Ben-Arie ***et al*** *(24) and Crookes and Grierson (65) showed that dissolution of middle lamella, and a gradual disintegration of fibrillar material throughout the cell wall is responsible for cell separation in apples, pears and tomatoes.*

Comparative account of structural changes in carrots during growth, storage and disease was recorded along with various fixing

methods by Devis and Gardon (67). Influence of processing on microstructure of carrot and beans were studied by Grote and Fromme (103).

Starch granules which are abundantly present in most of the unripe fruits and vegetables were well studied in relation to structural disintegration during ripening and storage by several workers. Striated structures on the surface of starch granules of banana were observed by Hidetsugu and Sugimoto (110). Formation of numerous pinholes on the surface of starch granules by the action of amylase was also evident. Internally these pores exhibited a terraced appearance. The starch granules however became fragile and broken into small irregular fragments on the 10th day of germination in red kidney beans (237) and broad beans (193).

II. EXTERNAL CONTROL OF RIPENING (POST-HARVEST STORAGE METHODS

Fruits and vegetables are highly perishable commodities and hence various techniques have been developed to preserve them. The various post-harvest treatments apart from processing fall in the perview of this survey. These treatments have several effects viz. extending or hastening ripening, curtail spoilage, prevent sprouting or physiological disorders, etc. They can be divided into:

Physical methods :	*Low temperature storage, controlled atmospheres, irradiation, hot water treatment, polymeric film storage.*
Chemical methods:	*Growth regulators (C_2H_2, C_2H_4, etc) waxing and other chemicals.*

PHYSICAL METHODS

Low temperature storage

Low temperature storage/refrigeration is the most effective

means of extending shelf-life of fresh horticultural produce and has been commercially exploited. Low temperatures reduce the rate of metabolism - delaying ripening of the host and growth of the pathogen. However, many tropical fruits develop chilling injury at low temperature. Respiratory pattern of mangoes was affected at 6°C and bananas held at 10°C for 1-2 week (229). Thomas and Oke (258) observed chilling injury in fruits held directly at 10°C whereas, a stepwise exposure to 20 and 15°C for 1 to 2 days respectively prior to storage at 10°C resulted in better flesh colour and organoleptic qualities. But, in tomatoes cyclical temperature regimes resulted in loss of fruit quality (115). Avocadoes developed chilling injurty at 6°C; removal of ethylene reduced the injury (164).

Capsicum (cv. Yolo wonder) developed chilling injury at 1.7°C held for 2 days. But the severity was decreased by holding at 5-10°C prior to storage (259). For varieties Kapiya Kalinkov and Zlaten, 7°C was the best storage temperature (86) and 10°C for Sumo and Quadrato cultivars (263). However, Cui-Zheng dong and coworkers (66) successfully stored green peppers at 0-1°C for one month.

Hydrocooling, forced air cooling, vacuum cooling, contact icing, evaporative cooling, etc. are some of the low temperature treatments given to fruits and vegetables.

Controlled/Modified atmosphere storage

The terms controlled atmosphere (CA storage) modified atmosphere (MA) storge and 'gas' storge are frequently used to imply the addition or removal of gases resulting in an atmospheric composition different from that of normal air. The levels of CO_2, O_2, N_2, C_2H_4 and CO in the atmosphere may be manipulated. CA generally refers to precise control of these gases, whereas the term MA is used when the composition of the storage atmosphere is not closely

controlled. The term gas storge is considered inappropriate (285).

In a CA storage the proportion of O_2 usually is lowered and/or that of CO_2 increased. Different commodities and cultivars vary in their tolerance to elevated CO_2/reduced O_2 at different temperatures. The optimum concentration of CO_2 and O_2 required to extend the storage life of fruits and vegetables is given by Kader and Morris (134). These differences among commodities or cultivars are attributed to structural (anatomical) rather than metabolic differences (134).

In general about 2% O_2 is the lower limit for most of the fruits and vegetables below which anaerobic respiration results in the development of off flavours. Tolerance limit for elevated CO_2 are more variable. In recent years considerable research has been done on CA storage of fruits and vegetables. A statistical account of research work on MA till the end of 1980 is reviewed by Kader (130).

Optimum concentration of elevated CO_2 and lowered O_2 are known to retard ripening and effectively extend the storage life of different commodities. Obviously this was achieved by the direct influence on the metabolic activities like respiration, ethylene production and inter-related events, manifesting in changes in colour, firmness, aroma, etc. Extended storage life of commodities under MA was also brought about by reduction of spoilage due to microbes (132a).

The present literature reviewed is restricted to the relevant research reported in fruits and vegetables in relation to the effects of MA on the above mentioned physico-biochemical changes.

Effect of MA on respiration

Kidd and West (144) were the first to show that apple fruit respiration decreased with decrease in external O_2 concentration. However, below 3 to 1% O_2 anaerobiosis occurs. The reduction in respiration rate by lowered O_2 is attributed not to the suppression of basal metabolism but to decreased activity of oxidases such as PPO, glycolic acid oxidase and ascorbic acid oxidase (243). The respiration of sweet potatoes (56,243) and bananas (50) were depressed by low O_2 concentration. The rate of CO_2 production was progressively reduced by lowering O_2 level to 2 or 1% in Idared apples (125).

Elevated CO_2 also reduces respiration rate but higher concentration (20%) result in CO_2 injury and accumulation of ethanol and acetaldehyde. Higasio and Ogata (111) observed a cyanide resistant respiration in tomatoes and bananas after 1 day treatment with 100% CO_2. Potato tubers treated with high CO_2 concentration showed a stimulated and pronounced respiration proportional to CO_2 concentration. This was accompanied by a decline in starch and of Glucose-6-P with increase in ATP (203).

A combination of elevated CO_2 and lowered O_2 has additive effect in suppressing respiration. CO added to CA also suppresses the respiration rate (131,135,289).

Effect of MA on ethylene production

Oxygen is essential for the conversion of ACC to ethylene (50). Contrary to this, CO_2 acts as a competative inhibitor of ethylene by binding to its receptor site (273).

Golden delicious apples stored under low O_2 (2.5%) accumulated ACC suppressing its conversion to ethylene (161). Similar

observations were made with McIntosh apple storage (32). Bartlett pears stored in 1% O_2 with elevated CO_2 showed suppressed ethylene production (59).

Elevated CO_2 can have varying effects on ethylene production depending on the commodity and conditions. At higher concentration it is known to increase ethylene production but, above the tolerance limit ethylene production is reduced in proportion to increased CO_2 concentration (58).

Effect on colour

Modified Atmosphere (MA) storage controls pigment changes; the loss of chlorophyll is retarded. Elevated CO_2 and reduced O_2 is known to reduce chlorophyll degradation and synthesis of carotenoids and anthocyanins in many commodities (41). Storing pears in 2% O_2 reduced the rate of chlorophyll loss (149). Citrus fruits stored in 5% CO_2 had the same effect (254) and in apples held at 2.5 to 4% O_2 (151) and 1% CO_2 + 1.5% O_2 (169). Goodenough **et al.,** *(98) reported retention of green colour in tomatoes stored in 5% O_2 + 5% CO_2 while bell peppers wrapped in plastic films also retained green colour (187).*

Effect on texture

MA maintains the firmness in fruits and vegetables. Elevated CO_2 has more advantageous effect on texture than lowered O_2. Lidster (166) observed a positive correlation between CO_2 concentration and fruit firmness in McIntosh apples. Knee (151) observed reduced soluble polyuronide formation and softening in apples stored at 2.5 to 4% O_2. Retention of apple fruit firmness by storing in low O_2 atmospheres has been reported by several workers (125, 159,162,169,241,284). The rate of softening of Kiwi fruits was reduced

in proportion to CO_2 level when stored under 2% O_2 + 3, 5 and 7% CO_2 at 0°C (12). Pears stored in 1% O_2 with or without CO_2 retained firmness (59). Toughening of vegetables can be prevented by CA storage. Bell peppers wrapped in plastic films retained their crisp texture (116,187), which is correlated with the action of plastic films in alleviating water stress (26).

The retention of firmness under CA storage indicates its effect on changes in cell wall polysaccharides and their related enzymes. Production of PG was prevented by storing under 5% O_2 + 5% CO_2 + 90% N_2 but was regained after removal from CA (98). Suppression in the activities of cellulase and PG was observed in Bell peppers stored in PE bags (26).

Changes in flavour

Optimum concentration of CO_2 and O_2 do not affect the *flavour of a commodity. Low O_2 treatments of Spartan apples did not affect the flavour (159) while Cox's orange pippin apples stored under 2% O_2 and 'G-D' varieties stored under 10 and 15% CO_2 showed improvement in organoleptic flavour (241,261). Lidster (166) observed suppression of total volatile production in apples under low O_2 storage (3% O_2 + 5% CO_2) but regained normal levels during reconditioning period. Very low levels of oxygen ($< 1\%$) and high CO_2 ($> 15\%$) lead to anaerobic respiration producing off flavours as noticed in bananas, apples, avocados, artichokes, strawberries, oranges, etc. (41).*

Changes in composition

It is well known that fruits preserved in a controlled gaseous environment are more acidic than those preserved in air. This may be the result of lowered respiratory activity, increased CO_2 fixation or the presence of a less active enzyme that converts malic acid

to pyruvate or oxalo acetate (132).

Higher titratable acids were observed in apples and 'Bartlett' pears stored under low O_2 (59,125,162). In potatoes stored under low O_2 (2.5%) inhibition of malate was observed (234). Retention of ascorbic acid in Chinese cabbage stored in 1% oxygen was reported by Wang (275).

Tanusai (254) and Kubo and Haginuma (156) observed higher total acid content and sugar during higher CO_2 storage of Satsuma mandarins and citrus Sudachi. High CO_2 favoured succinic acid accumulation but did not alter citrate in snap beans (44).

Pathological decay

Controlled atmosphere has dual effect on microbial decay: (a) It delays senescence and hence makes the host less susceptible to entry of pathogens or (b) it curtails the growth of the pathogen and reduces its virulence. However, unfavourable CA conditions induce physiological breakdown rendering it susceptible to pathogens. Generally O_2 levels below 1% and/or CO_2 levels above 10% are needed to suppress fungal growth (76).

***In vitro** fungal response to various atmospheric modifications varies with type and composition of the media (188,189,279). The advantages and disadvantages of different media have been reviewed (76,265). The response of fungi to varying concentration of O_2, CO_2 either alone or in combination are presented below:*

Effect oxygen: Requirement of O_2 for the growth/spore germination vary considerably among different fungi. A detailed review of the optimum concentration required by different fungi has been tabulated by El-Goorani and Sommer (77).

In many fungi growth occurs without molecular O_2 (279). O_2 below 2% suppresses significantly the growth of many fungi. Contrary to this ***Geotrichum candidum, Phytophthora cactorum, P. citrophtora, Monilinia fructicola*** *and* ***Rhizopus stolonifer*** *showed enhanced growth with 2 to 4% O_2 but not in anaerobic conditions (75,76,278). Increased O_2 concentration (above atmospheric level) is toxic for the growth of fungi. This is due to the formation of superoxide.*

Effect of CO_2: CO_2 below 5% is known to have little influence in retarding fungal growth. Since fungi have the capacity to utilize atmospheric CO_2, a slight increase in CO_2 (above 0.03%) is found to be stimulatory. But with increasing concentration the role of CO_2 changes from stimulatory to inhibitory (20). This inhibition is attributed to suppressed metabolic function at multiple locations. The concentration of CO_2 to inhibit growth and/or spore germination is listed by El-Goorani and Sommer (77).

Effect of low O_2 with high CO_2: Manipulation of both O_2 and CO_2 in storage atmosphere gave better inhibitory effect than their use singly. CO_2 at low levels ($< 5\%$) with 1 to 2% O_2 is stimulatory for fungal growth (188,189). Further, increase in CO_2 concentration is inhibitory both in liquid and solid media. Post-harvest pathogens under CA (10.5% CO_2 + 2% O_2) responded variedly with variation in temperature (288). Low O_2 with high CO_2 suppressed the growth of ***Botrytis alli, Rhizopus stolonifer, Penicillium expansum*** *(170),* ***Gleosporium album*** *(171),* ***Alternaria tenuis, Botry tis cinerae, Whelzlinia sclerotiorum*** *(2),* ***Erwinia, Pseudomonas flourescens*** *(277). But not* ***Rhizoctinia solani*** *and* ***Fusarium roseum*** *(2).*

Commercial status of CA/MA: Although research work on CA storage of several fruits and vegetables has been carried out, large scale use of CA is still limited to long term storage of apples and pears. CA or MA is used during transportation of strawberries, sweet cherries

and bananas. The recommended CA conditions for transport and/or storage of fruits and vegetables are given by Kader (130a). In all cases, the recommended temperatures vary between 0-15°C. Hence CA is almost universally accompanied by refrigeration or low temperature storage.

Hypobaric storage: *Also called sub-atmospheric pressure storage, low pressure storage (LPS or LP) or vacuum storage. In the storage environment the pressure, air, temperature and humidity are precisely controlled. It delays fruit ripening, flesh softening and chlorophyll degradation. It removes ethylene and reduces O_2 supply to the commodity. It is known to increase the shelf-life of many fruits and vegetables like apricots (226), mangoes (246), avacados (47), sweet peppers (154), limes (283), etc. It controls russet spot of lettuce and prevents formation of bitter principle (isocoumarin) in carrots (182). A list of commodities benefited by hypobaric storage over refrigeration is provided by Burg (48).*

Sub-atmospheric storage: *In this method of storage, a produce is maintained at a given temperature in a sealed container at a constant sub-atmospheric pressure, which is ventilated with air saturated with water vapour by continuously evacuating the container with a sealed vacuum pump (226). Extension in storage life of fresh produce is achieved by reducing the oxygen supply and thus respiratory rate of the produce, and ethylene and other gases are evacuated resulting in delayed ripening. The lower the pressure, greater is the delay in chlorophyll degradation and other ripening changes. The shelf-life of many fruits and vegetables has been extended by this method.*

Hot water treatment: *Hot water treatment is commonly used to control decay in fruit crops. In tomatoes (239) and mangoes (159) ripening was accelerated by HW treatment. It also reduced spoilage in mangoes (55,159,192,200). Hot water treatments have no protective action and recontamination by fungi is likely if storage sanitation is poor.*

High humidity storage : *Here the fresh fruits and vegetables are stored at RH, little higher than the recommended RH levels, but below saturation (100%) which may lead to increased decay (173). However, many vegetables and apples stored better at 98 to 100% RH (31,269). Produce stored under high RH retain greater crispness and firmness resulting from reduced moisture loss. Also, high RH with elevated temperature encourages wound healing by suberization in potatoes and sweet potatoes (73) and lignification in citrus fruits (121). All complications of recommended effective temperature and RH are compromised between physiologial, physical and pathological response of fresh commodities (101).*

Polymeric film storage : *The shelf-life of fruits and vegetables could be extended by storing in semipermeable PE or polystyrene bags; 30-50 μ thick films are usually recommended. In the package, both respiration and permeation occur simultaneously. Hardenberg (107) has reviewed the effects of plastic films in prevention of moisture loss and atmosphere control in keeping quality of fruits. The method has been tried for many commodities - tomatoes (15), bananas (230), avacados (3), etc. Kawada (137) has successfully extended the shelf-life of tomatoes, persimmons and grape fruit by 'Unipack' system (one to few fruits covered in LDPE bags).*

Irradition : *Gamma rays destroy the microorganisms. Passing ionizing particles through or in close proximity to sensitive portions of microbial cells, results in their death. But, radiation that are powerful enough to appreciably inhibit growth of pathogens are so high (200-400 krads) that they injure or kill the host before inhibiting pathogen. Optimum dosages can destroy microorganisms and insects without adversely affecting the quality. Irradiation is a useful growth inhibitor. Low doses ($\backsim$ 10 krads) can prevent cell division thus retarding sprouting in potatoes, sweet potatoes and onions. Loss of texture is the major disadvantage of irradiation (68). It has been used to retard ripening with increased level of*

carotenes in mangoes (256). The use of irradiation in preservation of horticultural crops is excellently reviewed by Paul Thomas (255) and Kader (132b).

CHEMICAL TREATMENTS

Growth regulators

Ethylene

Endogenous action of this hormone in enhancing ripening is well known. This has been utilized commercially to ripen fruits by exogenous application of ethylene. The commercial formulations - ethrel and ethephon (2-chloro ethyl phosphonic acid) which release ethylene are more commonly given as pre-harvest sprays or post-harvest dip or gaseous treatments.

Ethephon applications to apples before harvest significantly improved the red colour development of fruits (45,100,105). Ethephon (50 to 100 ppm) applied to maturing Carabao mangoes increased fruit retention on trees, accelerated fruit ripening and improved the chemical composition (54). Pre-harvest applications of ethephon to bell pepper plants (var. Yolo wonder and Paprika) increased the carotene and capsanthin development in fruits (148,287).

Green and yellow lemons treated with ethrel showed 2- to 3-fold increase in respiratory activity also, ethrel was more effective than ethylene in producing this effect (57). The respiratory climacteric in avocados was preponed by ethylene treatment (120). Ethrel (500 ppm) also increased the respiration rate in mangoes var. Dasheri (211), Chinese jujube fruits (133) and Chinese sour cherries (11).

In the above studies it was observed that ethylene also increased fruit softening without adversely affecting other chemical constituents.

The action of ethylene applied to fruits and vegetables has been studied by many workers. Earlier Burg (49) studied the interaction of C_2H_4, O_2 & CO_2 *in fruit ripening and revealed that* O_2 *is essential for ethylene association with a metal containing protein but competatively inhibited by* CO_2. *Mamedou* **et al.**, *(1976) demonstrated that in apples protein synthesis is activated by ethrel. In Satsuma mandarins chlorophyll b was preferentially degraded in ethylene treated fruits while in non-treated fruits chlorophyll a was predominantly degraded (235). Although, ethylene is known to act on membrane permeability no ultrastructural changes could be observed (184). Infiltration of calcium into apple fruits lowered the ethylene production rates but had no effect on respiration, titratable acids and soluble solids (228).*

Acetylene

Acetylene is also used to hasten fruit ripening. It can be released from calcium carbide and burning plant materials. It has been used to hasten ripening in bananas (141,225), citrus fruits (33, 268) and tomatoes (34,35). There are conflicting views regarding its effect on ripening. Mann and Dhillon (177) obtained accelerated and uniform ripening of Deshehari mangoes without quality impairment while Shanta Krishnamurthy and Gopala Rao (233) observed impairment of some chemical and organoleptic qualities in Pairi, Alphonso, Banganapalli, Totapuri and Langra varieties. In bananas, 1 ml/L of acetylene induced ripening similar to ethylene but lower concentrations (0.1 ml/L) failed to initiate ripening.

Other growth regulators

Growth regulators may have either hastening or delaying effect on ripening. 2,4-D hastened ripening in bananas and Guavas at 100 to 1000 ppm concentration (223,232), whereas 100 ppm concentration of 2,4,5 TP delayed ripening of Pairi mangoes (190). Abscicin is known to hasten ripening in oranges (64), banana slices (270) and mangoes (175,191).

The biological action of Indole acetic acid (IAA) and napthalene acetic acid (NAA) are similar. NAA is known to delay ripening in tomatoes (160), pineapples (248) and bananas (245). IAA inhibits PG activity in early stages of tomato ripening (160). GA and kinetin delayed ripening in mangoes (175) and pears (69). In general, auxins retard ripening. Repsonse of fruits to these chemicals depends on dosage, maturity and variety.

Waxing

Waxing or skin coating retards transpiration. Composition of wax emulsions vary and fungicides added to them give a beneficial effect. Wax emulsions are known to extend shelf-life of many fruits and vegetables. Storage life of mangoes (200), peppers and bananas (16) are extended with wax coating.

OBJECTIVES OF THE INVESTIGATION

Although many modern technologies have been developed to handle fruits and vegetables after harvest, they have meagre impact in India due to socio-economic and logistic considerations. Conventional methods dominate in fresh trade and processing industries of fruits and vegetables. A typical example is calcium-carbide treatment to hasten ripening in mangoes. Despite its extensive usage, scientific literature regarding the action of acetylene on fruit ripening are scanty. The available information are neither descriptive nor conclusive. Further, there is an 'expressed doubt' regarding the quality of Alphonso mangoes exposed to acetylene. Considering the importance of mangoes in India for both internal use and export trade, scientific knowledge regarding its effects on various physical, physiological & biochemical qualities of mangoes seems necessary. If the existing paucity of knowledge could be overcome, treatments supplemental or to complement or potentiate the effectiveness of acetylene treatment could be sought for. Hence, one of the objectives of present investigation is to evaluate various market and sensory qualitites of Alphonso mangoes treated with Acetylene.

In vegetables low quality has been accepted albeit with regret. The main reasons for low quality being improper method of post harvest handling and insufficient storage facilities. Added to this, localization of producing centres due to various agro-climatic conditions and the tropical climatic conditions prevailing in India aggravate deterioration of horticultural produce. This leads to high cost of commodities. Capsicum or bell pepper being one such, extending the storage life of capsicums with a simple low cost method was sought. The existing method for storage of capsicums is low temperature (7-9°C) supplemented with or without controlled atmosphere. This method is cost intensive and therefore not economically feasible in our country. In this connection a simplified method of MA (generated by the respiratory activity of the commodity) has been endeavoured to extend the shelf-life of the capsicums at ambient temperature.

CHAPTER 2

MATERIALS AND METHODS

The chapter covers various methods employed to evaluate the quality of Alphonso mangoes ripened by acetylene treatment and capsicums stored under MA. Studies were carried out for a period of 3 years (1982-85). The storage conditions adopted for mango and capsicums have also been described.

***MANGOES (Mangifera indica** L. var. Alphonso)*

Mature, green 'Alphonso' mangoes were harvested at early, middle and late part of the season, from a nearby orchard. Fruits were graded into floaters (sp. g. < 1) and sinkers (sp.g. > 1) based on their sp.gr. in water. To prevent fungal attack, fruits were dipped in 1000 ppm benlate solution for 5 min. and air dried.

Forty to fifty fruits were placed in each ventilated wooden box (23"x10"x13.5") lined with craft paper. Paper packets containing calcium carbide (CaC_2) were placed in each box. The fruits were covered with straw and craft paper. The boxes without CaC_2 served as control. Five replicates were kept under each group. The boxes were kept at ambient temperature (27±4°C) and RH (55±20%). The boxes with CaC_2 were kept in an unventilated and controls in a ventilated room separately. After the treatment period, CaC_2 packets and craft paper were removed and the fruits were allowed to ripen under ambient conditions in a separate ventilated room away from the control group. Observations were carried out periodically.

***BELL PEPPER** OR **CAPSICUMS** (**Capsicum annuum** var. Grossum: cult. Bullnose)*

Mature capsicums of first harvest were picked manually and transported to laboratory within 2 hrs. in ventilated wooden crates (23 x10 x 13.5 inches) lined with gunny cloth. Their stalks were trimmed to 1-1.5 cms with a razor blade.

The harvesting maturity was determined by subjecting the fruits of different sizes from 90 day old plants which are ready for first harvest to puncture test. Fruits from ten randomly selected plants were subjected to puncture test using hand magnus-taylor penetromer with the probe diameter of 9.6 mms.

Method of modified atmosphere storage (MA)

The fruits before storage were washed and pretreated with a solution containing 500 ppm Captan and 1000 ppm Streptocycline for 5 min. with 0.1% tween-80 as surfactant, for minimising the fungal and bacterial spoilage.

The fruits were thoroughly air dried. Fifty fruits were kept in each ventilated wooden crate. 250 gms of fused calcium chloride in petri dishes was placed on each crate to prevent condensation of moisture. Rubber tubes drawn from amidst the fruits were provided for taking out air samples (Plate 1).

Each crate was inserted into low density polyethylene bag (37.5 μ thickness with CO_2 permeability rate of 22 to 30 thousand cc/24 hrs/ meter/one atmosphere gradient at ambient temperature and pressure) and the opening of the polyethylene bag and rubber tubing was closed. Crates without the polyethylene cover were kept as control. All the crates were stored at ambient temperature and RH. Five replicates were kept under each group.

Plate 1. Commodity generated modified atmosphere chamber.

VISUAL OBSERVATION

(a) Colour

Change in surface colour of mangoes was evaluated by suitably modifying the method of Bondad and Pantastico (2) as green, turning or 50% yellow and edible ripe or 100% yellow. Capsicums were categorised as green - marketable and turning and/or ripe - unmarketable.

(b) Firmness (by finger feel)

Firmness of the fruits was evaluated subjectively by applying gentle pressure to the fruit held in the palm of the hand. The loss of firmness was catogerised as hard, turning soft and soft in mangoes and as firm and shrivelled in capsicums.

PHYSIOLOGICAL STUDIES

(a) Physiological loss in weight (PLW)

Ten fruits in each treatment were weighed at the beginning of the experiment and at regular intervals during storage. Their loss in weight was recorded and expressed as % weight loss from initial weight.

(b) Gaseous concentration inside MA chamber

The concentration of CO_2 and O_2 inside the MA chamber at different storage periods was measured by GC model 5580 (Dual column-Dual detector) conditions were : Thermal conductivity detector, carbowax column at 24°C, detector temperature 38°C, oven temperature 110°C,

injection temperature 100-155°C, dial oven temperature 3.5°C, current 100 milliamps, attenuator 8x and chart speed 1 cm/min.

Respiration

i) Non-destructive method

Rate of respiration of individual mangoes and capsicums was measured by the continuous current method (11). The amount of CO_2 evolved by individual fruits for one hour was calculated by formula

$$\frac{C\ x\ (x-y)}{Wxt}$$

where C is a constant 2.2, i.e. the ml CO_2 required to convert 1 ml of 0.1N $Ba(OH)_2$ to $BaCO_3$

x = titre value of blank

y = titre value of sample

W = weight of fruit in gms

t = time in hrs.

ii) Destructive method

Fifty grams of sliced capsicums (8±2 mm) at different storage periods was kept in a flask having provision to draw gas samples. Oxygen concentration inside the flask was measured after 30 min. by an orbisphere (model 2608) gas phase oxygen indicator. The percentage of oxygen consumed was calculated.

(d) Membrane integrity

Membrane integrity of the tissues at different ripening periods was determined by the leakage of K^+ ions (1). Fruits were cut into equal sized pieces. 20 g of tissue was placed in 40 ml distilled water and shaken gently for 30 min and filtered. The filtrate was assayed for K^+ concentration on a flame photometer.

TEXTURAL STUDIES

Mangoes

i) Whole fruit compression

This was done on Instron Food Texturometer (Model 1140). Whole fruits were compressed with a flat, circular 25.54 cm^2 cross section plunger. Force required corresponding to a deformation of 0.5 cms was recorded and expressed in gm per unit cross section area ($g.cm^{-2}$).

ii) Compression of cut fruits

(1) Test with General Foods Texturometer: Fruit slices (8±2 mm) were obtained with an automatic fruit slicer from both cheeks of the fruit. Compression test was carried out with a flat, cylindrical 13 mm diameter plunger to a final clearance of 2 mm. Each slice was tested at several points. Five fruits were tested for each treatment. The results were expressed as Compression force = kg/V, where kg = height of the peak; v = voltage used.

(2) Test with Instron Foods Texturometer: One cm thick and 2.54 cm dia. discs of mango flesh were used. Cylindrical flat plunger with 25.54 cm^{-2} cross section was used to a final clearance of 2 mm. The first peak or the point of major slope change was recorded and the results expressed as $g.cm^{-2}$.

(3) Magnus Taylor Puncture Test

This was carried out on Instron Universal Food Testing machine with a flat cylindrical plunger of 1.27 cms diameter to puncture mango flesh. The first peak was recorded and the results expressed as $g.cm^{-1}$ (3).

In all the above tests (conducted on Instron Texturometer) the

cross head speed was 5 cm min^{-1} and the chart speed was 10 cms/min. The orientation of fruits and slices were kept constant.

Capsicum

(a) Whole fruits

Methodology was standaridsed for capsicums. As the shape and surface of capsicum fruit is uneven, it is difficult to draw a dimensional data. Hence, weight of the fruits was taken for representation and Kramer shear tests were carried out. The peak forces from the force deformation curves were recorded.

Capsicums after removal of placenta were sheared across the vertical axis. Samples were kramer sheared at 100 mm min^{-1} with recording at 2:1 speed in the proportion mode. From the force deformation curves, peak force ($N.kg^{-1}$), work done ($J.Kg^{-1}$) and 1/2 peak width ($m.kg^{-1}$) were calculated.

(b) Cut fruits

(a) Test with Warner Bratzler Shear Press: Pieces of 1 cm x 4 cm were cut radially from capsicums and sheared in Warner-Bratzler Shear Press and their shear force expressed as $lbs.cm^{-2}$. Five fruits were taken under each sample.

(b) Test with Instron Food Texturometer: Capsicums were cut radially into 3-5 pieces, weighed and Kramer sheared on Instron Model 1140. From the resultant force and deformation curves, three parameters viz. peak force ($N.kg^{-1}$), work done ($J.kg^{-1}$) and 1/2 peak width ($m.kg^{-1}$) were measured as in the whole fruits.

CHEMICAL CONSTITUENTS

Chemical constituents of mangoes and capsicums were analysed in composite samples of 3-5 randomly selected fruits. Minimum of 3 replicates were analysed for each sample. The data were subjected to statiscal analysis. The mean values are presented. For some analysis freeze dried samples were used.

(a) pH

pH of the blended samples was determined on a Toshniwal pH meter with combination electrode using a Standard Buffer (pH 4.0).

(b) Titratable acidity

This was determined by AOAC method (16) with 0.1N NaOH and phenolpthalein as indicator. Acidity was expressed as % malic acid.

(c) Total soluble solids

The percent „ soluble solids was measured with Abbe's hand refractometer (0-50 range) after straining a few drops of blended sample through tissue paper and expressed as °Brix.

(d) Chlorophylls

Chlorophylls were estimated as described by Ranganna. (17) Chlorophylls were extracted in 80% acetone and phase partitioned in to diethyl ether. Absorbance of chlorophyll a and b was measured at 642.5 nm and 660 nm respectively using Bausch & Lomb Spectronic 20 colorimeter.

(e) Carotenoids

Carotenoids were estimated as described by Ranganna. (17) Carotenoids were separated into petroleum ether and their absorbance measured at 645 nm.

The various carotenoid components were separated according to the procedure of Tomes (23). Total carotenoids were extracted in cold acetone:n-hexane mixture 75:60 (v/v) in a Sorwall Omni Mixer and filtered through a sintered glass funnel using suction. The pooled extracts were freed of acetone by washing in distilled water and dried using anhydrous sodium sulphate.

A portion (25 ml) of the hexane layer was shaken with small volumes (10-15 ml) of 85% aq. methanol to separate free xanthophylls until the methanolic washings were colourless. The methanolic layers were pooled and made to a known volume.

To separate xanthophyll esters, the hexane upper layer was shaken overnight at room temperature in a rotary shaker with 25 ml of 20% KOH in 85% aqueous methanol. After separating the methanolic lower layer, the upper layer of hexane was reextracted with aqueous methanol to ensure complete removal of hydrolysed xanthophyll esters. Methanolic phase was pooled and made up to volume. The hexane phase, containing carotenes, was freed of methanol and alkali by washing with distilled water and dried over anhydrous sodium sulphate.

Relative concentrations of free xanthophylls, xanthophyll esters and β-carotenes were determined by absorbance at 436 nm in Beckman Model 35 spectrophotometer.

(g) Ascorbic acid (Vitamin C)

This was estimated by visual titration with 2,4-dichlorophenol-indophenol dye (14).

(h) Alcohol insoluble solids (AIS)

They were estimated by AOAC method (16). The residue (AIS) was washed with ethanol then with acetone and dried to constant weight in a vacuum oven at 45°C.

(i) Carbohydrate in AIS

100 mg of the AIS was hydrolysed in 72% sulphuric acid and the concentration was diluted to 10% by adding distilled water (20). Total sugars were then estimated by phenol-sulphuric acid test (5).

(j) Starch

Samples were extracted in ethanol to remove free sugars. Starch was hydrolysed using HCl and estimated as invert sugars (17).

% starch = % reducing sugars x 0.9

(k) Cellulose and hemicellulose

These were estimated in the freeze dried samples by the method of Southgate ***et al.****, (20).*

Samples were extracted in ethanol and the residue tested for starch. The starch free samples were extracted in hot water and then with 1N H_2SO_4*. These two filtrates were pooled and estimated for their free sugars by phenol sulphuric acid test representing the hemicellulosic fraction.*

The residue was then hydrolysed with 72% H_2SO_4*. The free sugars were estimated as mentioned earlier. This gives cellulose as glucose residues.*

(l) Pectin

Pectins were estimated from the AIS essentially by the method of Mc Cready and Mc Comb (13). 500 mg of AIS was added to 2 ml of ethanol and 100 ml 0.5% EDTA and pH adjusted to 11.5. It was stirred for 2 hrs. on a magnetic stirrer. Then the pH was lowered to 5 with

HCl, 0.2 ml pectinase enzyme was added and stirred for 3 hrs on a shaker. Volume was made up and filtered. Pectin in the filtrate was estimated as galacturonic acid residues.

(m) Free sugars

The samples were extracted with 95%, 80% and 65% ethanol successively on boiling water bath for 1 hr. each time and filtered. The filtrates were pooled, concentrated and estimated for free sugars by phenol sulphuric acid method (5). Reducing sugars were estimated according to Nelson (15).

(n) Ash content

The total ash content of the samples was estimated according to AOAC (16).

Potassium content in ash was determined by Ranganna's method (17). Ash obtained from 5 g dry weight of the samples was boiled in 10 ml conc. HCl and suitably diluted with double distilled water. The quantity of potassium was determined using a flame photometer. Standard graph was prepared from standard KCl solution.

(o) Epicuticular waxes

The waxes were extracted according to the method of Otmani and Coggins (6). Uniform sized fruits were weighed and then immersed successively in 3 beakers, each containing 500 ml of chloroform at 45°C for 20±2 sec. Chloroform-wax extracts for each replicate and treatment were pooled, filtered through Whatman No.1 paper, evaporated to dryness and weighed for total wax.

A known amount of the wax was dried under N_2, hexane was added to this and shaken. Soft waxes dissolved in hexane. Hard wax remained undissolved.

(p) Lipids

Lipids from the pulp of mangoes and capsicums were extracted, purified and analysed according to the procedure described by Mahadevappa and Raina (12). Ten g. of the dry samples were soaked in water overnight, homogenized and extracted thrice in 4 volumes of chloroform: methanol (2:1 v/v). The solvent layer was pooled and concentrated. Lipid was redissolved in chloroform and washed twice with 0.74% aqueous KCl. The chloroform layer was dried over anhydrous sodium sulphate and finally taken in a known volume of chloroform.

Neutral (NL) and Polar Lipids (PL) were resolved by TLC. Neutral lipids were separated using petroleum ether:solvent ether:acetic acid (80:20:1) solvent system in which, PL remained at the origin. The resolved NL (excluding the origin) were scrapped off extracted in chloroform: methanol (2:1), dried and weighed.

Fatty acid methyl esters of neutral and polar lipid fractions were prepared by the method of Kates (10) and were analysed by GLC on Packard Gas Chromatograph Model 237 with flame ionisation detector. Nitrogen flow was 20 ml/min. 7 ft x 1/8" column with 10% w/w DEGS coated on chromosorb W, at 180° was used. Peaks were identified by comparing with authentic standards.

(q) Acetylene estimation

Initial studies have indicated that with the type of column material available during the investigation, it was not possible to separate acetylene and ethylene by GLC. It was therefore necessary to standardise a method for estimation of acetylene colorimetrically in mango samples. A colorimetric estimation of acetylene was developed based on the procedures described by Reid and Salmon (18) and Feigl (7) to suit our experimental conditions as described below.

Reagents:

(A) 1.5 g cupric chloride and 3 g ammonium chloride in 20 ml conc. ammonia, diluted to 50 ml with water.

(B) 5 g hydroxylamine hydrochloride in 50 ml water.

Ammoniacal cuprous solution: 1 ml of solution (A) + 2 ml of solution (B) - freshly prepared.

Standard acetylene solution: was prepared by bubbling acetylene released from 10 g of calcium carbide into 100 ml water for 15 min., 1 ml saturated solution contained 1180 µg C_2H_2 (7).

Standard curve: was prepared by developing the colour with graded concentrations of saturated acetylene solution in water. Colour was developed as follows:

1 ml of acetylene solution + 1 ml 20% Tween-80 + 1 ml water mixed with 3 ml of ammoniacal cuprous solution. Brick red colour developed was measured at 570 nm in Spectronic-20 colorimeter. Tween-80 was added to hold the red cuprous acetylide in suspension as a protective colloid.

Estimation of acetylene in mango pulp: 20 g of mango pulp (from acetylene treated fruits) was homogenised with 100 ml ethyl alcohol. The alcohol was immediately filtered and the filtrate tested for acetylene. The recovery of externally added acetylene was also carried out.

BIOCHEMICAL STUDIES

Enzymes

(a) Pectinase

Enzyme extracts were prepared by suspending 3 g acetone powder in 25 ml 0.1M phosphate buffer (pH 7.8) and stirred for 2 hrs at ice cold temperature. The filtrate served as the enzyme source.

Pectinase assay was done by viscometric method (25). 20 ml of 1% purified pectin solution was prepared in 0.1M sodium citrate - citric acid buffer (pH 4.0). 2 ml enzyme source was added and incubated at 40°C for 30 min. Boiled enzyme solution served as control. Flow time of the reaction mixture was determined by using Ostwald Viscometer. Percentage reduction in viscosity was calculated by the formula

$$\frac{T - T_1}{T} \times 100$$

where T *- flow time in sec /ml of control*

T_1 *- flow time in sec/ml of reaction mixture*

(b) Cellulase

Enzyme extract was prepared as mentioned above. Enzyme activity was measured by the formation of sugars from cellulose as substrate. Sugars formed were estimated by DNS method (22).

Cellulase activity was assayed as follows: Enzyme solution (0.5 ml) + citrate buffer pH 4.8 (1 ml) were added to paper strips (cellulose) and incubated for 1 hr at 50°C. Then 2 ml of DNS reagent was added and kept in a boiling water bath for 5 min cooled, diluted and colour developed was measured at 540 nm against reagent blank. Reducing sugars as glucose were measured and quantified by the standard graph.

Metabolic experiments

The radio-labelled compounds (U-^{14}C aspartate, U-^{14}C malate, 1,5-^{14}C citrate and ^{14}C acetate) were suitably diluted in 0.4M mannitol to give 1.8 µci/0.05 ml. 0.05 ml of the radio-labelled compound was injected with a syringe into the fruit through the stalk region and vacuum infiltrated. The amount of label entering in to each fruit was calculated by substracting the counts of syringe wash from the total counts taken initially in the syringe for injecting into the fruit (19).

The fruits injected with labelled compounds were kept in disiccators individually fitted with an inlet for air and outlet for CO_2. The $^{14}CO_2$ released was trapped for 7.5 hrs in 3N NaOH by applying suction (24). The fruits were then removed from desiccators and checked for microbial contamination. The pulp of sound fruits was homogenised and 25 g of pulp fixed in alcohol. Amino acids, organic acids and sugars were extracted thrice in 90, 70 and 60% simmering alcohols for 60, 30 and 20 min respectively. The alcohol extracts were pooled, concentrated to 10 ml and centrifuged. The clear supernatant was separated into amino acids, organic acids and sugar fractions by ion exhcnage chromatography. Each fraction was suitably concentrated, 0.5 to 2.0 ml of which was taken in 15 ml scintillation medium and counted in a Beckman LS 100C liquid scintillation system. The $^{14}CO_2$ fraction collected in NaOH was precipitated with $BaCl_2$ as $Ba^{14}CO_3$ and analysed for radio activity (19).

FLAVOUR ANALYSIS

(a) Capillary GCMS

Volatiles were extracted from the blended mango pulp in water by vacuum steam distillation at 80 ± 5°C. The distillate was extracted into methylene chloride by partition method and dried over anhydrous sodium sulphate and concentrated. Concentrated volatile fraction was analysed by capillary GCMS, on VG Micromass 70-70 using 25M carbowax 20M capillary silica fused column. Ionisation energy was 20eV, trap current 200 μA, source temperature 200°C, accelerating voltage was 54.02 KV with a resolution of 1000 and scan speed of 0.7 sec/decade. Temperature programme was 60°-5-6-180°-10 with Helium flow of 0.5 kilopounds/cm^2.

Compounds were identified by interpretation of their mass spectral data followed by comparison with the mass spectra of authentic samples (4,21).

(b) Experiment with ^{14}C labelled linoleic acid

Semi-ripe mango fruits of both control and treated groups were injected with 0.05 ml of ^{14}C linoleic acid in 0.4M mannitol solution. The fruits were allowed to metabolise for 7 hrs. After which the pulp of the fruits was homogenised. One hundred g of the pulp was vacuum steam distilled to collect the volatiles. A known volume of steam distillate was added to scintillating medium and counted on Beckman LS 100C liquid scintillation system and % incorporation of ^{14}C linoleic acid into volatile fraction of mangoes was calculated.

PATHOLOGICAL STUDIES

(1) Microbial spoilage

The nature of spoilage during ripening and storage of mangoes and capsicums was recorded. Fungi responsible for spoilage were isolated and identified.

(2) Estimation of surface mycoflora

Capsicums without any treatment, pre-treated with 500 ppm Captan + 1000 ppm. Streptocycline and fruits kept under MA after pretreatment were studied for their surface mycoflora by serial dilution technique.

(3) In vitro studies

***In vitro** studies were carried out on **Alternaria tenuis** and **Fusarium oxysporum** which were originally isolated from infected capsicums stored in MA.*

Medium

Potato dextrose agar (PDA) medium was prepared by the standard method.

Storage environment (MA)

MA was created ***in vitro*** *in an air tight steel chamber of vacuum oven (10 litre capacity) having an inlet and outlet for gases, fitted with flow meters and control valves. The chamber was evacuated and* CO_2 *and* O_2 *in the required proportion were released from cylinders using flow meters.* N_2 *was used as a filler gas. The gas concentrations used were:*

(a) 7.5% O_2 *with 3.5%, 4.0%, 4.5% and 5.0%* CO_2 *respectively, and*

(b) 3.5% CO_2 *with 5.5%, 6.5%, 7.5% and 8.5% of* O_2 *respectively.*

The control groups were stored in air.

Spore suspension

Spore suspensions of ***A. tenuis*** *and* ***F. oxysporum*** *were made by flooding 7-day old cultures with sterile distilled water. The spore concentration was adjusted to 3000/ml counts by hemocytometer.*

(a) Spore germination test

The suspensions were diluted from 10^{-1} *and* 10^{-7} *to standardise optimum spore concentration for spore germination tests (8). 0.1 ml of spore suspension of suitable dilution was pipetted in to petri dishes (100 mm) containing 20 ml of solidified PDA and then uniformly spread using a 'hockey stick'. The seeded dishes were incubated in MA chambers at ambient conditions. The dishes were removed after the treatment period and counted for number of colonies by a colony counter.*

(b) Mycelial growth test

The fungicidal/fungistatic effect of MA on the linear mycelial extension was measured according to Follstad (8). Inoculum plugs (5 mm dia.) were taken from the margin of 5-day old culture of the fungus maintained on PDA. They were inoculated on to the centre of petridishes containing 20 ml solidified PDA medium. The dishes were incubated in

MA chambers at ambient temperature and humidity. Linear measurements of colonies in cms. were taken at the end of storage period. The % inhibition or stimulation under MA was calculated by comparing with respective controls stored in air.

SENSORY EVALUATION

(a) Mangoes

The study was conducted on 3 harvest maturities (early, middle and late) and two categories (floaters and sinkers) under each maturity except in late harvest group. The fruits were evaluated at ripe stage. Ripening stage of samples was determined by examining the fruits regularly by a small panel.

A descriptive quality profile procedure (score card Fig.1) was developed and used to evaluate the ripe fruits for individual quality attributes of whole fruits and slices of each sample. Data were compiled and analysed by two-way and three-way analysis of variance to compare quality characteristics between floaters and sinkers, control and treated and among 3 maturities. Significant findings were interpreted in relation to optimal level of quality for individual attributes.

(2) Capsicum

Sensory evaluation of capsicums was carried out after harvest and at the end of storage period for control group. MA stored fruits were evaluated on 13th day soon after removal from MA chamber and at the end of their storage period (16 days). Their quality was assessed by a panel of 10 judges and the results subjected to statistical analysis.

Fig. 1 SENSORY EVALUATION SCORE CARD

Name:

(A) DESCRIPTIVE EVALUATION OF MANGOES— WHOLE FRUIT

Evaluate each sample through the descriptors in the scales below by crossing and writing the sample code at any position judged appropriate

Colour and appearance

Green, yellowish green	Glossy, greenish yellow	Glossy appealing yellow, reddish yellow, orange yellow	Slightly dull	Dull, shrivelling, not appealing, black spots, discoloration

Odour (Smell)

Green unripe	characteristic weak, slightly unripe	characteristic strong	characteristic less fresh, slightly overripe	Overripe, foreign/ off odour odour

Firmness (Finger feel)

Hard	Slightly hard	Firm optimal	Tending to soft	Soft

Overall quality

Excellent	Very good	Good	Fair	Poor

Indicate defectives in each sample, if any, by writing the sample code above the appropriate descriptiors given below:
Discolouration, black spots, shrivelling

Signature

(B) DESCRIPTIVE EVALUATION OF MANGOES CUT PIECES

Evaluate each sample through the descriptors in the scales below by crossing and writing the sample code at any position judged appropriate

Colour

Uniform, deep yellow, orange yellow, reddish yellow	Uniform, less deep yellow	Non-uniform pale/ yellowish white, white patches, discolouration

Compactness (Visual)

Compact, clean-cut-edge	Not so compact, frayed cut-edge	Not compact, centre softening

Texture

Hard	slightly hard	firm, smooth	slightly soft	mashy

Aroma

Raw, greenish	Typical, weak, slightly unripe	Typical, full intense	Typical, less fresh, slightly overripe	Over ripe, off/foreign

Taste

Raw, sour	Slightly sour, unbalanced	Optimally sweet, balance	Not sour, less sweet, unbalanced	Flat, bland, off/foreign

Overall quality

Excellent	Very good	Good	Fair	Poor

Indicate defectives in each sample, if any, by writing the sample code above the appropriate descriptors given below:
Non-uniform colour, discolouration, white patches, centre softening, foreign/off aroma, foreign/off taste.

Signature

STRUCTURAL STUDIES

(i) Light microscopy

Slices (1 sq.cm.) of mangoes (which includes epicarp and mesocarp) and capsicums (including epicarp, mesocarp and endocarp) at identical regions from both control and treated fruits were cut and fixed in formalin-acetic acid-alcohol (FAA) (5:5:9) for 24 hrs. and stored in 70% alcohol after thorough washing in the same solution. Serial sections of preserved tissues were obtained by microtomy according to Johanson (9).

The tissues were dehydrated with tertiary butyl alcohol and embedded in paraffin (MP 52-56°C). Sections 10-15 μ thick were cut using rotary microtome. They were stained for the cell walls following Foster's tannic acid - Ferric chloride method (9). Slides were mounted in DPX mountant and oven dried.

Sections were observed under binocular light microscope, unsectioned tissues were observed for topographical changes under stereo binocular microscope. Photomicrographs of required stages were taken.

(ii) Scanning electron microscopy

Samples used for SEM were either freeze dried (6) or fixed in FAA followed by osmium tetroxide treatment. They were dehydrated in acetone series, air dried and mounted.

The mounted samples were gold sputter coated on Polaron spotter (300-500 angstrom thick) and observed under Cambride Stereoscan 150, operated both at 10 and 20 KV.

CHAPTER 3

ACCELERATED RIPENING OF MANGOES BY ACETYLENE

Acetylene released from calcium carbide is extraneously used to ripen 'Alphonso' mangoes. It is not a novel method but, is used extensively in commercial trade. Although it is evident that acetylene induces uniform and early ripening in mangoes, controversies exist regarding the quality aspects. This is mainly due to paucity of scientific knowledge regarding effect of acetylene treatment. The present investigation accomplishes the ripening changes in 'Alphonso' mangoes exposed to acetylene at a commercial dosage. Several quality attributes like colour, texture, taste, aroma and associated chemical and biochemical changes induced by acetylene have been discussed in the chapter.

INTRODUCTION

India ranks first in the world in production of mangoes. Mango crop in India is ca 60% of the total area of fruit cultivation. Alphonso variety of mangoes are highly priced and most preferred both for table and processing purposes. But, they suffer from inherent qualities of non-uniform and delayed ripening. Ethylene and hot water are known to induce early and uniform ripening in mangoes. However, they aggravate spoilage and hence not commonly employed. In commercial trade calcium carbide is used widely in India and many other countries to achieve this. In addition, Calcium carbide is also used to ripen bananas, citrus fruits and tomatoes. There are only few scientific reports on the effect of calcium carbide on fruit ripening and mangoes in particular. The existing reports are controversial. Some have reported improvement of organoleptic qualities while others have reported impairment of organoleptic qualities. The present work therefore envisages the effect of CaC_2 treatment on the physical, biochemical and organoleptic qualities of Alphonso mangoes.

RESULTS

Mangoes (var. Alphonso) can be induced to ripen early and uniformly by exposure to acetylene. The effect of exposure of mango fruits to acetylene (in the form of CaC_2) was studied over a period of 3 seasons i.e. 1982, 1983 and 1985. To elucidate the response of mangoes to acetylene, various chemical, physical and physiological studies were carried out at different ripening stages. The results are presented below.

PRELIMINARY STUDIES

Preliminary studies were carried out to ascertain the advantage of commercial dosage and duration of exposure of mangoes to acetylene. The possibilities of reducing the concentration and duration of acetylene exposure were also studied.

Fruits were exposed to CaC_2 concentration of 1 g/kg (T_1) along with 2 g/kg (T_2) for a period of 4 days and subsequently, ripened under ambient conditions. Results are presented in Table 1. It is evident from the table that the treated fruits were superior to control fruits in marketability. In all the groups, sinkers were better than floaters. In view of the better performance of T_2 over T_1, another experiment was conducted to reduce the duration of exposure with group T_2 only.

Fruits were exposed to 2 g/kg. CaC_2 for a period of 2 days and 4 days and then allowed to ripen under ambient conditions. Their

Table 1. Quality of mangoes exposed to acetylene: effect of concentration*

Observations	Raw fruits	Ripe fruits* Control		T_1		T_2	
		floaters	sinkers	floaters	sinkers	floaters	sinkers
TSS (°Brix)	7	11	16	11	19	13	13
Acidity (% Malic acid)	2.47	0.55	0.18	0.75	0.22	0.31	0.17
Carotenoids pulp (µg/100 g f.wt.)	578	11,292	12,998	7,769	9,044	7,463	9,719
% Marketability (on 8th day)	-	17	22	59	100	91	100
Overall sensory quality (scale 1 to 5)	-	2.8	3.2	2.4	2.6	2.6	2.8

* T_1 Fruits exposed to 1 g/kg CaC2 for 4 days; T_2 - Fruits exposed to 2 g/kg CaC_2 for 4 days; Ripe stage for control floaters is 17 days for control sinkers 14 days and for sinkers and floaters of group T_1 and T_2, 7 days.

behaviour is depicted in Table 2. Even here, the treated fruits were superior (52.6% for Group 1 and 96.4% for Group 2) in marketability to control fruits (13.0%) on 8th day. Among the two treatments, 4 days exposure was more effective than 2 days exposure.

The above experiments revealed that CaC_2 at the concentration of 2 g/kg for a period of 4 days was optimum to achieve highest marketability. This is also the concentration used in commercial trade of mangoes. Hence, detailed studies were conducted with the above said treatment. Further, its effect was studied on fruits at 3 harvest stages (early, middle and late part of the season) and for floaters and sinkers. The abundance of sinkers in early, middle and late harvests was 55, 58 and 90% respectively. Hence, floaters were not considered in late harvest group.

Table 2. Quality of mangoes exposed to acetylene: Effect of duration.

Observation	Raw fruits	Ripe fruits* Control	Group 1	Group 2
pH	2.54	4.51	4.53	4.56
TSS (°Brix)	7.0	19.0	17.0	17.0
Acidity (% malic acid)	2.29	0.13	0.17	0.15
P.L.W (%)	0.0	10.50	10.55	10.28
Chlorophylls-Peel (mg/100g)	113.90	20.26	19.99	8.32
Carotenoids-Peel (µg/100)	-	5,989	6,364	12,869
Carotenoids-Pulp (µg/100g)	1,565	11,799	7,209	7,594
Texture**		without peel		
Compression force (kg/v)	7.01	0.88	0.65	0.57
		with peel		
	8.88	0.93	0.78	0.59
Marketability (%) on 8th day	-	13.0	52.6	96.4
Overall sensory quality (Score 1 to 10)	-	6.8	5.3	6.3

Group 1 - Fruits exposed to 2g/kg CaC_2 for 2 days; Group 2-Fruits exposed to 2g/kg CaC_2 for 4 days; * Ripe stage for control floaters is 17 days for control sinkers 14 days and for sinkers and floaters of group 1 and 2,7 days; ** measured with General Foods Texturometer.

Changes in colour and appearance

Alphonso mango fruit which is dull, olive green in colour at raw stage turns bright orange yellow at ripe stage. During normal ripening, the change in peel colour is slow and not uniform. This colour change is influenced by many factors like harvest date, maturity and storage conditions. For each harvest, observations were divided into green, 25% yellow, 50% yellow and 100% yellow at different time intervals after harvest. 100% yellow indicates fully ripe marketable mangoes and their percentage in control and treated groups is given in Fig.2.

With acetylene treatment, days required to develop full ripe colour (100% yellow) was reduced to 7 days. In control group, the floaters ripened by 17 days and sinkers by 14 days. Whereas in treated

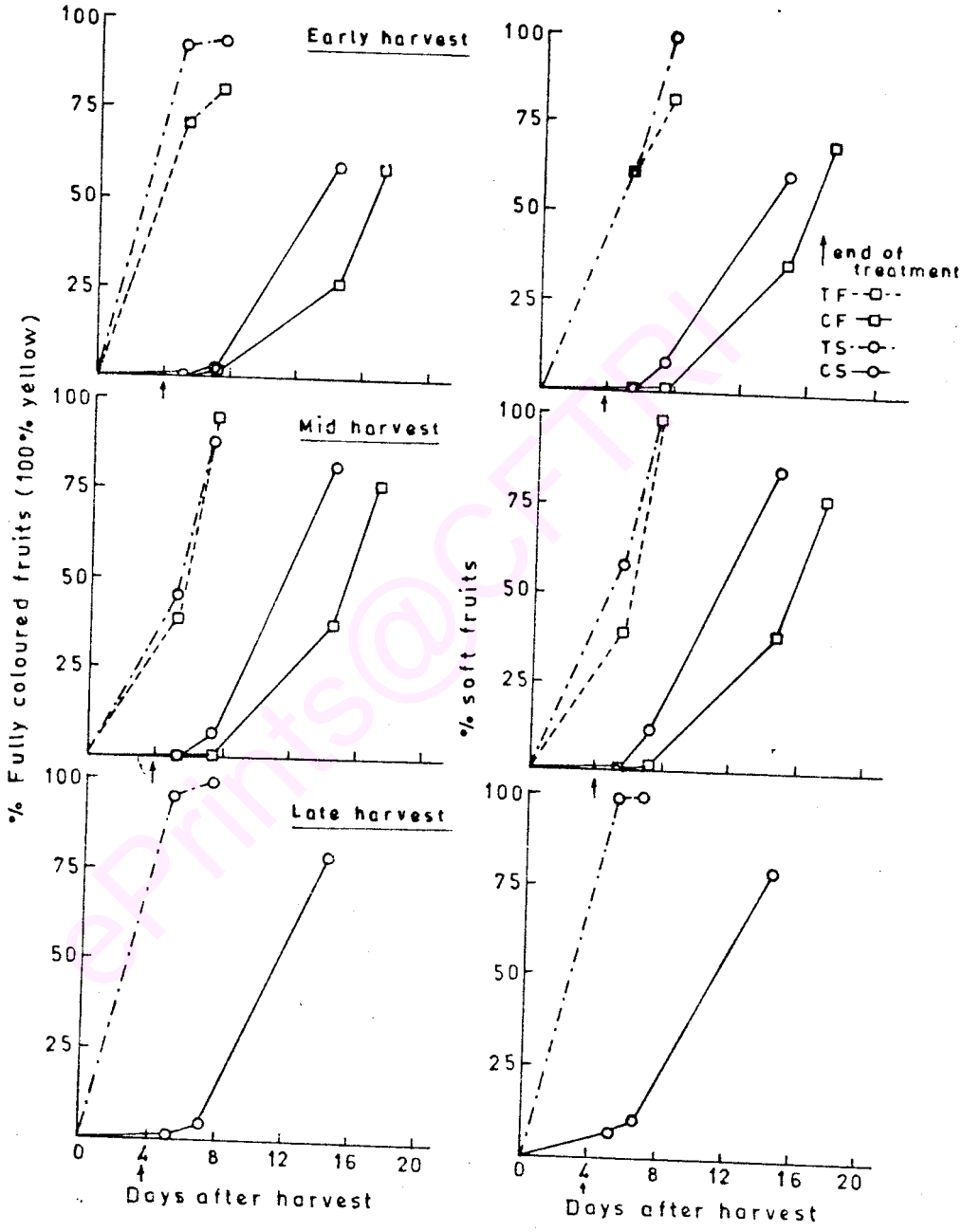


Fig. 2 The percentage of fully ripe coloured and ripe fruits in acetylene treated mangoes

group, both floaters and sinkers ripened on 7th day. Also, the treated fruits developed uniform bright yellow skin colour (Plate 2). The percentage of fully ripe coloured fruits was 60 - 81.4% in control group even after 14 - 17 days, compared to 81.7 to 100% in treated group on 7th day. The percent ripening was higher in sinkers than floaters in both control and treated groups (Fig. 2).

Changes in fruit firmness

Mangoes soften during ripening and hence fruit firmness (as sensed by fingers) is also an index to assess ripening. Control fruits (65 - 85%) attained edible softness by 14-17 days. Acetylene treated fruits softened early with almost all fruits (100%) attaining edible softness by 7th day (Fig. 2).

Rate of softening increased with increase in harvest maturity. Also, sinkers of control group of all the 3 harvests softened earlier (14 days) than floaters (17 days). Both floaters and sinkers of treated group however softened by 7th day. The changes in fruit firmness accompanied changes in colour of the fruit.

INSTRUMENTAL TEXTURE MEASUREMENT

The subjective changes in firmness by finger feel were confirmed by instrumental texture studies. Table 3 presents the results of the three tests carried out at the edible ripe stage. Compression force required for whole fruits decreased from early to mid-harvest and from floaters to sinkers. Control floaters were more firm than treated floaters.

The compression of mango discs revealed a reverse trend with respect to harvest maturity, the early harvest group requiring lesser force than mid-harvest group. In both the harvests however, the control fruit discs offered more resistance to compression force than treated.

a - 4th day immediately after exposure

b - 7th day at edible ripe stage

Plate 2. Acetylene treated 'Alphonso' mangoes

Table 3. Instrumental texture measurements in mangoes treated with acetylene at edible ripe stage*

Method	Category	Early harvest		Mid harvest	
		Control	Treated	Control	Treated
			g/cm^2		
1. Whole fruit compression	Floaters	117	88	87	89
	Sinkers	85	88	66	78
2. Disc compression	Floaters	334	232	382	295
	Sinkers	294	214	316	310
3. Magnus-Taylor Puncture	Floaters	670	444	588	546
	Sinkers	552	464	430	520

* Ripe stage for control floaters is 17 days, for control sinkers 14 days and for sinkers and floaters of treated 7 days.

In control group, flaoters required higher force than sinkers while this difference was narrowed by treatment. Magnus Taylor puncture test also revealed a similar trend. In general control fruits were more firm than treated fruits and fruits of floaters group more firm than sinkers group.

Physiological loss in weight (PLW)

Loss in weight of mangoes increased with increase in storage period. This trend was similar in all the 3 harvest groups. Floaters of both control and treated groups lost more weight than their respective sinkers at ripe stages. Control fruits lost more weight (11-13%) than treated (8-10%) at the end of ripening (Fig. 3).

Respiration (whole fruits)

Mangoes exhibited climacteric pattern of respiration. Control fruits showed a pre-climacteric depression on 5th day and climacteric peak on 9th day. There was declining trend in respiration rate during the post-climacteric period (Fig. 4).

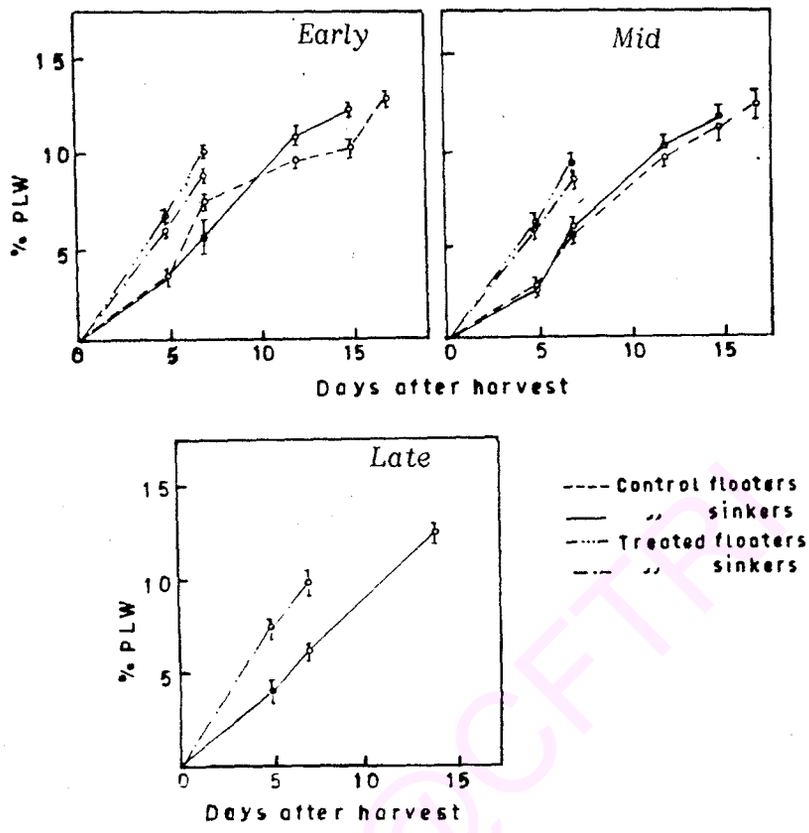


Fig. 3 Changes in physiological loss in weight of acetylene treated mangoes

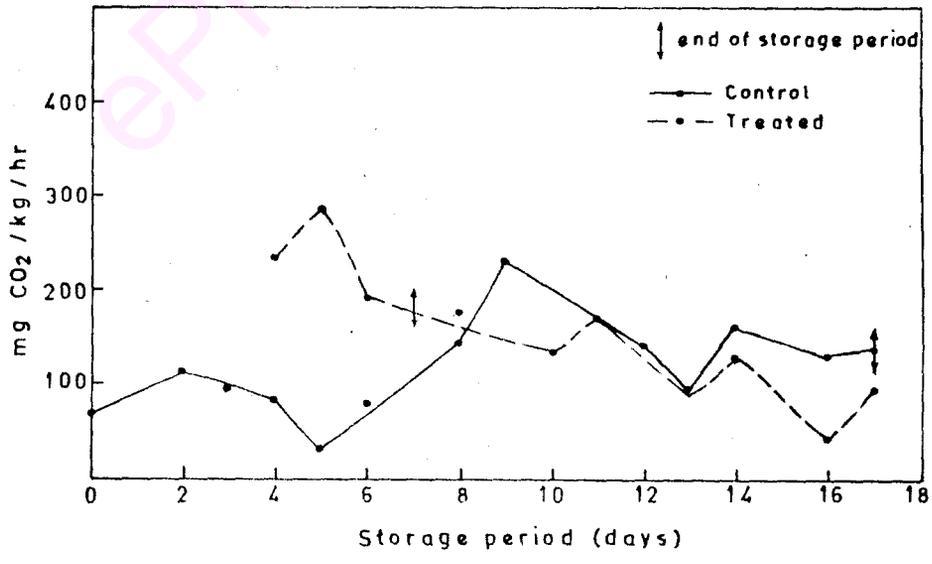


Fig. 4 Respiratory pattern of mangoes treated with acetylene

In treated fruits there was an increase in respiration rate on 4th day and reached the climacteric peak on 5th day. There was a gradual decline in respiration during the post-climacteric period. This preponement in climacteric peak (by 4 days) was also associated with 20% increase in CO_2 evolution. Thus there was preponement of the climacteric peak in respiration due to acetylene treatment. This was also associated with accelerated ripening changes.

Membrane integrity

Integrity of the membranes was measured by the rate of K^+ ion efflux from the fruit slices and the results are presented in Fig. 5. There was increased leakage of K^+ ions with advance in ripening. In the acetylene treated fruits, there was 2-fold increase during the 7 day ripening period. In the control fruits, the amount of K^+ leaked out of the tissues was lower than in treated fruits.

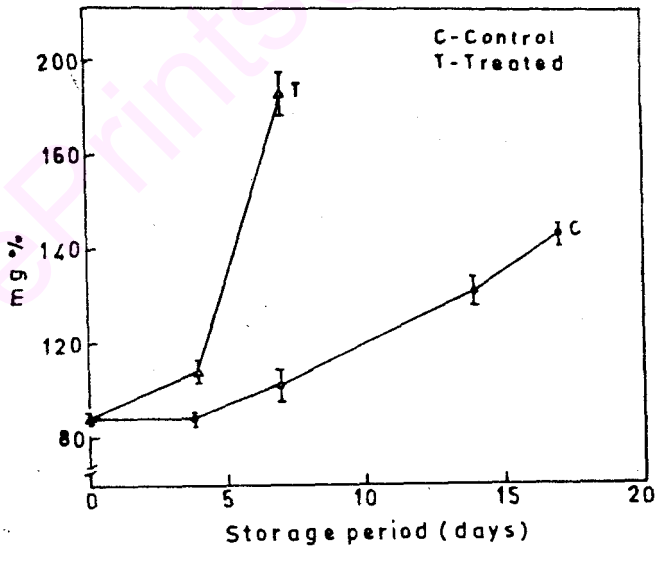


Fig. 5 Potassium (K^+) efflux in mangoes treated with acetylene

CHEMICAL CONSTITUENTS

Changes in pH

The pH of mangoes increased during storage. The pH of raw fruits decreased with advance in harvest date. The ripe fruits of treated group had slightly lower pH than their respective control fruits (Table 4).

Table 4. Changes in pH of acetylene treated mangoes

Harvest	Category	Treatment	Days after harvest				
			0	5	7	14	17
Early	Floaters	Control	3.5	3.3	3.7	4.0	(4.6)
		Treated	3.5	3.6	(4.2)**	-	-
	Sinkers	Control	3.45	3.4	3.7	(4.0)	-
		Treated	3.45	3.55	$(4.2)^{NS}$	-	-
Mid	Floaters	Control	3.15	3.5	3.9	4.2	(4.9)
		Treated	3.15	3.8	(4.6)***	-	-
	Sinkers	Control	3.10	3.7	4.2	(4.8)	-
		Treated	3.10	3.9	(4.6)*	-	-
Late	Sinkers	Control	3.10	3.10	4.6	(4.8)	-
		Treated	3.10	4.0	(4.3)***	-	-

Figures in parenthesis separately for each harvest and category are compared for significant difference: NS-not significant; * 5% level; ** 1% level; *** 0.1% level.

Changes in acidity

Acidity in mangoes, irrespective of treatment decreased considerably during ripening (Table 5). The acid content at harvest was higher in early harvested fruits compared to late harvested fruits and so was their corresponding loss during ripening. Treatment further reduced acidity of fruits.

Table. 5 *Changes in acidity*[+] *of acetylene treated mangoes*

Harvest	Category	Treatment	Days after harvest				
			0	5	7	14	17
Early	Floaters	Control	3.93	2.39	2.29	1.95	(0.30)
		treated	3.93	0.65	(0.39)***	-	-
	Sinkers	Control	4.07	3.65	3.0	(0.36)	
		Treated	4.07	0.65	(0.32)**	-	-
Mid	Floaters	Control	3.93	3.20	2.80	1.63	(0.19)
		Treated	3.93	0.59	(0.26)**	-	-
	Sinkers	Control	3.87	2.56	1.29	(0.19)	-
		Treated	3.87	0.45	(0.30)***	-	-
Late	Sinkers	Control	2.95	2.05	1.44	(0.19)	-
		Treated	2.95	0.60	(0.26)***	-	-

+ % Malic acid; Figures in parenthesis separately for each harvest and category are compared for significant difference
** Significant at 1% level; *** Significant at 0.1% level.

Changes in total soluble solids (TSS)

The TSS of mangoes ranged from 6-9% at harvest and increased to 16-20% at ripe stage (Table 6). The TSS was higher in sinkers than in floaters both in control and treated groups at all stages of ripening. The TSS of ripe fruits was slightly higher in treated than in control fruits.

Carotenoids in peel

The changes in the content of carotenoids in the peel during ripening of control and treated mangoes are presented in Fig. 6 and their composition at ripe stage is given in Table 7.

There was increased synthesis of carotenoids during ripening, transforming a dull, olive green skin colour into bright, orange yellow skin. Sinkers accumulated more carotenoids than floaters irrespective

Table 6. Changes in total soluble solids[+] of acetylene treated mangoes

Harvest	Category	Treatment	Days after harvest				
			0	5	7	14	17
Early	Floaters	Control	7.5	7.5	9.0	12.0	(17.0)
		Treated	7.5	18.0	(18.0)*	-	-
	Sinkers	Control	9.0	10.0	15.0	(20.0)	-
		Treated	9.0	20.0	(20.0)^{NS}	-	-
Mid	Floaters	Control	6.0	6.0	15.0	16.0	(16.0)
		Treated	6.0	18.0	(19.0)***	-	-
	Sinkers	Control	7.5	12.0	14.0	(16.0)	-
		Treated	7.5	19.0	(20.0)**	-	-
Late	Sinkers	Control	8.0	16.0	18.0	(18.0)	-
		Treated	8.0	18.0	(18.0)^{NS}	-	-

+ °Brix; *Figures in parenthesis separately for each harvest and category are compared for significant difference: NS-not significant; * 5% level; ** 1% level; *** 0.1% level.*

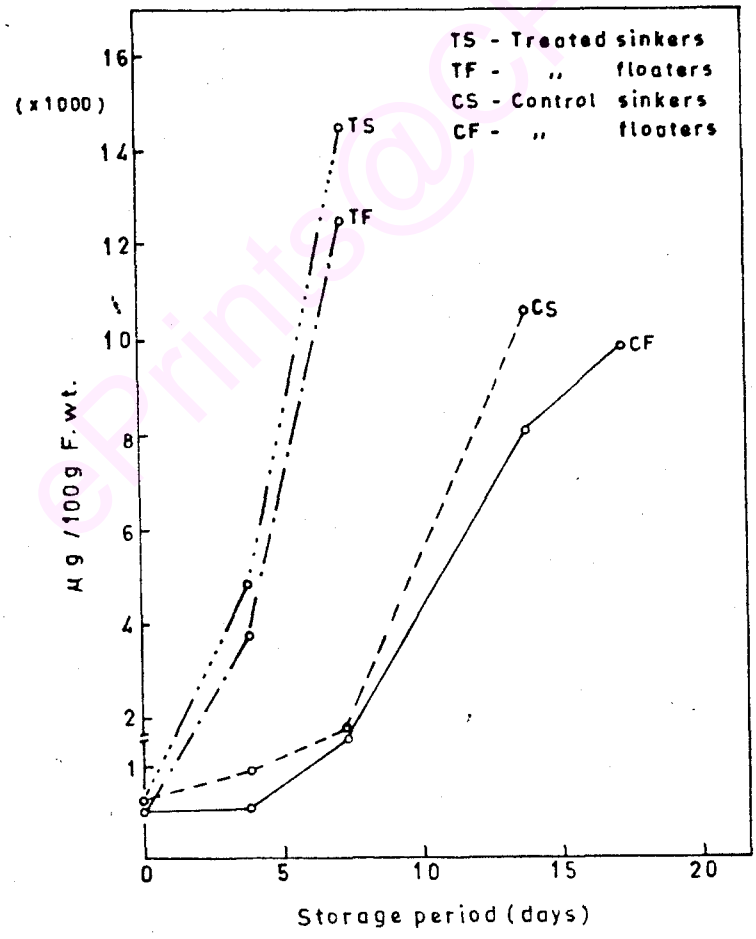


Fig. 6 Carotenoids in the peel of mangoes treated with acetylene

Table 7. Carotenoid composition of ripe mangoes§

Category	Treatment	Xanthophylls	Xanthophyll esters	β-carotenes	Total carotenoids
Floaters	Control	809	2,603	6,479	(9,893)
	Treated	451	4,165	8,099	(12,707)***
Sinkers	Control	694	2,962	6,942	(10,599)
	Treated	566	4,686	9,256	(14,510)*

§ as μg/100 gm f.wt.; Figures in parenthesis separately for each category are compared for significant difference; *** Significant at 0.1% level; * Significant at 5% level.

of treatment. Treatment induced increased accumulation of carotenoids, than in control fruits at all stages; their content being 20-26% higher at ripe stage.

β-carotenes were dominant among carotenoids in all fruits at ripe stage followed by xanthophyll esters and xanthophylls. The treated fruits were found to accumulate more of xanthophyll esters while untreated fruits had more of xanthophylls than treated fruits (Table 7).

Carotenoids in pulp

There was 10-20 times increase in carotenoids in the pulp of mangoes during ripening, converting pale greenish pulp of raw fruits into orange colour at ripe stage. Carotenoid content of raw fruits increased with maturity and harvest (i.e. it was higher in sinkers than in floaters and increased from early to late harvests). The carotenoid accumulation in treated group although very rapid and high in initial stages was lower than control fruits at edible ripe stage. The amount

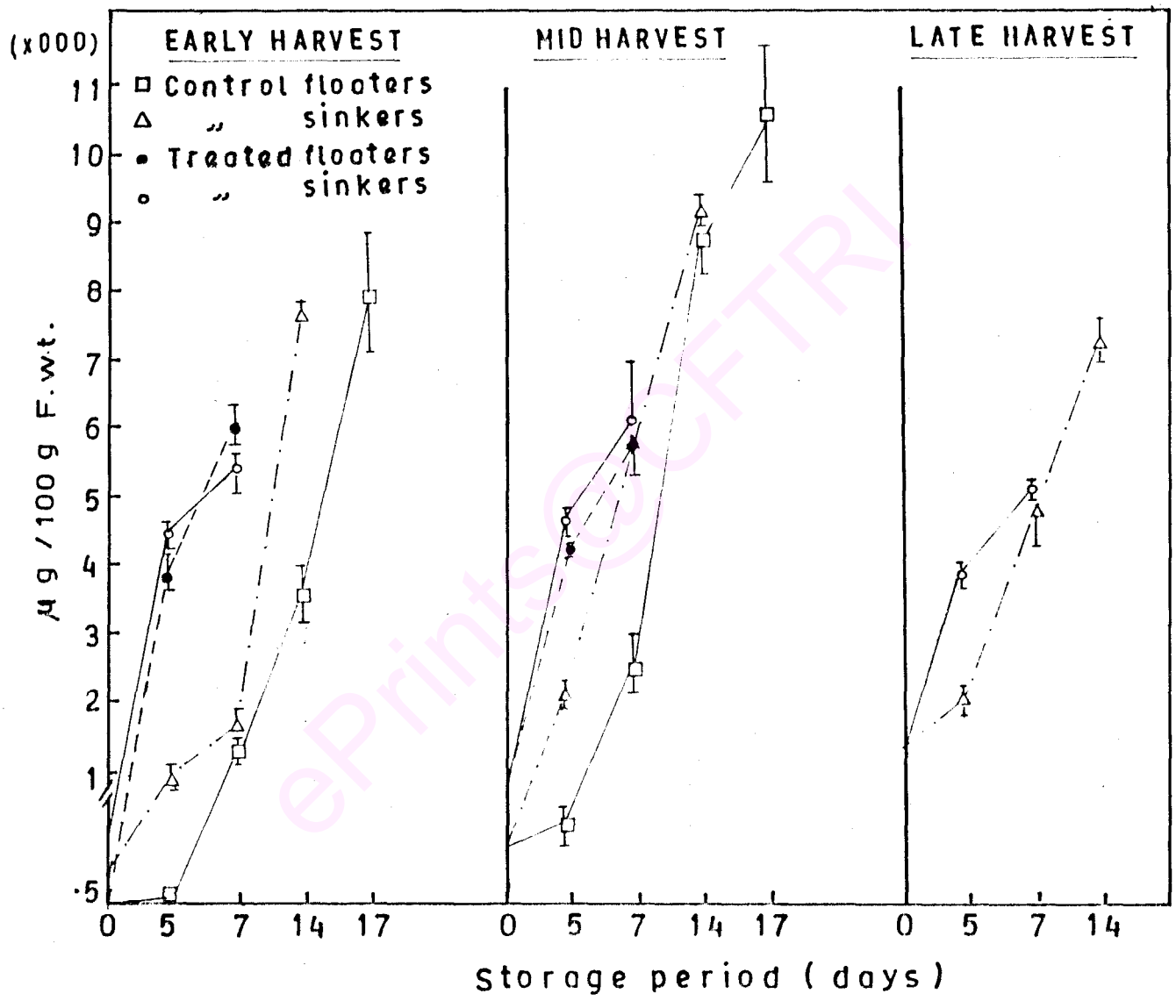


Fig 7. Carotenoid content in the pulp of acetylene treated mangoes

of carotenoids at harvest was higher in the late harvest group than corresponding early and mid harvest fruits. But, it was lower than them at ripe stage (Fig. 7).

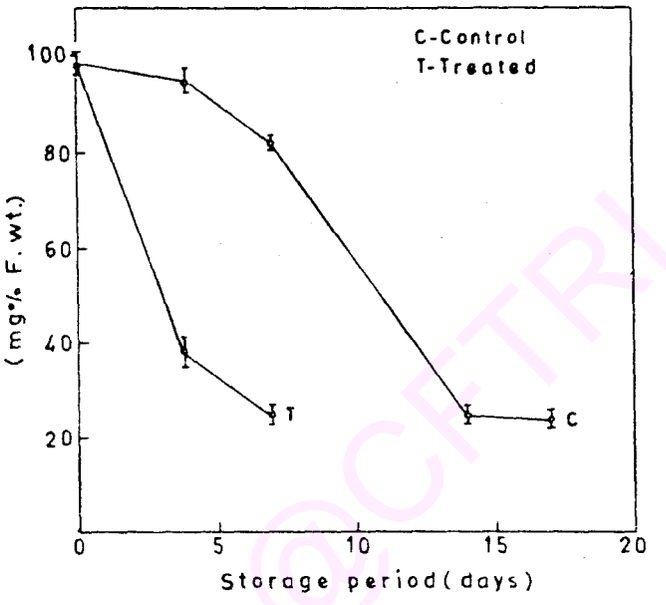


Fig. 8 Changes in ascorbic acid content in acetylene treated mangoes

Ascorbic acid

Vitamin C content which was inappreciable quantities in raw mangoes decreased during ripening from 98 to 25 mg% (Fig. 8). The content of ascorbic acid was higher in case of treated than in control fruits at edible ripe stages.

Alcohol insoluble solids (AIS)

The alcohol insoluble solids mainly constituting the cell wall material and starch showed a significant decline during ripening of

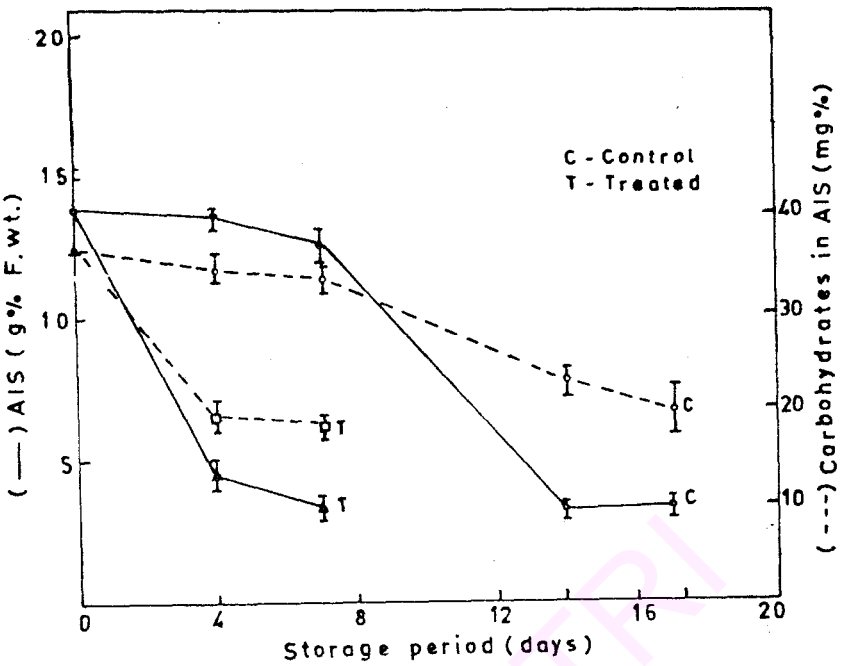


Fig 9. Changes in AIS and their carbohydrates in acetylene treated mangoes

mangoes from 13.0% in mature raw fruit to only 3.18% in ripe mangoes (Fig. 9). At ripe stage, the AIS content of treated fruits was comparable to that in control fruits.

Carbohydrates in AIS

The carbohydrates of AIS, in terms of glucose residues, are presented in Fig. 9. They showed a gradual decline during ripening from 37 to 20 mg%. In case of treated fruits, the reduction was rapid but their content was comparable to control at ripe stage.

Starch

Mango fruits at harvest contain considerable amount of reserve carbohydrates as starch (22.0 g% d.wt). There was significant hydrolysis of starch to form sugars during ripening. At the end of 17 day ripening period, the starch was almost completely hydrolyzed.

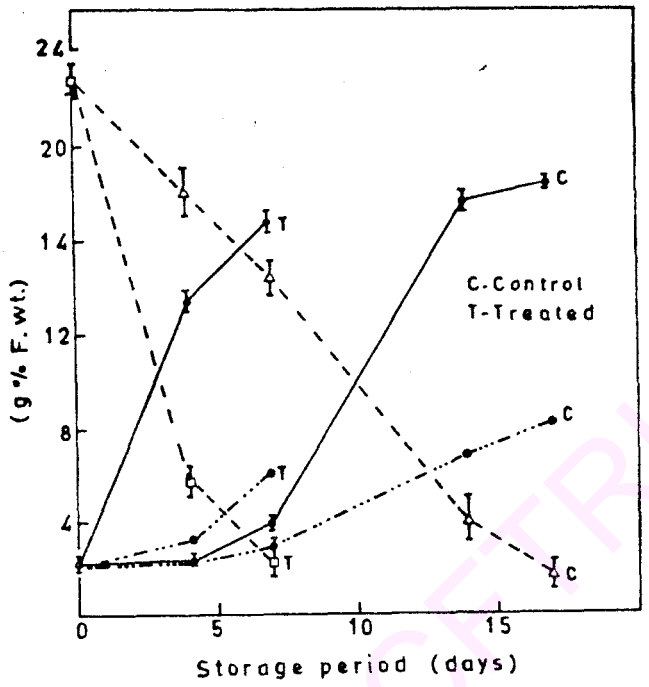


Fig. 10. Changes in starch (--), total sugars (—) and reducing sugars (-...-) in acetylene treated mangoes

In acetylene treated fruits, by the end of 4-day treatment period, 75.4% of the starch was hydrolysed. The amount of starch present in them at ripe stage was comparable to control fruits (Fig. 10).

Changes in sugars

Free sugar formation was considerable during storage. The content increased from 2 to 18% during ripening. In treated fruits, free sugar formation was rapid during treatment period but meagre during the post treatment period ultimately resulting in less free sugars than in control fruits at ripe stage (Fig. 10).

The reducing sugar fraction increased during ripening in both control and treated groups. Reducing sugars were in significantly lower amounts in treated fruits than in control fruits at their ripe stages (Fig. 10).

Changes in pectin

Total pectin content decreased slightly during ripening from 2.0 to 1.06 g % (Fig. 11). Pectin breakdown showed a similar trend in treated fruits and they decreased to 0.98 g% at their ripe stage (7th day).

Ash content

The ash content of mangoes (expressed on dry weight basis) increased considerably during ripening. In treated fruits, the increase was not in par with that of control resulting in significantly lower ash content at ripe stage (Table 8).

Table 8. Ash content of mangoes treated with acetylene*

Treatment	Days after harvest				
	0	4	7	14	17
Control	1.31 0.81	1.39 ±0.03	1.48 ±0.04	2.59 ±0.34	(2.85) ±0.13
Treated	1.31 ±0.18	2.10 ±0.12	(2.06)*** ±0.07	-	-

* g % dry wt. Mean ± S.D.
Compared for significant difference: *** - 5% level

Potassium content in ash of mangoes did not change significantly during ripening in both control and treated fruits (Table 9).

Lipids

The lipid content in the pulp of control and treated fruits rose considerably during ripening (Table 10). In treated fruits, the rate

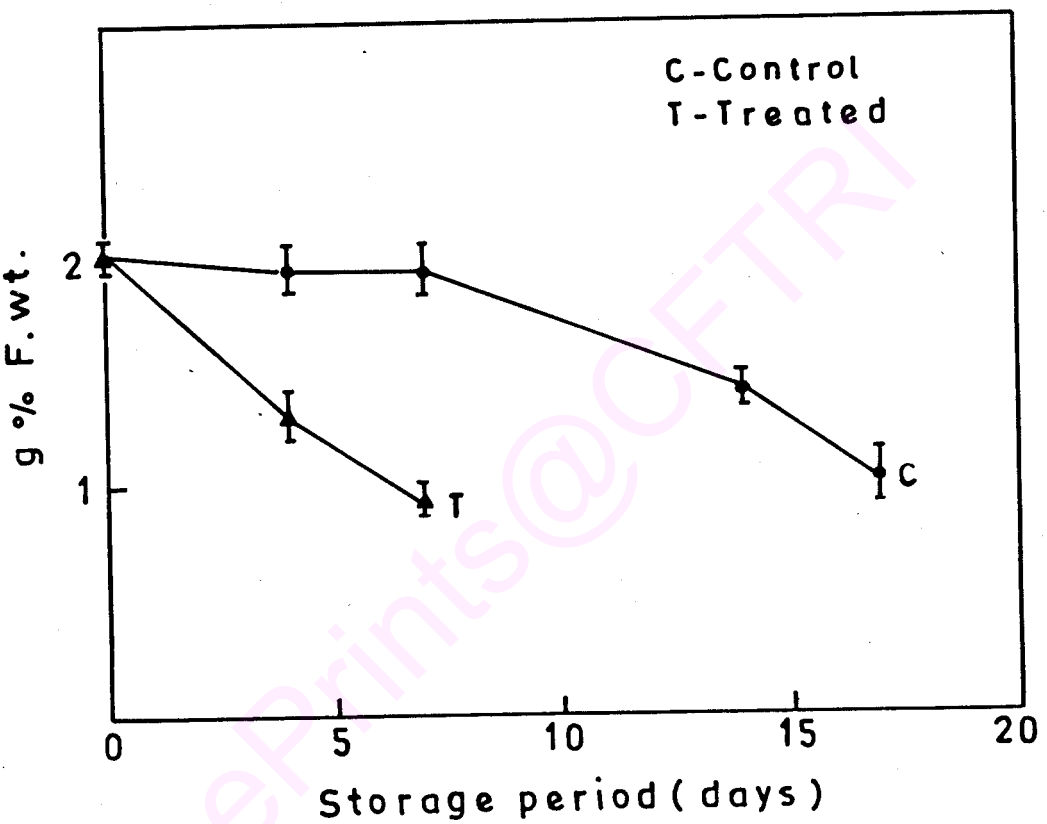


Fig. 11 Changes in pectin content of acetylene treated mangoes

Table 9. Potassium* content of acetylene treated mangoes

Treatment	Days after harvest				
	0	4	7	14	17
Control	10.11 ±0.07)	10.00 ±0.02	9.88 ±0.19	10.23 ±0.02	(10.23) ±0.09
Treated	10.11 ±0.07	10.00 ±0.04	(10.00)^{NS} ±0.01		

* mg/g dry wt. Mean ± S.D.
( ) Compared for significant difference: NS - not significant.

of lipid synthesis which was high during the treatment period was suppressed during the post-treatment period resulting in less lipids at ripe stage compared to ripe control fruits.

Table 10. Total lipids content in pulp of mangoes treated with acetylene (g % dry wt.)

Treatment	Days after harvest				
	0	4	7	14	17
Control	1.91 ±0.04	1.96 ±0.06	2.08 ±0.13	4.37 ±0.18	(5.03) ±0.29
Treated	1.91 ±0.04	3.49 ±0.32	(3.29)* ±0.08		

( ) are compared for significant difference; * Significant at 5% level.

The relative proportion of neutral and polar lipids also varied during ripening. The neutral lipids increased during ripening from 57-86% with concomitant decrease in polar lipids from 43 to 14%. Interestingly, in treated fruits neutral lipid content was lower (71%) but polar lipid content was higher (29%) compared to control ripe

fruits (Table 11).

Table 11. Relative proportion of neutral and polar lipid fraction in acetylene treated mangoes at ripe stage*

Lipid composition	Raw	Ripe	
		Control	Treated
		%	
Neutral lipids	57	86	71
Polar lipids	43	14	29

* Ripe stage for control is 17 days & treated is 7 days.

Fatty acid composition

Twelve fatty acids were detected in the neutral and polar lipids of mango pulp. In raw fruits, palmitic acid was the most abundant (62.3%) followed by oleic (12.1%), and linoleic acids (6.0%), palmitoleic acid was found in traces. During ripening, there was considerable decrease in the content of palmitic and considerable increase in palmitoleic and oleic acids. Linolenic and myristoleic acids also showed an increased content during ripening (Table 12).

During ripening of treated fruits, the trend remained similar. But their content of palmitoleic, linolenic and arachidic acids was considerably higher than in control ripe fruits. The content of oleic acid was however lower. Myristic acid occurred in trace amounts in all the samples. The fatty acid composition of both neutral and polar lipids in all groups showed a similar trend.

Acetylene estimation

Acetylene estimation was done as described in methodology. By this method, a brick red colour developed in the presence of acetylene. The sensitivity of the method was in the range of 45 to 240 µg (within which it followed Beer's law). The standard graph obtained is given in Fig. 12. The colour was stable for an hour in the concentration range of 45 to 190 µg acetylene. Above this concentration,

Table 12. Fatty acid profile of lipids in acetylene treated mangoes

Fatty acid	Neutral lipids* (%)			Polar lipids* (%)		
	Raw	Ripe		Raw	Ripe	
		Control	Treated		Control	Treated
Lauric ($C_{12:0}$)	0.7	1.6	0.5	Tr	0.5	0.5
Myristic ($C_{14:0}$)	Tr	Tr	Tr	Tr	Tr	Tr
Myristoleic ($C_{14:0}$)	1.5	2.7	1.8	1.6	4.2	3.4
Palmitic ($C_{16:0}$)	62.3	25.1	26.5	56.3	25.8	28.9
Palmitoleic ($C_{16:1}$)	Tr	39.0	43.5	Tr	32.0	35.7
Stearic ($C_{18:0}$)	2.4	Tr	Tr	0.5	1.7	Tr
Oleic ($C_{18:1}$)	12.1	22.0	10.4	11.1	20.3	10.0
Linoleic ($C_{18:2}$)	6.0	-	1.6	7.1	4.8	3.3
Linolenic ($C_{18:0}$)	2.0	4.5	9.7	3.7	6.3	11.9
Arachidic ($C_{20:0}$)	1.7	Tr	3.9	-	-	3.8
Eicosenoic ($C_{20:1}$)	6.3	-	-	11.8	Tr	-
Unknown	5.0	5.1	2.1	1.5	4.4	2.5
Ratio of US/S	0.5	2.6	2.2	0.6	2.5	1.9

*Tr = Traces (< 0.5%), US - Unsaturated fatty acids; S-Saturated fatty acids.

precipitation occurred within 10 min.

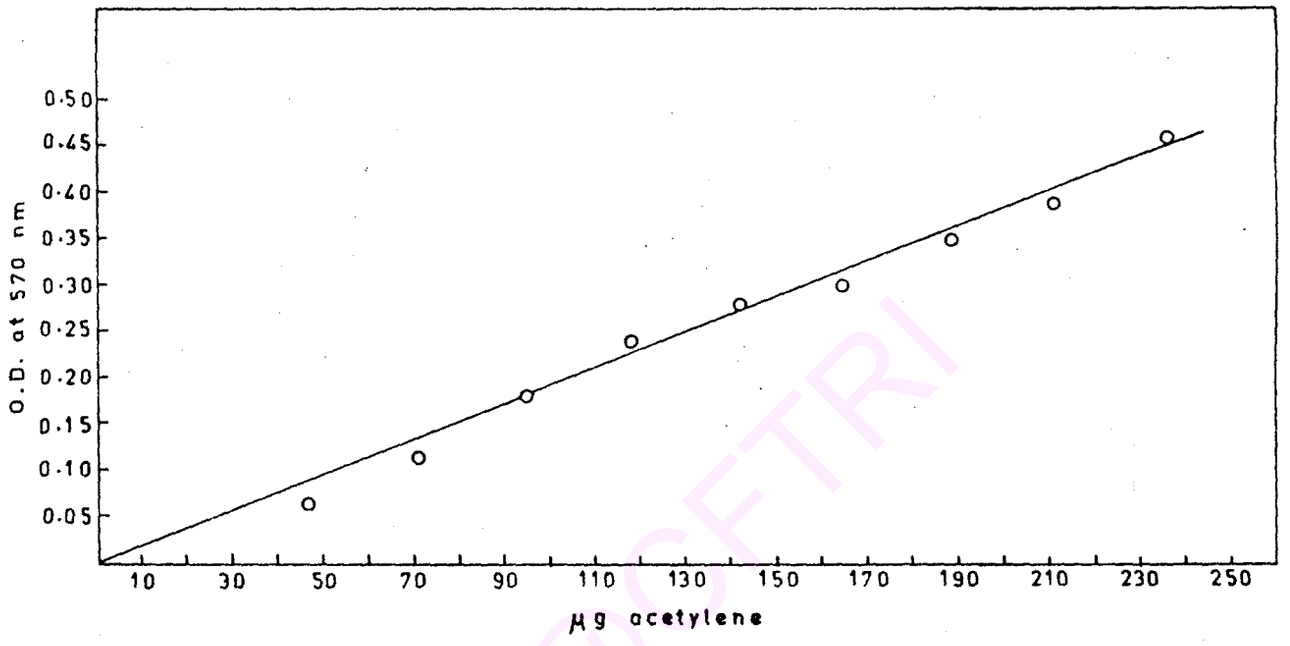


Fig. 12 Standard graph of acetylene

Acetylene could not be detected in the pulp of mangoes exposed to CaC_2 (acetylene) atmosphere for a period of 4 days (Analysis was carried out immediately after the treatment). The recovery of externally added acetylene from mango pulp was only 50-60%. This was due to the escape of acetylene during the analysis procedure.

BIOCHEMICAL STUDIES

Studies with ^{14}C-labelled acids

The intermediary metabolism was studied using labelled $U^{14}C$-aspartate, malate and acetate. The labels were injected into treated fruits soon after acetylene treatment (4th day). For comparison, control fruits were also injected (they were still greenish and could be compared with raw fruits). The ripe stage for treated fruits was

on 7th day and for comparison the control fruits were also analysed on the same day (their pulp was slightly turning yellow). The results obtained are presented in Table 13.

Table 13. Distribution of % radio activity in various fractions of acetylene treated mangoes injected with C^{14}-labelled acids

Label	Days+	Treatment	Amino acid (AA)	Organic acid (OA)	Sugar	CO_2
$U^{14}C$-aspartate	4	Control	9.43 ±5.78	22.0 ±7.18	21.7 ±5.71	48.1 ±14.32
		Treated	7.00 ±2.50	16.0^{NS} ±6.48	11.4* ±4.54	65.5^{NS} ±8.18
	7	Control	7.6 ±0.66	27.0 ±0.35	14.1 ±1.17	51.3 ±0.34
		Treated	6.2* ±0.75	9.4*** ±0.46	9.8*** ±0.37	74.8*** ±0.96
$U^{14}C$-malate	4	Control	4.2 ±2.42	34.7 ±8.29	19.3 ±4.38	41.7 ±9.12
		Treated	2.0^{NS} ±0.92	58.5* ±19.18	8.3** ±2.02	31.1^{NS} ±12.50
	7	Control	1.4 ±1.29	66.2 ±5.0	9.2 ±2.18	23.3 ±2.49
		Treated	2.8^{NS} ±0.43	60.7^{NS} ±4.65	9.3^{NS} ±1.47	30.3^{NS} ±0.28
$U^{14}C$-acetate	7	Control	6.3 ±3.05	15.0 ±2.14	15.2 ±4.91	63.3 ±9.34
		Treated	2.0^{NS} ±1.31	27.0^{NS} ±11.04	15.5^{NS} ±10.84	54.4^{NS} ±20.28

+ Fruits on 4th day - raw in control; semi-ripe in treated; Fruits on 7th day - turning in control; ripe in treated; Figures for control and treated fruits under each column separately for each label and day have been compared for their significant difference: NS-not significant; *-5% level; **-1% level; ***-0.1% level.

Labelled aspartate was metabolised faster in treated fruits than control resulting in more CO_2 evolution. But the incorporation of ^{14}C-aspartate into sugar and OA fractions was lower in treated than in control fruits. Metabolism of malate was higher incontrol than treated fruits (as can be seenfrom higher counts remaining in OA fraction). Its incorporation into sugar fraction in treated fruits was lower on 4th day but was comparable to control fruits on 7th day. Incorporation of ^{14}C-acetate into AA fraction was less in treated than in control but was similar for sugar fraction in both cases.

In general, the label incorporation into sugar fraction from various sources was significantly lower in treated compared to their respective control fruits.

FLAVOUR ANALYSIS

Capillary GCMS analysis

Aroma constituents of ripe Alphonso mangoes treated with acetylene and untreated control fruits were isolated by vacuum steam distillation. The aroma samples obtained were examined by capillary GC-MS (electron impact). The mass spectra obtained were identified based on their fragmentation pattern and by comparison with authentic references. Their capillary GLC pattern has been presented in Fig.13 and the components in Table 14.

The GLC pattern revealed the presence of fewer compounds than reported. This may be due to limitations in the methods adopted for extraction and identification. Major qualitative difference could not be noted between the 2 samples. (Slight variation in retention time of the two samples is due to difference at the point of injection).

The most abundant compound identified was an ester, ethyl 3-hydroxybutanoate, which occurred in significantly higher amounts compared to other compounds. Other major peaks identified were aldehydes-furfural and trans-2-hexenal, carbonyls-4-methoxy,2,5-dimethyl 2,4-furan-3-one and 3 - methylbutane-2-one, alcohol-cyclohexen-1-ol, lactone-γ-butyro lactone and acids-acetic, hexanoic and octonoic acids.

There were however differences in the relative concentrations of the aroma constituents in control and treated fruits. In treated fruits the concentrations of furfural and γ-butyrolactones was significantly lower whereas the concentrations of 2,5-dimethyl-4-methoxy and 2,4-furan-3-one and octanoic acid were significantly higher than in control fruits (Table 14).

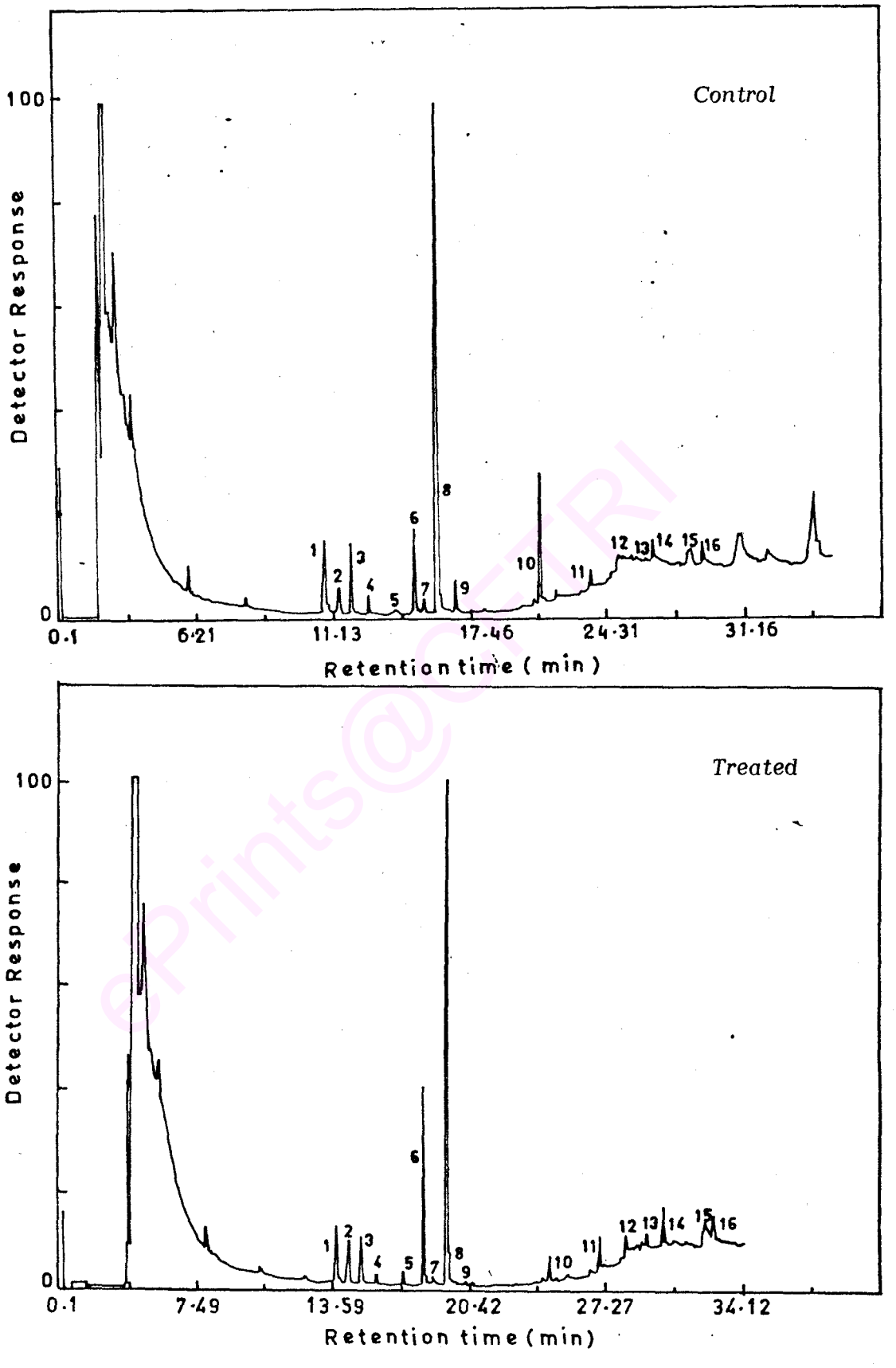


Fig. 13 Capillary GLC of the aroma constituents in acetylene treated ripe mangoes

Table 14. Relative concentrations of aroma constituents in acetylene treated Alphonso mangoes

Peak No.	Compound	Relative %	
		control	treated
1.	Furfural	7.23	4.10
2.	Unidentified	1.65	3.15
3.	Cyclohexen-1-ol	4.82	3.47
4.	Trans-2-hexenal	0.60	Tr.
5.	Unidentified	Tr.	1.10
6.	2,5-dimethyl-4-methoxy-2,4-furan-3-one	8.56	14.84
7.	Unidentified	0.90	0.63
8.	Ethyl-3-hydroxybutanoate	53.38	55.92
9.	3-methylbutane-2-one	2.23	1.89
10.	γ-butyrolacetone	13.57	1.26
11.	Acetic acid	1.20	1.26
12.	Hexanoic acid	Tr.	1.57
13.	Unidentified	Tr.	0.94
14.	Octanoic acid	1.35	4.73
15.	Unidentified	2.41	3.15
16.	Unidentified	2.03	1.89

Experiment with ^{14}C linoleic acid

Linoleic acid is a precursor of aroma volatiles in fruits. It gets converted into hexenal, hexanol and related compounds. The steam distillate from the pulp of ripe fruits injected with ^{14}C-linoleic acid was measured for the apparent ^{14}C-hexanol and hexenal fraction and the results are presented in Table 15.

Table 15. Distribution of radio activity in aroma fraction of acetylene treated mangoes

Label	*Treatment*	*Aroma fraction (% counts)*
U^{14}C-linoleic acid	*Control*	*1.09 ±0.14*
	Treated	*0.41** ±0.02*

***Significantly different at 1% level.*

It is seen from the table that the conversion of ^{14}C-linoleic acid into aroma fraction was inhibited considerably in treated fruits.

PATHOLOGICAL ANALYSIS

Spoilage pattern

All the mangoes were pretreated with benlate solution (1000 ppm) which significantly reduced spoilage during ripening in both control and treated groups. The cumulative spoilage is given in Table 16.

Spoilage increased with increase in date of harvest. Fungi were the major destructors, however, in the late harvest group insect damage was high. Exposure to acetylene did not aggravate spoilage. Important fungi identified were: ***Aspergillus niger, Diplodia natalensis, Colletotrichum gloeosporoides*** *and* ***Phoma*** *sp.*

Table 16. Cumulative spoilage of acetylene treated mangoes[+]

Harvest	Category	Treatment	Days after harvest			
			5	7	14	17
Early	Floaters	Control	1.0 ±0.58	Nil	Nil	(1.0) ±0.58
		Treated	1.0 ±0.20	(1.0)^{NS} ±0.29	-	-
	Sinkers	Control	Nil	Nil	Nil	-
		Treated	3.0 ±0.26	(3.0)** ±1.26	-	-
Mid	Floaters	Control	Nil	Nil	Nil	(1.0) ±0.58
		Treated	Nil	(2.0)^{NS}	-	-
	Sinkers	Control	Nil	Nil	(3.0) ±0.76	-
		Treated	1.0 ±1.00	(2.0)^{NS} ±1.15	-	-
Late	Sinkers	Control	2.0 ±0.58	4.0 ±1.04	(7.5) ±1.26	- -
		Treated	1.5 ±0.29	(3.5)** ±0.76	- -	- -

+ as %; Mean ± SD; Figures in parenthesis separately for each harvest and category are compared for significant difference; ** Significant at 1% level; NS-Not significant.

SENSORY QUALITIES

Acetylene treated fruits developed good colour at the end of 4 day treatment. Evaluation of these fruits by a small panel of 8 members indicated that aroma development was negligible and the taste sour. Hence they were again evaluated on 7th day (ripe stage). The sensory qualities of treated and control mangoes were studied at their edible ripe stages (7th day for floaters and sinkers of treated group, 14th day for control sinkers and 17th day for control floaters). Sensory qualities of fruits were compared between harvest maturity, floaters and sinkers and between control and treated (Tables 17 and 18).

In treated fruits the mean score was slightly higher with respect to external colour and appearance and they were more soft than control fruits. In the cut fruits, the treated were more soft

in texture and less intense in aroma. In both control and treated groups, sinkers were better in colour, appearance and odour (Table 17).

Table 17. Effect of maturity, treatment and category on sensory quality attributes of mangoes

Quality attributes	Harvest maturity		Treatment		Category	
	Early	Middle	Control	Treated	Floaters	Sinkers
Whole fruit						
Colour and appearance	3.59	3.52	3.16^{c}	3.95^{d}	3.32^{e}	3.79^{f}
Odour	3.66	3.84	3.71	3.79	3.49^{e}	4.01^{f}
Softness (finger feel)	4.65^{a}	5.46^{b}	4.65^{c}	5.45^{d}	4.93	5.16
Overall quality	3.21	2.99	3.05	3.21	2.81^{e}	3.39^{f}
Slices						
Colour	3.71	3.64	3.94	3.40	3.73	3.62
Compactness	4.30	4.30	4.24	4.36	4.31	4.28
Texture	4.74^{a}	5.47^{b}	4.85^{c}	5.37^{d}	5.12	5.10
Aroma	4.06^{a}	4.75^{b}	4.84^{c}	3.95^{d}	4.28	4.52
Taste	4.11^{a}	5.65^{b}	4.91	4.85	4.89	4.87
Overall quality	3.19	2.89	3.04	3.03	2.77^{e}	3.31^{f}

Mean score 1 to 5-unripe to optimally ripe quality; 5 to 9-optimally ripe to overripe quality; Figures with different superscripts in the same row separately for maturity, treatment and category are significantly different ($P< 0.05$).

Overall quality grading in the control group showed agreement between whole and cut fruits whereas, in the treated group it was lower for cut fruits. Sinkers scored higher in overall quality for whole as well as cut fruits than floaters and the early harvest fruits scored better than mid harvest fruits (Table 17).

When only sinkers were considered, the overall quality of both whole and cut fruits decreased from early to late harvest groups (Table 18).

Table 18. *Effect of maturity and treatment (sinkers only) on sensory quality attributes of mangoes*

Quality attributes	Harvest maturity			Treatment	
	Early	Middle	Late	Control	Treated
Whole fruits					
Colour and appearance	3.82	3.76	3.36	3.53	3.77
Odour	3.97	4.05	3.59	3.97	3.77
Softness (finger feel)	4.84	5.50	5.75	4.84^{c}	5.89^{d}
Overall quality	3.52	3.25	2.87	3.22	3.22
Slices					
Colour	3.73	3.50	3.58	3.90^{c}	3.31^{d}
Compactness	4.34	4.20	4.01	4.09	4.29
Texture	4.75	5.44	5.61	5.17	5.57
Aroma	4.13	4.91	4.42	4.99^{c}	3.98^{d}
Taste	4.20^{a}	5.54^{b}	5.52^{b}	5.04	5.14
Overall quality	3.32^{a}	3.30^{a}	2.68^{b}	3.17	3.03

Mean scores: 1 to 5 - unripe to optimally ripe; 5 to 9 - optimally ripe to overripe quality. Figures with different superscripts in the same row separately for maturity and treatment are significantly different at ($P < 0.05$).

In overall quality of the whole fruits, control and treated fruits did not differ from each other. But the treated fruits scored higher for softness. The mean score for pulp colour and aroma was significantly higher in control whereas treated fruits scored higher for compactness, texture and taste. However, there was compromise between control and treated fruits in overall quality attribute.

DISCUSSION

Non-uniform and delayed ripening is a perennial problem that exists in commercial trade of mangoes. In India mangoes are commercially ripened by acetylene (released from calcium carbide) treatment for both table and processing purposes. The present study revealed that 2 g/kg for 4 days (commercial dosage) was optimum to induce maximum effect in Alphonso mangoes. Lower dosage tried by us and higher dosages tried elsewhere (63) did not show any improvement over this.

Treatment with acetylene effectively halved the ripening period of Alphonso mangoes with the development of attractive peel colour (Fig. 21) and optimum softness without aggravating spoilage. Treatment with ethylene (as ethrel or ethephon) and hot water, alone or in combination, are also known to induce early ripening (44,65) . These treatments however increased spoilage (65) which is a limitation to fresh trade industry. Accelerated ripening of mangoes induced by ethylene or ethrel and hot water treatment has been reported by several workers (15,44,48). Hot water treatment alone induced 75-80% of the fruits to ripen while the rest spoiled (65). In the commercial practice, ethrel and hot water treatments are not widely used due to logistic considerations whereas CaC_2 is almost universally used in all the major mango producing countries because of the simplicity and economic feasibility of the treatment. It is also used to ripen bananas (39,60) citrus fruits (7,67) and tomatoes (8,9).

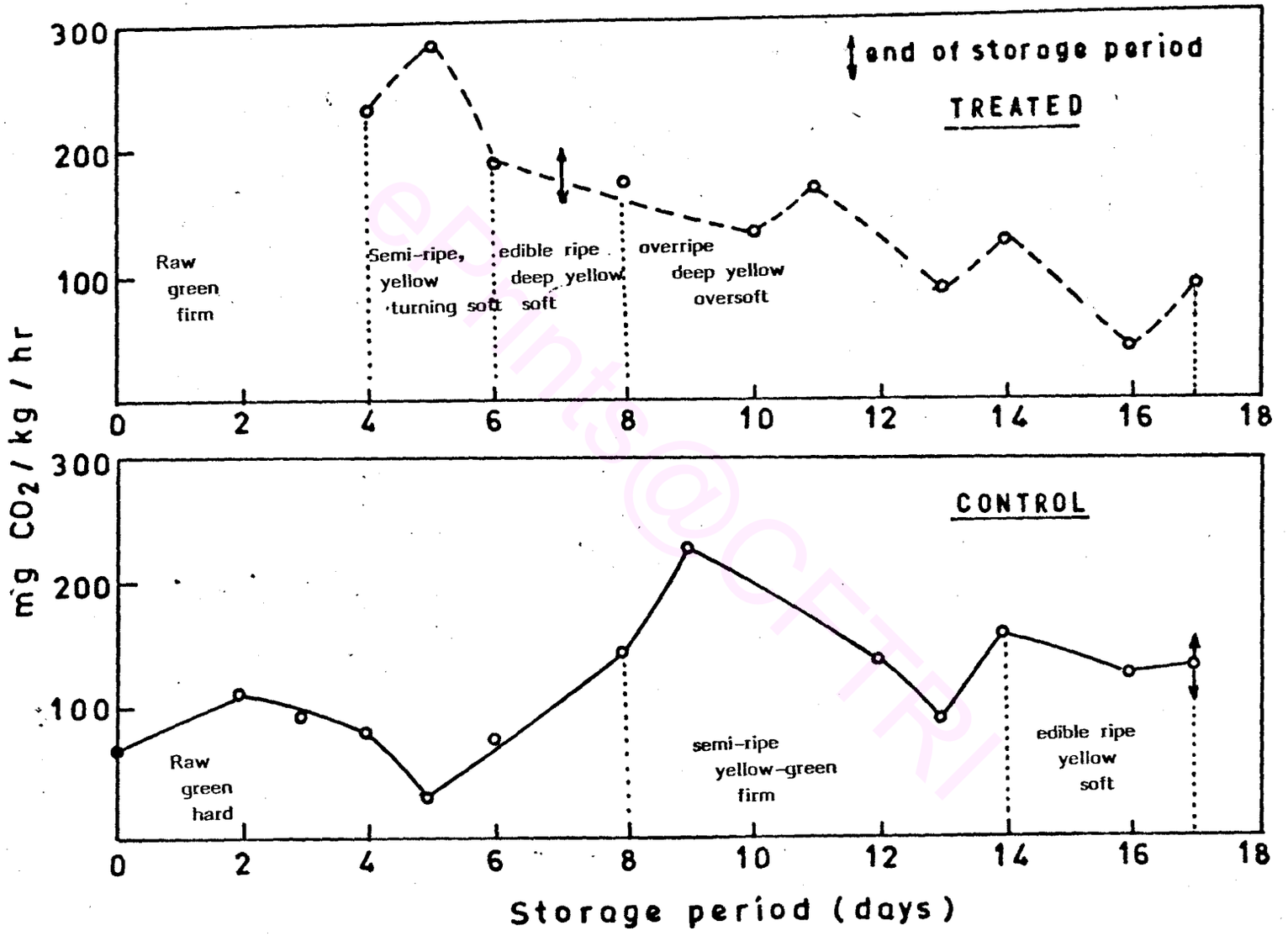


Fig. 21 Accelerated ripening of mangoes by acetylene treatment.

Colour and its associated changes. *Surface colour is the primary visual index of ripening for marketing. Effective degreening of mangoes was brought about by acetylene by increased synthesis of peel carotenoids (22-26%) culminating in attractive and complete orange yellow skin colour within a short period of 7 days. Similar improvement in peel colour has also been reported by hot water (43, 62) and ethylene (44) treatments. Ethrel both as pre-harvest application (16,12,27,30) and post-harvest treatment (11, 12, 17) accelerated chlorophyll degradation in many fruits. The* ***in vivo*** *degradation of chlorophyll, however has not been well understood.*

Acetylene treatment was less effective in inducing the synthesis of pulp carotenoids unlike in ethrel and hot water treatments in mangoes (44). Acetylene treatment simulates to a lesser extent low temperature (10°C) ripening of mangoes wherein, less amounts of carotenoids were synthesized (40). During acetylene treatment there was slight increase in temperature. Temperature upto 30°C however, is known to favour or unaffect carotenoid synthesis (19,28). Further research is essential to explain this unique effect of acetylene on carotenoid metabolism. The amount of carotenoid formation in the pulp seems to influence the development of characteristic aroma and flavour in mangoes. The intensity of aroma and flavour are proportional to carotenoid content in several varieties of mangoes (52,60).

Respiration and related changes. *Respiration indicates metabolic status of ripening in climacteric fruits. Mangoes being climacteric fruits achieve this phenomenon irrespetive of the stage of development (42)). The major events occurring during pre-climacteric and post-climacteric are discussed keeping climacteric peak as a reference point for comparison. Acetylene treated fruits exhibit climacteric peak 4 days earlier than the untreated fruits. Preponement of respiratory climacteric was also recorded in mangoes treated with ethylene and/or hot water (65) and in avocados treated with ethylene (35) . Based on flesh softening and change in peel colour, fruits were considered "just ripe" at respiratory climacteric (5th day). Edible ripeness was attained 2 days after the*

climacteric in treated fruits whereas in control it extended upto 8 days. A similar time lag of 10-20 days between respiratory climacteric and ripening was recorded in many climacteric fruits (31,32). Thus acetylene treatment not only preponed the climacteric but also the post-climacteric changes. This preponement of climacteric also accompanied the accelerated rate of CO_2 production (by 20%). In treated fruits the $^{14}CO_2$ respired from ^{14}C-aspartate was also significantly higher than in control fruits indicating accelerated metabolic activity in treated fruits. Ethylene also increased the respiratory activity (by 2 to 3 fold) of lemons (17) Dasheri mangoes (56), Chinese jujube fruits (37), and Chinese sour cherries (2).

Although the relative importance of ethylene production or inhibition by acetylene treatment had been envisaged, it is regretted that ethylene could not be measured in the present study due to lack of necessary facilities.

Climacteric rise in respiration may be the consequence or cause of many physico-biochemical changes (58, 59) .Whether preponement of respiratory climacteric by acetylene also accompanied these ripening changes faithfully is the subject of further discussion under the light of our experimental results.

Change in surface colour from green to yellow was almost completed during pre-climacteric period (4 days) in acetylene treated fruits (Plate 2a). The control fruits did not completely degreen even during the post climacteric period (17 days). In both cases it is indicative that pigment changes were not governed by respiratory climacteric. It has been observed in bananas (51) and pears (26) that no parallelism occurred between loss of chlorophyll or synthesis of carotenoids with respiratory climacteric.

Changes in fruit softening were similar to changes in colour with respect to respiratory climacteric. Fruit softening in pears (70)

and honey dew melons (54) also showed no correlation with respiratory climacteric. Softening as indicated by pressure measurement of acetylene treated mangoes went hand in hand with decrease in AIS, pectin and starch content. Breakdown of pectin, starch and cellulose are known to be activated early in the climacteric and play a role in early softening of fruits (4.55,69).

Carbohydrates are the principal sources of energy and major respiratory substates in fruits and vegetables. Sharp decrease in starch content preceeds respirtory rise in acetylene treated mangoes. This may be due to either de novo synthesis or activation of hydrolytic enzymes as suggested by Young **et al.**, (73) for banana fruits. This conversion is an essential factor in the formation of sugars which impart sweetness and to maintain the sugar acid balance in pulp which in turn influences the flavour expression in mangoes. Intense aroma of control fruits could also be correlated with higher amounts of sugars and low titratable acids. Relationship between aroma and chemical constitutents like sugars and acids was emphasized in aroma development of apples (62).

Chemical constituents. In acetylene treated fruits changes in chemical constitutents such as acidity, pH, TSS, AIS, pectin, ash content, etc. followed the normal feature of ripening. But their rate of change was accelerated along with the preponement of respiratory climacteric. Such an early change in chemical constituents was also induced by ethylene in many fruits (35,37,56).

As mentioned in the results, it was not possible to detect acetylene in the pulp of mango fruits exposed to acetylene. This may be due to the possibility that

- no acetylene had entered the fruit from the surrounding atmosphere,
- acetylene if entered might have been metabolised into toulene and benzene as reported earlier (36).
- concentration of acetylene in the mango pulp was far below the limit of detection.

Tracer studies with ^{14}C-labelled acetylene might have helped in this direction. Our attempts to procure ^{14}C-acetylene were however not successful.

Texture and its associated changes. *Changes from hard, crisp and dry texture of raw fruit into soft, succulent and juicy ripe fruit during ripening are essential for fresh market as well as for processing. These changes were induced early in acetylene treated fruits. The most crucial information of textural breakdown is loss of cohesiveness, moisture and loss of cell membrane integrity ultimately leading to cell separation and rupture of cell walls. In mangoes, moisture loss by PLW correlates negatively with shear stress. This decreased failure stress and increased moisture loss appears to indicte ripeness in mangoes as in melons and apples (21 ,53, 57). The loss of mechanical strength and cohesiveness is probably due to lessening of mechanical strength (intactness) of cell walls and weakening of binding matrix of cellular tissues.*

The change in cell wall composition, enzymes involved and mode of degradation in fruits are excellently reviewed (6 ,41). The present attempt to account the chemical cause for textural degradation supports their findings. The decrease in mechanical strength and cohesiveness is well sequenced with ripening change and duration of ripening both in treated and control fruits. Early ripening induced by acetylene accompanied the change in texture also. The loss of alcohol insoluble solids,& their carbohydrates in cell wall partly explains the reduction in mechanical strength to puncture the tissue. Further, significant reduction of starch and pectin with advance of ripening were found to be mainly responsible for loss of cohesiveness and cell separation respectively. Cell walls tend to 'round off' when starch, which constitutes 22% of total weight of mesophyll cells, is hydrolized resulting in increase of intercellular space and loss of cohesiveness. Similar results were well documented in potato (57). Pectin degradation causes dissolution of middle lamella and ultimately cell separation. This has been well correlated with decrease in shear stress for rupture. The importance of

pectin in cellular organisation and its role in textural breakdown evidenced in our results support the findings of several research worker in fruits (6,41). Early decrease in mechanical characteristics of texture profile in alphonso mangoes treated with acetylene resembled accelerated textural breakdown recorded in the irradiated mangoes and peaches (1).

It was also confirmed by the histological evidences wherein loosening and rupture of cells with enlarged intercellular spaces, disappearance of starch granules and loss of cell integrity due to cell wall breakdown occurred during the course of ripening. These histological alterations occurred to a lesser extent in control fruits and extended over the ripening period of 17 days. The high rate of cell wall breakdown displayed in the parenchymatous mesocarp cells may mainly be responsible for rapid and pronounced textural changes in acetylene treated fruits. This radical loss of cell wall organisation responsible for softness in texture was displayed more clearly by SEM studies. In control ripe fruits, normal separation of cells along the middle lamella was more common, leading to separated intact cells which may collectively offer more resistence. In treated fruits however the high degree of cell wall breakdown resulted in more softness at ripe stage. Intact cells are known to offer greater resistance resulting from mechanical support due to cell turgidity (10,46).

Ripening or senescence of detached plant organs is accompanied by loss of membrane integrity. Membrane can influence the texture by affecting the cell turgidity. Leakage of ions from tissues can be correlated to membrane integrity of a system (45,49,71) Acetylene treatment enhanced the leakage of K+ ions from mango tissues during ripening. This was correlated to loss of structural integrity in cell walls. Our results confirmed the findings in apple fruit tissues (25) which exhibited increased K+ leakage with advancement of senescence. Similarly peppers and citrus fruits in seal packaging maintained more firmness and this was correlated to less leakage of electrolytes and free amino acids (5).

Aroma and its associated changes. *The appearance of the food although evokes the initial response, it is the flavour that ultimately determines its acceptance or rejection by consumers. Application of acetylene led to accelerated colour change, softening and related chemical changes however there was less accumulation of aroma components.*

Acetylene treatment although increased the accumulation of carotenoids in peel, their levels in the pulp was significantly reduced. Less carotenoids reflect poor development of aroma by (i) reduced availability of carotenes as substrates for aroma development, their amount being proportional to intensity of aroma and flavour in the mango fruits (52). Similarly lack of intense red colour at harvest resulted in poor development of all aroma constituents in tomatoes (75). (ii) Colour also has psycho-physical relationship with sensory flavour quality (18).

Linoleic and linolenic acids are known to be precursors of C_6 *aldehydes and alcohols which are reported to be the major aroma components of alphonso mangoes (24). In mangoes (24), bananas and apples (23, 67) production of aldehydes could be correlated to the amounts of linoleic and linolenic acids. Drawert* **et al.,** *(23) reported enzymatic formation of 2-hexenal and hexanal from linoleic and linolenic acids in bananas, apples, pears and grapes. He also showed that no aldehydes were produced if the enzymes were inhibited. The higher amounts of linoleic and linolenic acids remaining unconverted in acetylene treated fruits explains lower aroma synthesis in them. This may be due to inhibition or suppression of lipid oxidation enzymes. Further, the amount of total lipids itself was significantly lower in acetylene treated fruits.*

Kazeniac and Hall (38) demonstrated the conversion of ^{14}C *linolenic acid into Cis-3-hexenal, trans-2-hexenal and cis-3-hexanal in tomatoes. The conversion of* ^{14}C *linoleic acid into aroma components was quantitatively reduced in case of acetylene treated fruits. This partly explains reduced aroma development in treated fruits as flavour development depends on multiple pathways with wide source of substrates.*

The ratio of palmitic to Palmitoleic acids has been correlated with the aroma of alphonso mangoes (3). The ratio during ripening varied from 0.89 for table ripe to 0.83 for overripe fruits. Similar correlation was observed in polar lipid fraction in both control and treated fruits in the present studies. Neutral lipids gave much lower values. However, raw mangoes gave much higher ratio which was due to the presence of trace amounts of palmitoleic acid.

Significant reduction in the ripening period by C_2H_2 treatment might also be the cause for reduced aroma synthesis. Flavour compounds are produced during the climacteric and post-climacteric period of ripening in a cyclic manner (29). The treated fruits ripened 2 days after their respiratory climacteric. The control fruits however, ripened with a time lag of 8 days after their respiratory climacteric with full intense aroma. This time lag of a bout 8 days after the climacteric rise may be an essential factor for full aroma development. In apples a time lag of 10-20 days and in bananas a minimum of 10 days after climacteric peak was necessary for the production of most of the volatile compounds (22) and this appears to be a normal case in climacteric fruits. With ethrel treatment however, there was rapid increase in levels of aroma volatiles with increasing ripeness in Kiwi fruits (74).

Alphonso mango aroma is a complex combination of hundred and odd compounds (24 34). The detection of fewer compounds in our studies by GC-MS than reported earlier may be due to limitations in the method of extraction and identification employed. There was however, no major qualitative difference in the aroma profile between control and acetylene treated fruits. Ethyl -3-hydroxy butanoate was the most abundant compound identified. This compound has been reported in the volatile fractions of baladi and alphonso mangoes (24) and other tropical fruits like pineapple (20) woodapple (50) and passion fruit (72). It occurred in higher amounts in Baladi than in Alphonso variety and

is known to impart the mild, woody, resin like note of Baladi mangoes along with related esters.

4 methoxy-2,5-dimethyl-3,4-furan-3-one which occurred in considerable amount in acetylene treated mangoes, has been reported to be a constituent in canned mangoes (33). Engle and Tressel (24) reported C_6 aldehydes and alcohols to be the major components in alphonso mangoes. Trans-2-hexen-1-al could be identified along with cyclohexen-1-ol in both control and treated fruits. It can be seen that hydrocarbons such as myrcene, ocimene, caryophyllene which are reported (24) to be abundantly present in Alphonso mango aroma could not be separated in our experiments due to limitations mentioned earlier.

Thus there were no qualitative differences in the range of compounds identified between control and acetylene treated fruits. It is the quantity of aroma synthesised along with various other factors described which may be responsible for low intensity of aroma in acetylene treated fruits. However this distinction did not diminish the overall quality and acceptability. The mode of action of acetylene on aroma development needs to be elucidated.

Organoleptic qualities. The various physico-chemical ripening changes were reflected in the organoleptic qualities of the ripe fruits. The acetylene ripened mangoes developed attractive peel colour and appearance. Acetylene is also used to degreen bananas (39,60) tomatoes (8, 9) citrus fruits (7,67) and mangoes (47,63). The development of flesh colour and flavour was however less in treated fruits. But this did not affect their marketability, the sensory panelists rating them equal to control fruits in overall qualities. Difference was observed in varietal response to acetylene in mangoes. Dasheri (47) and Bangalora (63) varieties showed improved organoleptic qualities while impairment of qualities by acetylene treatment has been reported in Alphonso, Banganapalli, Pairi, Totapuri and Langra varieties (64a). It was also observed during the investigation that maturity at harvest was an important factor influ-

encing the effects of acetylene. Immature fruits treated with acetylene were completely bland in taste and aroma although they developed yellow peel colour (data not presented). The advantage of acetylene to ripen immature and culled mangoes being commercially exploited by traders leads to low quality fruits in the market.

Acetylene appears to have varying effects on different ripening processes. The role of acetylene in coordinating the sequence of ripening changes may be by:

- *simulating the action of ethylene but to a lesser degree,*
- *initiating increased production of endogenous ethylene,*
- *accelerating processes that proceed slowly in absence of endogenous ethylene as in non-climacteric fruits,*
- *by the synergistic action of all the three.*

The results obtained lead us to speculate that acetylene may act as a ripening hormone like ethylene but with lower efficiency especially on certain metabolic pathways. Infact the biological activity of acetylene was far below that of ethylene as assayed in pea hypocotyls (13) and bananas (14).

CHAPTER 4

EXTENSION OF SHELF LIFE OF CAPSICUMS BY MA STORAGE

With an aim to extend the storage life of capsicums (bell peppers) at ambient temperature, the potential use of commodity generated modified atmosphere was tried. The results were more encouraging than envisaged and they have been presented in this chapter. The preliminary experiments to standardise the optimum modified atmosphere (MA) storage conditions are also tabulated. The market and sensory qualities of capsicums were quarantined after harvest, and at the end of MA storage. The various quality attributes of capsicums were evaluated and correlated with physical, physiological & chemical changes. The results obtained have been discussed to elucidate the interaction of storage environment with capsicums.

INTRODUCTION

Capsicums or bell peppers (Var. bull nose) like many other vegetables are a luxury item in India due to high cost which can be afforded by a small percentage of the population. The high cost of the vegetable is manily due to its localised production and high perishability resulting in short shelf-life. The post harvest losses in bell peppers are mainly due to material loss and to a lesser extent nutritional losses. These may include loss of weight, colour, bruise, decay , etc.,at various stages from harvest to consumption. The expected shelf life of Capsicum depends upon post harvest handling and storage environment. To date cool storage (~ 7 - 9°C) is the only known technique economically feasible for short term storage of fresh Capsicums. Use of CA as a supplement to cool storage has also been beneficial. In India, the prevailing tropical climate and lack of adequate cold storage facilities shorten the shelf life of capsicums to only 6-8 days at ambient temparature (27±4°C). Hence, there is need to extend the shelf-life of Capsicums at ambient temp. to extend the market season and to create distant markets. In this connection, storage of Capsicums in self generated MA seems to have great potential to extend its shelf-life. The present investigation envisages the optimization of conditions and the quality of Capsicums stored under this method.

RESULTS

The potential use of modified atmosphere (MA) to extend the storage life of capsicums at ambient temperature (27 ± 4°C) and RH (50-75%) was studied. The optimum horticultural conditions for capsicum and period of storage were standardised. Further, the quality of capsicums during extended period of MA storage was evaluated by various physico-chemical studies.

OPTIMIZATION OF MA STORAGE CONDITIONS

Horticultural conditions

Harvest maturity of capsicums is based on firmness and colour of the fruit which can be categorised as:

Immature	light green in colour, slightly dull appearance and soft and flexible by finger feel.
Optimally mature	Deep green, smooth and shiny, hard and crisp in firmness.
Over mature	Deep green colour slightly changing to red, hard in firmness.

Immature fruits tend to shrivel very fast while over mature fruits ripen early becoming unmarketable. In commercial trade fruits of all maturity stages occur in a lot.

Commercially capsicums are hand picked at raw, green condition. After harvest, fruits are loaded into gunny bags (~80 kgs/bag) and transported by lorry along with other vegetables. A minimum time lag of 18-24 hrs occurs between harvest and marketing. Suitability of market samples of capsicums for MA storage was tested and the results are given in Table 19 and 20.

Physiological loss in weight (PLW) was rapid in the air stored fruits at the rate of 2% for every 24 hrs and 12% of the weight was lost at the end of 6 days storage period. In contrast, fruits stored in MA showed significant reduction in PLW, there was 50% reduction in PLW in 5 days-MA stored fruits on 6th day (Table 19). The reduction increased with increase in storage period under MA. This reduction was observed during the post storage period also.

Table 19. The physiological loss in weight of capsicums (market samples)

MA storage (days)	Days after harvest 2	3	4	5	6
Control*	4.08	5.83	7.53	10.11	12.91
2	2.15	3.80	5.50	7.82	10.62
3	-	2.66	4.15	6.21	8.82
4	-	-	3.06	6.09	8.24
5	-	-	-	3.26	6.02

* Controls were air stored.

Heavy loss (55.3%) of capsicums due to microbial spoilage was recorded in the air stored fruits. However, fruits stored under MA showed 10% reduction in spoilage at the end of 8 days. Spoilage decreased with increase in storage period in MA. The reduction in spoilage was well reflected in increased percentage of marketable fruits (Table 20).

Table 20. Cumulative spoilage and marketability of capsicums after 8 days of storage in MA.

MA storage (days)	Spoilage (%)	Ripening + Shrivelling (%)	Marketability (%)
Control *	55.3	28.2	16.6
2	49.9	6.1	44.0
3	45.2	2.8	52.0
4	46.6	0.1	53.3
5	44.2	1.6	54.2

* Control fruits were air stored.

The results of this initial trial with market samples revealed that by MA storage 5 days extension in shelf-life of capsicum could be achieved with 54.2% marketable fruits as comparedto 16.6% in case of control group. Microbial decay was the major limiting factor for further extension. This could be attributed to bad handling at harvest and transportation practices. Hence, it was felt necessary to obtain capsicums directly from the field. All the experiments described hereafter were conducted with capsicums harvested from the field.

Harvest maturity

Fruits at different stages of maturity on individual plants were tested for their texture by Hand Magnus Taylor Pressure Tester. Optimally mature fruits measured 4 to 5.3 lbs/sq. inch below which the fruits were considered as immature.

Storage period

Fruits were stored under MA (as described in methodology) for a period of 3 to 13 days. The results are presented in Table 21.

Table 21. PLW, spoilage and marketability of MA stored capsicums (after 15 days)

MA storage (days)	PLW (%)	Microbial spoilage (%)	Ripening + shrivelling(%)	Marketability (%)
Control*	17.93	23.7	48.8	27.5
3	17.73	9.8	58.1	32.1
5	13.43	24.1	37.0	38.9
7	13.28	18.0	22.3	59.7
10[a]	10.02	2.5	12.5	85.0
13[b]	8.91	3.2	6.8	90.0

* *Control samples are marketable upto 8 days only. Observations are continued further to compare with MA stored fruits; a - Sample stored under MA for 10 days is termed* T_1; *b - and for 13 days is termed* T_2.

The PLW was reduced by MA storage, the reduction was significant with prolonged storage periods. The PLW was halved in 13 days MA stored fruits (on 15th day). Spoilage after 15 days storage was 23.7% in case of control and 2.5 and 3.2% in case of 10 and 13 days MA stored fruits respectively. The marketability after 15 days storage was 27.5% for control and 85 and 91.6% respectively for 10 days and 13 days MA stored fruits. Remaining fruits were either shrivelled or ripened.

Shortening the time lag between harvest and storage, coupled with post-harvest treatments efficiently extended the storage life of capsicums with high percentage of marketable fruits. This was achieved by reduction in spoilage and maintenance of quality. Since

the benefits of MA extended upto 13 days, further extension in storage period upto 21 days was tried. Observations are as follows:

Table 22 Cumulative spoilage of capsicums stored under MA

MA storage (days)	days after harvest 3	6	8	10	13	16	19	21	23
						%			
Control	2.0 ±1.78	4.0 ±2.02	10.0 ±1.7	19.0 ±1.7	24.0 ±3.92	26.0^a	30.0^a	30.0^a	35.0^a
10 (T_1)				6.0 ±0.75	10.0 ±1.39	14.9^b	24.0^b	26.0^b	27.0^b
13 (T_2)					6.5	10.2^b	20.2^c	40.0^c	40.0^c
16						18.0^c	30.0^a	31.5^a	34.0^a
19							20.8^c	47.5^d	50.0^d
21								26.0^b	39.0^c
(S.E.)						±2.33	±1.48	±1.32	±1.99
DF						(8)	(10)	(12)	(12)
				Mean ± S.D.			Mean*		

*Means with different superscripts under each column differ significantly ($P < 0.05$)

The spoilage increased with increase in storage period under MA beyond 13 days (Table 22). On 16th day of storage the spoilage was 26% in control fruits compared to 14.9 and 10.2% in T_1 and T_2 respectively. Compared to T_1 and T_2, 16 days MA stored fruits showed 3 fold increase in spoilage at the end of their storage period. Spoilage increased further with increase in MA storage period upto 21 days. The nature of spoilage by CO_2 injury was characterised by discolouration of skin followed by browning. At advanced stages these regions were infected with putrifying bacteria which reduced capsicums into a mass of putrified tissue.

With increase in spoilage the percentage of marketable fruits also declined (Table 23). Control fruits were marketable upto 7-8 days with 85-90% marketability (Plate 3). In freshness, the MA stored fruits soon after removal from MA were comparable to freshly harvested capsicums (Plate 4) and were marketable upto 3 days after removal from MA i.e. 13 days for T_1 and 16 days for T_2 with 87.0 and 84.8%

Plate 3. Air stored capsicums at the end of storage (8th day)

Plate 4. MA stored capsicums immediately after removal from MA.

on 10th day

i. Air stored ii. MA stored (T_1)

on 13th day

i) Air stored ii) MA stored (T_2)

Table 23 Marketability of capsicums stored under MA

MA storage (days)	Days after harvest 3	7	10	13	16	19	21	23
				%				
Control	94.0 ±4.07	85.0 ±2.44	35.0 ±2.57	23.0 ±9.87	8.0^{a}	5.0^{a}	0	0
10 (T_1)			92.5 ±2.8	87.0 ±3.10	68.7^{b}	46.7	33.7^{a}	17.3^{a}
13 (T_2)				90.5 ±3.51	84.8^{c}	60.2^{c}	47.0^{b}	34.0^{b}
16					70.8^{d}	38.9^{d}	25.0^{c}	19.7^{a}
19						59.2^{c}	11.8^{d}	10.5^{c}
21							59.0^{e}	43.3^{d}
S.E. (DF)					2.46 (8)	±2.53 (10)	±1.39 (10)	±1.41 (10)
	Mean ± S.D.					Mean*		

* Means of the same column with different superscripts differ significantly ($P<0.05$)

marketability (Plate 5). Extended storage in MA resulted in decreased marketability due to increased spoilage. The results were confirmed during 3 seasons of which two experiments have been presented in Tables 23 a and b.

QUALITY OF CAPSICUMS STORED IN MA

Under MA storage extension in storage period upto 13 and 16 days including the post storage life of 3 days was achieved with acceptable quality and minimum spoilage. (Beyond 3 days of post storage period the marketability sharply declined and hence may not be economical). Therefore detailed studies were restricted to 10 days (T_1) and 13 days (T_2) MA storage periods only. The parameters studied are dealt under separate headings as given below.

All the observations/analyses were carried out in capsicums soon after harvest and on 8th day (at the end of marketable period) in control fruits. MA stored fruits were analysed immediately after removal from MA (i.e. on 10th day for T_1 and 13th day for T_2) and

(Table 23a) Spoilage and marketability of MA stored capsicums

Sample	Days after harvest	Shrivelling %	Turning %	Microbial spoilage %	Marketability %
Control	8	0.6	2.0	1.5	95.9
Control	10	8.0	7.0	5.0	80.0
T_1		0.0	0.0	1.0	99.0
Control	13	6.5	1.5	16.5	65.5
T_1		2.9	1.6	5.0	90.5
T_2		0.0	0.5	0.9	98.9
Control	16	15.5	14.5	19.2	50.8
T_1		10.0	1.5	8.0	80.5
T_2		5.0	1.0	4.5	89.5

(Table 23b) Spoilage and marketability of MA stored capsicums

Sample	Days after harvest	Shrivelling %	Turning %	Microbial spoilage %	Marketability %
Control	8	1.0	0.3	3.5	94.2
Control	10	17.1	6.7	10.7	65.5
T_1		1.5	1.5	6.3	90.7
Control	13	57.8	4.4	14.7	23.1
T_1		8.1	7.3	6.6	78.0
T_2		0.0	0.1	3.9	96.0
Control	16	56.5	20.4	16.0	7.1
T_1		13.3	2.0	16.7	68.0
T_2		2.1	2.0	10.9	80.0

Plate 5. Post MA storage life of capsicums

on 13th day – *MA stored* (T_1) **on 16th day** – *MA stored* (T_2)

at the end of their marketable period (13th day for T_1 and 16th day for T_2).

For some parameters, control fruits were analysed beyond 8 days for comparison with MA stored fruits. In some experiments only T_2 fruits were analysed as there was no difference between the two (T_1 and T_2) treatments.

Physical studies

Gas concentration in MA

There was a linear decrease in O_2 and gradual increase in CO_2 concentration till 10th day of storage in MA chamber. Later the gas concentration maintained a constant level till 16th day. Oxygen depletion inside the chamber was rapid compared with the increase in CO_2 concentration. The CO_2 and O_2 concentration inside the chamber on 10th day and 13th day were 3.68 + 7.73% and 4.68 + 6.45% respectively (Fig. 14).

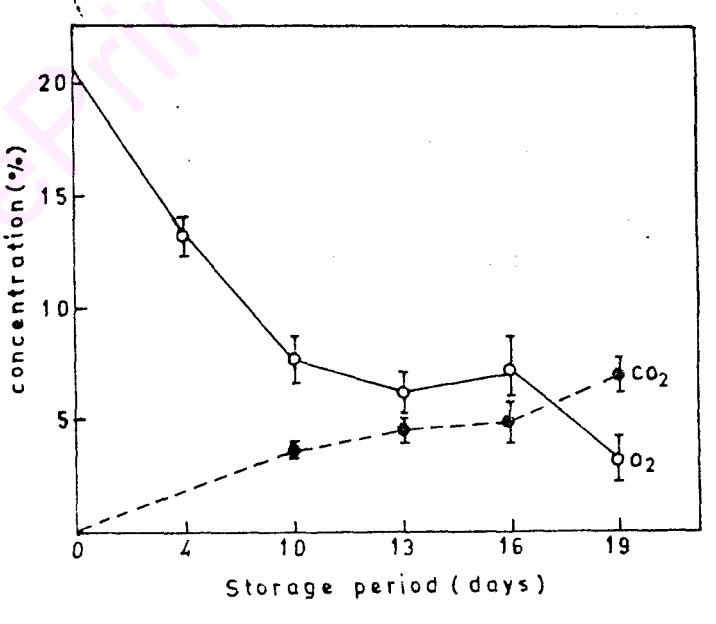


Fig. 14 Concentrations of CO_2 and O_2 inside the MA chamber

Temperature and RH

Temperature inside the MA chamber was slightly (±0.5°C) higher or lower than the ambient temperature (Fig.15). The RH inside the MA chamber varied from 85-100% while it varied from 59-75% in the ambient atmosphere (Fig. 15).

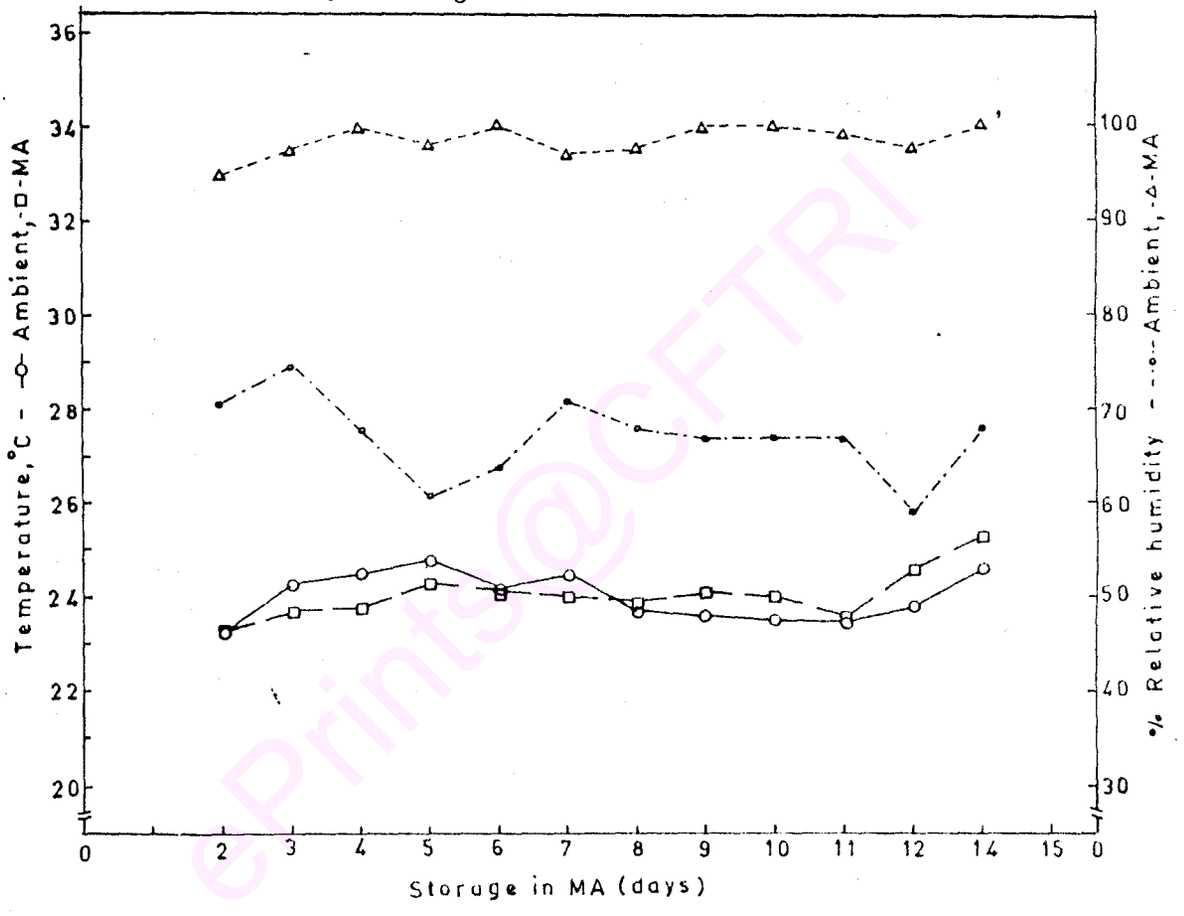


Fig. 15 Temperature and RH inside the MA chamber.

Ripening pattern

The deep green coloured capsicum fruits turn bright red upon ripening and are then unmarketable. Number of ripened fruits increased with advance in storage period. Elevated CO_2 and decreased O_2 in MA had a decisive role on ripening. On 16th day, 23% of the control fruits ripened compared to only 6 and 5% of T_1 and T_2 fruits (Table 24).

Table 24. Ripening of capsicums stored in MA

Sample	Days after harvest 3	6	8	10	13	16	19	21	23
	% red fruits								
Control	3.0 ±1.26	9.0 ±1.32	7.0 ±1.0	11.0 ±1.23	22.0 ±1.23	23.0^a	26.0^a	26.0^a	18.0^a
T_1	-	-	-	1.5 ±0.21	3.0 ±0.50	6.0^b	19.3^b	20.0^b	25.7^b
T_2	-	-	-	-	3.0	5.0^b	10.0^c	20.0^b	9.4^c
S.E. (DF)						±0.31 (8)	±0.78 (10)	±0.92 (12)	±0.99 (12)
	Mean ± S.D.						Mean*		

* Means of the same column not followed by common superscript differ significantly according to Duncan's New Multiple Range test (P 0.05).

Shrivelling

Capsicums shrivel rapidly during storage and hence lose their crisp texture. Shrivelled capsicums have poor marketability. Shrivelling in air stored fruits was low at initial days of storage. Later it went up to 39% by 10th day. During MA storage, shrivelling was completely eliminated. However, during the post storage period, the fruits showed gradual increase in shrivelling rate. The percentage of shrivelled fruits was very high (33%) in case of control on 16th day compared to 10.4% in T_1 and 3% in T_2 fruits. Thus in MA storage shrivelling was sigificantly suppressed (Table 25).

Table 25. Visible rate of shrivelling of capsicums stored in MA

Sample	Days after harvest 3	6	8	10	13	16	19	21	23
	%								
Control	1.0 ±1.58	2.0 ±0.03	5.0 ±0.95	30.0 ±3.01	31.0 ±3.81	33.0^a	39.0^a	44.0^a	47.0^a
T_1				0.0	2.0 ±1.3	10.4^b	18.0^b	20.3^b	30.0^b
T_2					0.0	3.0^c	9.8^b	13.0^c	16.5^c
S.D. (DF)						±0.50 (8)	±0.62 (10)	±1.42 (10)	±1.13 (12)
				Mean ± S.D.			Mean*		

*Means of the same column not followed by a common superscript differ significantly according to Duncan's New Multiple Range Test ($P < 0.05$).

Instrumental texture

(a) Whole fruits: Fruits were subjected to Kramer Shear testing at different storage periods. From the deformation curves obtained, peak force (indicating tender/toughness), area under curve (representing work done toshear) and peak width at half peak force (i.e. 1/2 peak width which may indicate thickness of compressed mass) were calculated.

Except peak force, other parameters were a linear function of sample weight with correlation coefficients of 0.39, 0.92 and 0.98 respectively for peak force (kg.), curve area (cm^2), and 1/2 peak width (cm). The discrepancy that peak force had a poor correlation whereas 1/2 peak width had very good correlation with sample weight was due to the ill defined peak. The peak force for low weight samples was higher than for high weight samples. This was correlated by the factor F.

F = weight of the sample/mean weight.

The corrected peak force had a good correlation with sample weight (r = 0.94). The correction factor essentially makes up the effects mainly on peak forces, other parameters remain in the order qualitatively (Fig. 16).

Table 26 presents data on peak force ($N.Kg^{-1}$), work done ($J.Kg^{-1}$) and 1/2 peak width ($m.Kg^{-1}$) after correction. Inspite of visual differences for work done and 1/2 peak width, control and treated fruits were not different from each other ($P > 0.05$).

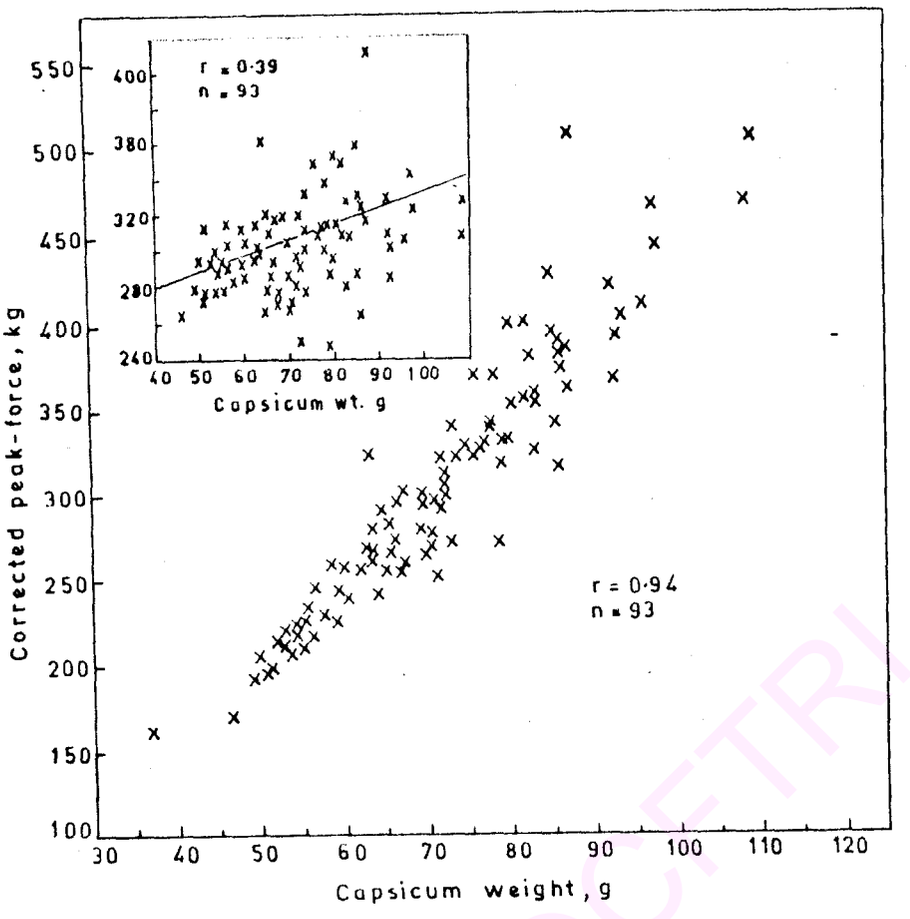


Fig. 16 Corrected peak force from the deformation curves of capsicums subjected to Kramer Shear testing (inset is the original peak force obtained)

*Table 26. Instrumental textural quality of capsicums (whole fruits) stored in MA**

Parameter	*Sample*	*Days after harvest* 6	10	13	18
Peak force $N.Kg^{-1}x10^4$	*Control*	*3.95*	*4.02*	*4.48*	*4.03*
	T_1		*4.32*	*4.08*	*4.06*
	T_2			*4.23*	*4.19*
Work done $J.Kg^{-1}x10^2$	*Control*	*5.83*	*6.98*	*5.47*	*6.82*
	T_1		*6.82*	*5.81*	*6.12*
	T_2			*7.38*	*7.25*
1/2 peak width $m.Kg^{-1} \times 10^{-1}$	*Control*	*2.10*	*2.48*	*1.91*	*2.61*
	T_1		*2.36*	*2.12*	*2.27*
	T_2			*2.53*	*2.52*

*Mean of 5 replicates

It was concluded that weight of the sample had a profound effect on the parameters studied. As it was difficult to get a representative sample which would fit into the Kramer shear cell it was decided to conduct textural studies with slices of capsicum.

(b) Fruit slices: The texture of radial pieces of capsicums at different storage periods was measured on Warner-Bratzler shear press and the results are presented in Table 27.

Table 27. Instrumental texture quality of capsicums (fruit slices) stored in MA

Sample	Days after harvest						
	0	4	6	8	10	13	16
	Shear force (lbs)						
Control	0.32 ±0.05	0.53 ±0.07	0.94 ±0.03	0.99 ±0.01	1.64 ±0.02	0.99 ±0.02	1.85 ±0.04
T_1	0.32 ±0.05	0.53 ±0.07	0.94 ±0.03	0.99 ±0.01	0.55 ±0.04	0.94 ±0.03	0.98 ±0.02
T_2	0.32 ±0.05	0.53 ±0.07	0.94 ±0.03	0.99 ±0.01	0.55 ±0.04	0.60 ±0.01	0.96 ±0.05

Freshly harvested fruit pieces offered least resistance to the shear force applied. But it increased with advance in senescence. The firmness of fruits from harvest till the end of marketability ranged from 0.32 - 0.99 lbs. Fruits stored under MA fall within this range upto 16 days of storage. The low shear force indicating maintenance of textural quality by MA storage.

The results were confirmed on Instron Model 1140 by Kramer shear test (Table 28). Of the 3 parameters studied, 1/2 peak width reflected better the texture of capsicums. It generally increased with storage time for control as well as MA stored samples. But control fruits on 10th day recorded significantly higher peak width (0.45 $m.Kg^{-1}$). MA stored fruits on 10th day and 13th day were comparable to freshly harvested fruits in their texture measurements (0.40 $m.Kg^{-1}$).

Peak force and work done did not show significant differences for all the samples ($P > 0.05$).

Table 28. Instrumental texture quality of capsicums (fruit slices) stored in MA

Sample	Time (days)	Fruit wt. (g)	1/2 peak width $m.Kg^{-1}$	Peak force $N.Kg^{-1}$	Work done $J.Kg^{-1}$
Control	1	85.7^{a}	0.40^{a}	32948^{a}	1282.2^{a}
	10	85.5^{a}	0.45^{c}	31524^{a}	1292.5^{a}
T_1	10	34.0^{a}	0.41^{a}	36884^{a}	1465.8^{a}
	13	85.2^{a}	0.43^{b}	36121^{a}	1434.6^{a}
T_2	13	87.4^{a}	0.40^{a}	33293^{a}	1392.6^{a}
	16	84.5^{a}	0.43^{b}	37631^{a}	1480.3^{a}
F		9.11^{NS}	7.89**	3.73**	1.19^{NS}
SEm		1.28	0.007	1912.3	76.6
d_f		28	28	28	28

**$P < 0.01$; NS- Not significant ($P > 0.05$); Samples with a different superscript in a column are different from each other ($P < 0.05$); Means were separated using Duncan's New Multiple Range Test.

Physiological parameters

PLW

PLW increased steadily with increase in days of storage in control fruits whereas MA storage adversely affected the PLW. PLW decreased with increase in storage period in MA with corresponding decrease in percentage of shrivelled fruits. But the PLW increased during the post storage period. However, their PLW was always significantly lower than in control fruits. Control fruits lost 11.56% of their weight on 7th day compared to 11.93% and 11.5% on 13th and 16th days for T_1 and T_2 respectively (Table 29).

Table 29. Effect of MA storge on physiological loss in weight of capsicums

Sample	Days after harvest								
	3	5	7	10	13	16	19	21	25
	%								
Control	5.3 ±0.47	7.63 ±1.07	11.56 ±1.27	16.77 ±0.77	22.68^a	26.28^a	27.38^a	33.53^a	35.47^a
T_1	-	-	-	6.48 ±1.52	11.93^b	14.60^b	16.38^b	23.23^b	25.43^b
T_2	-	-	-	-	5.60^c	11.59^b	18.29^b	20.76^b	24.67^b
	Mean ± S.D.				Mean*				

*Means of the same column not followed by a common superscript differ significantly according to Duncan's New Multiple Range Test (P 0.05).

Respiration

Whole fruits : Capsicums exhibited a non-climacteric pattern of respiration (Fig. 17). Control fruits showed a decline in respiration from harvest till the end of their marketable period (7 days) from 63.0 to 36.5 mg CO_2/Kg/hr. In MA stored fruits the decline was more steep measuring 14.85 and 20.59 mg CO_2/kg/hr respectively for T_1 and T_2 at the end of their marketable periods. Upon further storage the rate of respiration increased in both control and treated groups. Thus during the marketable period the rate of CO_2 evolution was suppressed by MA storage.

Fruit slices (as % oxygen consumed) : There was increased oxygen consumption till the end of storage by the control fruits (Fig. 17). It increased from 4.70 to 7.86% till 6th day. The oxygen consumption was greatly suppressed (∽ 50%) in MA stored capsicum. The suppression was higher in T_2 than in T_1. Also, the oxygen demand was lower even during the post storage period in case of MA stored fruits.

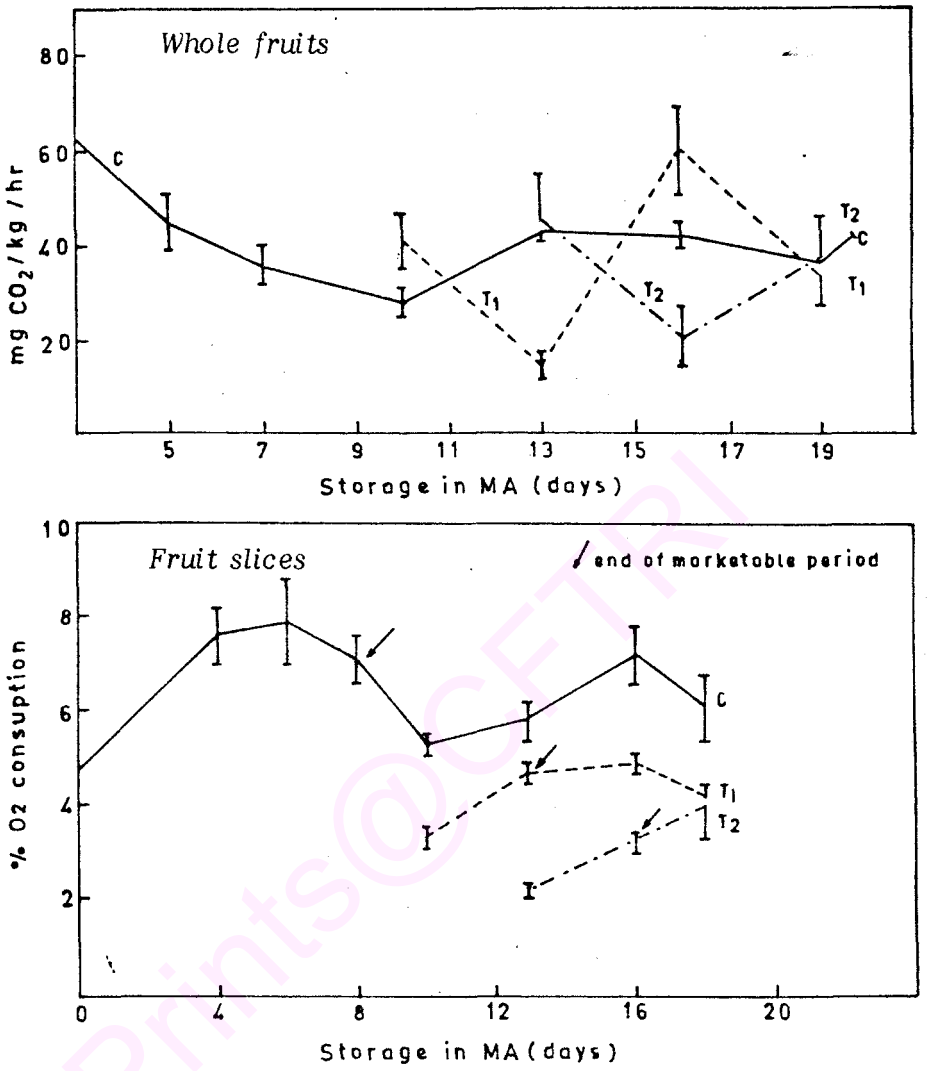


Fig. 17 Respiratory patterns of capsicums stored in MA (C = air stored control; T_1 = 10 days in MA; T_2 = 13 days in MA)

Chemical analysis

By MA storage the shelf-life of capsicums could be successfully extended compared to the air stored controls. The effect of MA on quality of capsicums was ascertained by various chemical analyses.

TSS and pH

There was no significant change in TSS and pH of capsicums during storage. TSS varied between 4.0 to 4.5% and pH between 5.8 to 6.0 both in control and MA stored capsicums (Table 30).

Table 30. Effect of MA storage on chemical constituents of capsicums

Sample	Days after harvest	TSS °Brix	pH	Ascorbic acid mg/100 g F.Wt.	Free sugars g % f.wt.
At harvest	0	4.0	5.8	80	1.88
Control	8	4.5	5.5	115	1.60
T_1	10	3.5	5.9	100	2.00
	13	4.5	5.8	156	1.60
T_2	13	3.5	6.0	115	1.80
	16	3.5	5.9	164	1.72

Ascorbic acid

The ascorbic acid content of capsicums increased after harvest from 80 to 115 mg/100 g. MA favoured the formation of ascorbic acid both during storage and upon removal to air. The ascorbic acid content increased by ∽50% by the end of their storage period (Table 30).

Total free sugars

Total free sugars varied between 1.88 to 1.60 g % during storage (Table 30). In T_1 and T_2 fruits it varied between 1.60 to 2.00 g % during or after MA storage.

Chlorophyll

Chlorophyll is an essential factor for greeness & an index for

raw, fresh condition of capsicums. Chlorophyll decreased during the storage period. Control fruits lost 2.15 mg% of chlorophyll in 8 days period. The degradation was low in MA stored fruits (1.23 to 1.85 mg %) in 13 to 16 days (Table 31).

Table 31 Chlorophyll content of capsicums stored under MA (mg/100 g f.wt)

Sample	Chlorophyll mg/100 g f.wt. Days after harvest 0	8	10	13	16
Control	61.43 ±2.67	59.28 ±3.47	50.32 ±2.49	46.43^{a} ±0.52	40.20^{a} ±2.91
T_1	61.43 ±2.67		61.25 ±4.71	60.02^{b} ±1.80	57.99^{b} ±3.38
T_2	61.43 ±2.67			60.66^{b} ±1.29	59.81^{c} ±6.01

Mean ± S.D. Mean ± S.E.

Means not followed by a common superscript differ significantly ($P < 0.05$).

Alcohol insoluble solids

AIS content decreased during storage of capsicums (Table 32). The decrease was slightly higher in MA stored fruits compared to control fruits.

Table 32. Effect of MA on cell wall components of capsicum

Sample	Days after harvest	AIS g %	Cellulose µg/mg d.wt	Hemicellulose µg/mg d.wt.	Pectin g % d.wt.
At harvest	0	43.69	160.5	25.86	1.90
Control	8	45.04	170.8	22.45	1.90
T_1	10	46.55	160.2	24.00	1.60
	13	39.13	210.0	19.89	2.30
T_2	13	37.23	180.8	23.14	2.25
	16	37.66	190.9	20.89	2.35

Cellulose

Cellulose which mainly constitutes the cell wall material did not show much change (Table 32). Its content varied from 160 to 170 µg/mg AIS in control fruits and from 160 to 210 µg/mg in case of MA stored fruits.

Hemicelluloses

The content of hemicelluloses decreased during storage of capsicums. The content was 25.86 mg/g AIS at harvest and decreased to 22.45 mg/g by 8th day for air stored controls. In MA stored fruits the decrease was slightly rapid after removal to air resulting in 19.89 and 20.89 mg/g in T_1 and T_2 fruits on 13th and 16th days respectively (Table 32).

Total pectin

Air stored fruits did not show any change in pectin content during their marketable period (Table 32). The pectin content of MA stored fruits was slightly higher than control fruits at the end of marketable period.

Ash content

There was slight increase in the ash content of capsicums during storage. It increased from 6.98 to 7.82 g % on dry weight basis during 8 days storage in air. In MA stored fruits it varied from 7.14 to 9.0 g % (Table 33).

The potassium content in capsicums remained almost constant during storage both in air and in MA (Table 33).

Table 33. Ash content and potassium concentration in MA stored capsicums

Sample	Days after harvest	Ash content g % d.wt.	Potassium in ash mg/g
At harvest	0	6.98	10.77
Control	8	7.82	10.77
T_1	10	7.55	10.57
	13	8.34	10.57
T_2	13	7.14	10.38
	16	9.00	10.38

Epicuticular waxes

Accumulation of epicuticular waxes on the surface of fruits continued even after harvest till the end of storage period in control fruits (Table 34). It increased from 6.85 to 8.42 mg/100 g fruit. On the contrary there was inhibition in the accumulation of epicuticular waxes druing MA storage. The decrease was more pronounced with prolonged period of storage under MA. The content of waxes at the end of 13 days and 16 days storage period was 6.60 and 4.59 mg/100 g in T_1 and T_2 respectively.

The relative percentage ofsoft waxes in the epicuticular waxes was higher than hard waxes at all stages of storage both in control and treated fruits. The proportion of soft waxes decreased considerably during storage beyond the marketable period in control fruits. In T_1 fruits also there was marked decrease in the soft waxes beyond their marketable period. However, T_2 did not show much of a difference during their storage period (Table 34).

Table 34. Effect of MA storage on the epicuticular wax and their hard and soft wax content in capsicums

Treatment	Storage (days)	Total Epicuticular wax	Soft wax	Hard wax
		mg/100 g F.wt.	%	%
Control	0	6.85	68.75	31.25
	8	8.42	67.57	32.43
	13	6.00	44.20	55.80
T_1	10	6.14	63.35	36.65
	13	6.60	66.18	33.82
	16	3.62	54.88	45.12
T_2	13	4.90	63.40	36.60
	16	4.59	63.16	36.84

Total lipids

The lipid content of capsicums increased slightly during the storage period in control fruits, but did not show much change in case of MA stored fruits both in T_1 and T_2 (Table 35). Content of lipids was higher in T_2 compared to T_1 fruits.

Group of neutral lipids formed a higher proportion (68.8%) of the lipids at harvest but decreased considerably (to 41.2%) during 8-day storage in control fruits. In case of MA stored fruits, the decrease was ∩ 46% and NL were 36.87% on 16th day of storge. The remaining percentage of the total lipids comprised of polar lipid group.

Table 35. Lipid content of capsicums stored under MA

Sample	Storage (days)	Lipid content (% wt.)
Control	0	1.9 ± 0.05
	8	2.3 ± 0.15
T_1	10	1.72 ± 0.09
	13	1.76 ± 0.02
T_2	13	2.08 ± 0.04
	16	2.03 ± 0.05

Fatty acid composition

Only T_2 group was analysed for fatty acids since no difference was observed between T_1 and T_2 fruits. Thus, T_2 fruits were analysed at the end of their storage period. Eleven fatty acids were detected in NLand PL lipid fractions of capsicum (Table 36). Of these palmitic, stearic and oleic were predominant at harvest. After 8 days storage, the control fruits showed notable decrease in palmitic and increase in oleic acids. In general short chain, saturated fatty acids (10:0, 12:0, 16:0) decreased while long chain, unsaturated fatty acids (18:1, 18:2, 18:3) increased during storage. Myristic, palmitic and stearic acids did not show much change.

In treated fruits the trend remained same. But with intensified effect i.e. the decrease in palmitic and increase in oleic acids were much higher than in control fruits. The freshly harvested fruits gave lower US/S fatty acid ratio indicating less unsaturation which increased during storage. However, the ratio was similar between 8 days air stored and 16 days MA stored fruits. Between NL and PL fraction the latter gave higher values in all the samples.

Table 36. Fatty acid composition of lipids of capsicums stored in MA

Fatty acid	Neutral lipids*			Polar lipids*		
	C_1	C_2	C_3	C_1	C_2	C_3
	%			%		
Capric ($C_{10:0}$)	5.9	2.1	2.2	-	Tr	Tr
Lauric ($C_{12:0}$)	2.1	1.0	0.7	-	0.9	Tr
Myristic ($C_{14:0}$)	3.5	3.2	2.9	0.5	0.6	1.1
Palmitic ($C_{16:0}$)	37.3	31.8	29.4	29.0	26.5	23.9
Palmitoleic ($C_{16:1}$)	2.1	2.3	1.8	Tr	Tr	Tr
Stearic ($C_{18:0}$)	12.2	11.5	12.3	13.5	13.6	11.8
Oleic acid ($C_{18:1}$)	17.6	21.3	30.4	29.8	31.8	25.5
Linoleic ($C_{18:2}$)	4.0	6.3	9.3	22.0	18.7	24.3
Linolenic ($C_{18:3}$)	2.7	2.8	2.3	3.2	3.9	5.8
Eicosenoic ($C_{20:1}$)	8.7	12.0	4.0	1.7	2.1	2.9
Unknown	3.9	5.8	4.6	-	1.5	4.0
Ratio US/S	0.6	1.0	1.1	1.3	1.4	1.7

* Tr = Traces (0.05%); C_1 = freshly harvested; C_2 = control on 8th day; C_3 = T_2 on 16th day; US=Unsaturated fatty acids & S=Saturated fatty acids.

Biochemical changes

Studies with ^{14}C-labelled acids

U ^{14}C-aspartate : In freshly harvested capsicums the aspartate conversion into organic acid (OA) fraction was 16.5% and CO_2 44.3%. But, 37.9% remained unconverted. After 8 days storage 82.2% of aspartate got metabolised and major incorporation was into the OA fraction (56.6%) while, CO_2 respired was low (24.2%) (Table 37).

During MA storage, the fruits had suppressed metablism, 40.1% of labelled aspartate remaining unconverted. Conversion into CO_2 fraction was higher (38.7%) compared to OA fraction (18.9%). During

Table 37. Distribution of U ^{14}C-Aspartate in to various fractions of MA stored capsicums

Sample	Storage period (days)	Amino acid (AA)	Organic acid (OA)	Sugar	Carbon dioxide (CO_2)
Control	0	37.9^a	16.5^a	1.1^a	44.3^a
	8	17.8^b	56.6^b	1.1^a	24.2^a
T_2	13	40.1^a	18.9^a	2.1^a	38.7^a
	16	19.8^b	64.9^b	1.1^a	14.0^a
S.E. (DF=8)		±9.00	±4.47	±0.43	±9.93

Any two means of the same column not followed by a common superscript differ significantly ($P < 0.05$)

the post storage period aspartate metabolism was enhanced, 80.2% getting converted into various fractions. CO_2 respired decreased to 14.0% on 16th day of storage and, OA fraction recorded highest counts (64.9%). In all cases, sugar accumulation was very low (1-2%) (Table 37).

U ^{14}C-Malate: At harvest, 59.3% of the labelled malate was metabolized into other fractions (Table 38). 27.3% counts were released as CO_2 and 11.9% accumulated in AA. Sugars receiving a meagre fraction (1.3%). Upon storage for 8 days in air, the malate conversion was further reduced to 30.9%, CO_2 and AA fractions also receiving lower counts than in freshly harvested fruits.

MA storage further reduced the malate metabolism (71.3% remaining unconverted in OA fraction). During the post storage period, the malate metabolism was further reduced (73.2% remaining in OA fraction). Amino acids received 50% less counts than on 13th day. In all cases sugars receiv ed least counts (0.7 to 1.5%).

Table 38. Distribution of U ^{14}C-Malate in to various fractions of MA stored capsicums

Sample	Storage period (days)	Amino acid (AA)	Organic acid (OA)	Sugar	Carbon-dioxide (CO_2)
Control	0	11.9	59.3	1.3	27.3
	8	7.3	69.1	0.8	21.8
T_2	13	16.0	71.3	0.7	11.7
	16	8.5	73.2	1.5	16.6
SE (DF=8)		±2.38	±6.23	±0.27	±4.96

Means of the same column do not differ significantly ($P > 0.05$)

1-5 ^{14}C citrate : Citrate metabolism showed a similar trend with that of malate. In both control and MA stored fruits, citrate metabolism lowered upon storage. Only ∽ 50% ofthe citrate getting converted into other fractions (Table 39).

Table 39. Distribution of 1-5-^{14}C Citrate in to various fractions of MA stored capsicums

Sample	Storage period (days)	Amino acid (AA)	Organic acid (OA)	Sugar	Carbon-dioxide (CO_2)
Control	0	9.3	46.2	0.4	43.9
	8	6.1	54.6	1.3	37.8
T_2	13	10.3	56.4	0.6	32.0
	16	5.7	66.4	1.0	26.7
SE (DF=8)		±1.44	±8.03	±0.40	±6.56

Means of the same column do not differ significantly (P > 0.05)

At harvest 53.8% citrate was metabolised of which a major portion (43.9%) was evolved as CO_2. Upon 8 days storage, citrate metabolism decreased, only 45.5% getting metabolised. The counts in AA and CO_2 fractions showed a corresponding decrease.

MA stored fruits showed a similar trend, on 13th day they were comparable to freshly harvested fruits and on 16th day to those of 8th day controls. However, the counts remaining in OA were higher (56.4 and 66.4% on 13th and 16th days respectively) than in control fruits. Sugar fractions in all cases contained least counts (0.4 - 1.3%) and CO_2 respired decreased with advance in storage period.

Pectinase activity : The activity of pectinase varied between 5.51 and 5.78 %/mg protein during 8 day storage period in control fruits. In MA stored fruits, its activity was comparatively lower both during and after MA storage (Table 40).

Table 40. Effect of MA storage on pectinase activity in capsicums

Sample	Days after harvest				
	0	*8*	*10*	*13*	*16*
			% activity/mg P.		
Control	*5.51 ±0.65*	*5.78 ±0.01*			
T_1	*5.51 ±0.65*		*4.23 ±0.55*	*4.88 ±0.49*	
T_2	*5.51 ±0.65*			*4.96 ±0.41*	*4.81 ±0.65*

Cellulase activity : Activity of this enzyme was low in capsicums at harvest. Its activity increased slightly during storage. By storing the fruits further, its activity increased to 20 on 16th day. Fruits storedin MA although recorded an increase in cellulase activity, its rate was significantly reduced compared to air stored controls. There was 50% inhibition in its activity in the T_2 fruits on 16th day (Fig. 18).

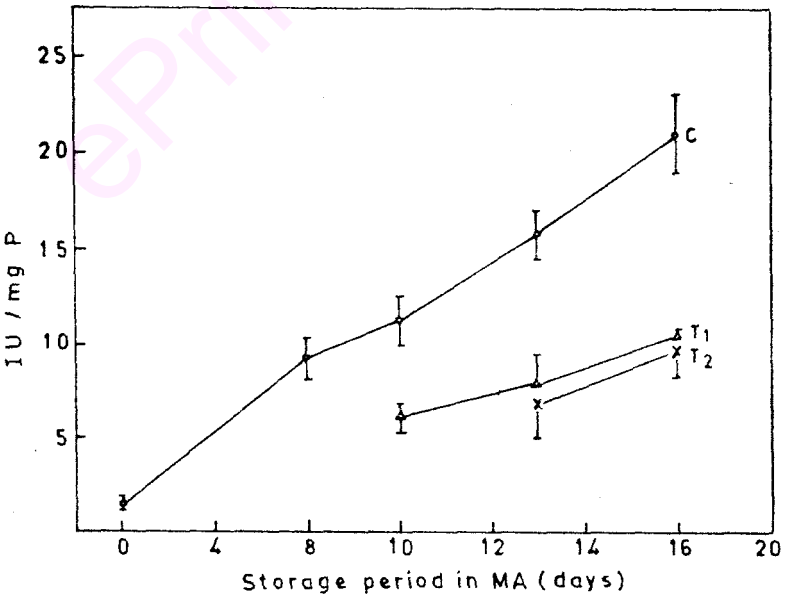


Fig. 18 Effect of MA storage on cellulase activity in capsicums

Pathological studies

Fungal diseases recorded

The major fungi identified were **Fusarium** spp. (viz. **F. oxysporum, F. solani** and **F. semitectum**), **Alternaria tenuis, Colle totrichum** sp., **Rhizopus** sp. and **Aspergillus niger.** Bacterial spoilage was accounted to **Erwinia carotovora.**

Surface mycoflora

The surface spore load of capsicums was tested after 13 days storage. Control fruits without any treatment recorded 86 viable colonies. Fruits treated with fungicidal and bactericidal solution (500 ppm captan + 1000 ppm streptocycline) recorded 63% reduction in viable fungal colonies while fruits pretreated with fungicide and stored in MA recorded about 99% reduction in viable fungal colony. Fungal sp. recorded are given in Table 41. Thus there was suppression of fungal pathogens with fungicides. MA storage further reduced the viability of pathogens of capsicums.

Table 41. Influence of MA on surface mycoflora of capsicums (after 13 days)

Fungi	No. of colonies at 10^{-1} dilution		
	Control	Pretreated *	Pretreated * + MA
Fusarium sp.	61	24	0
Alternaria sp.	22	5	0
Rhizopus sp.	2	2	0
Aspergillus niger	1	1	1
Total	86	32	1

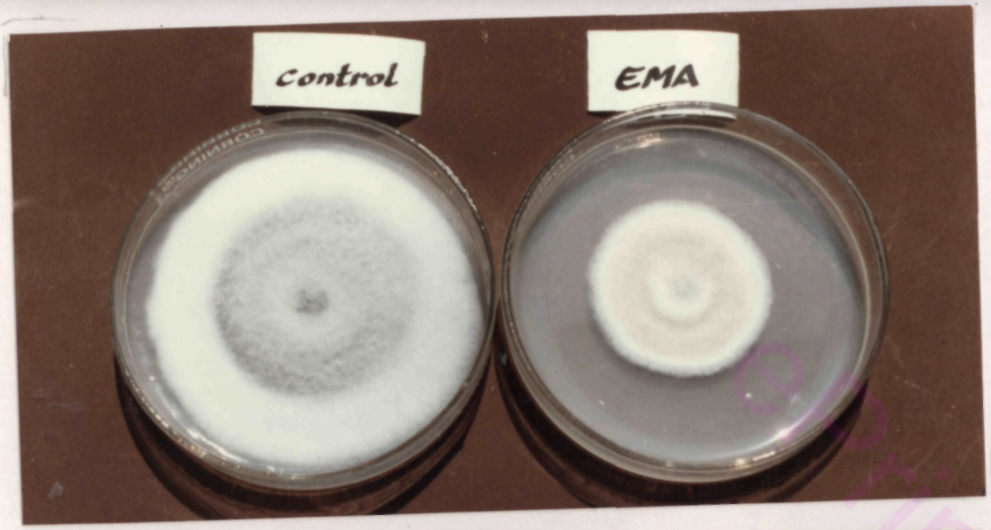
* Pretreated with 500ppm Captan + 1000ppm streptocycline

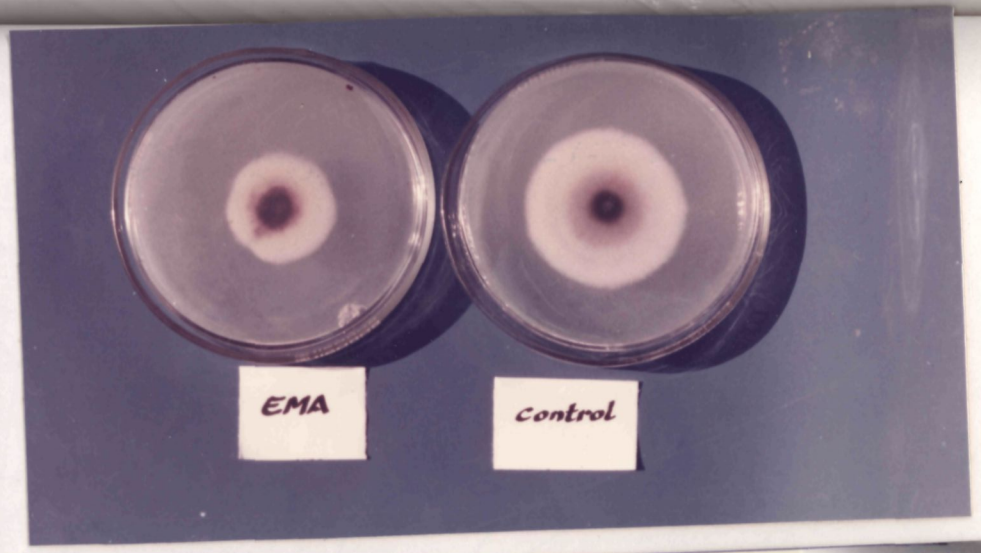
In vitro effect of MA on fungal pathogens of capsicum : The ***in vitro*** *effect of depleted* O_2 *with increased* CO_2 *on two major pathogenic fungi of capsicums viz.* ***Fusarium oxysporum*** *and* ***Alternaria tenuis*** *were studied. The concentration of gases selected were based on the effective concentration of* O_2 *and* CO_2 *inside the MA chamber on 10th and 13th day of capsicum storage.*

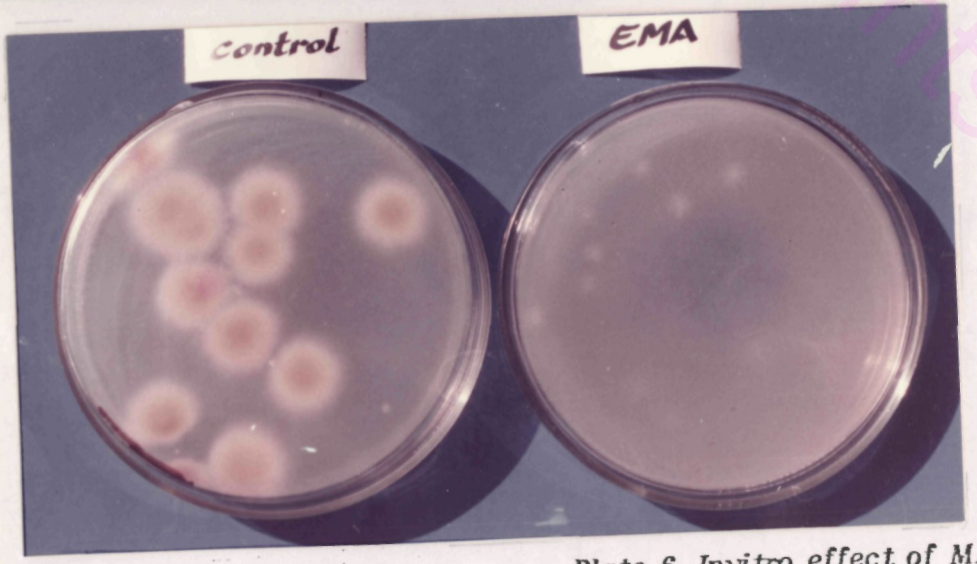
Effect of CO_2 *on mycelial growth (with oxygen kept constant at 7.5%): There was a linear decrease in percentage inhibition of* ***Fusarium*** *with increase in* CO_2 *concentration from 3.5 to 5.0%. Decrease in* CO_2 *to 3.5 and 4.0% was more effective. The percentage inhibition declined sharply with increase in duration of exposure (Fig. 19).* ***Alternaria*** *also showed reduced mycelial growth with decreased* CO_2 *concentration. However unlike in* ***Fusarium,*** *it was more effective even under prolonged storage period (Fig. 19).*

Effect of O_2 *(with* CO_2 *kept constant at 3.5%) : In* ***Fusarium*** *there was increase in the percentage of inhibition with decreased* O_2 *concentration. The* O_2 *concentration of 5.5 to 6.5 with 3.5%* CO_2 *was found to be more effective in reducing mycelial growth for a prolonged period. Maximum inhibition (40%) was recorded in the lowest concentration of* O_2 *(5.5%) on the 7th day (Fig. 19). In* ***Alternaria,*** O_2 *concentration of 6.5 to 7.5% with 3.5%* CO_2 *was found to be more critical and exhibited 50 and 56% inhibition respectively on 7th day. The lowest concentration of* O_2 *(5.5%) was also effective to a lesser extent (Fig. 19).*

Comparatively low O_2 *concentrations tried were more effective in reducing the rate of mycelial growth in* ***Alternaria*** *than in* ***Fusarium.*** *In both the fungi the percentage inhibition obtained was highest on 7th day coinciding with log phase of fungal growth (Plate 6).*







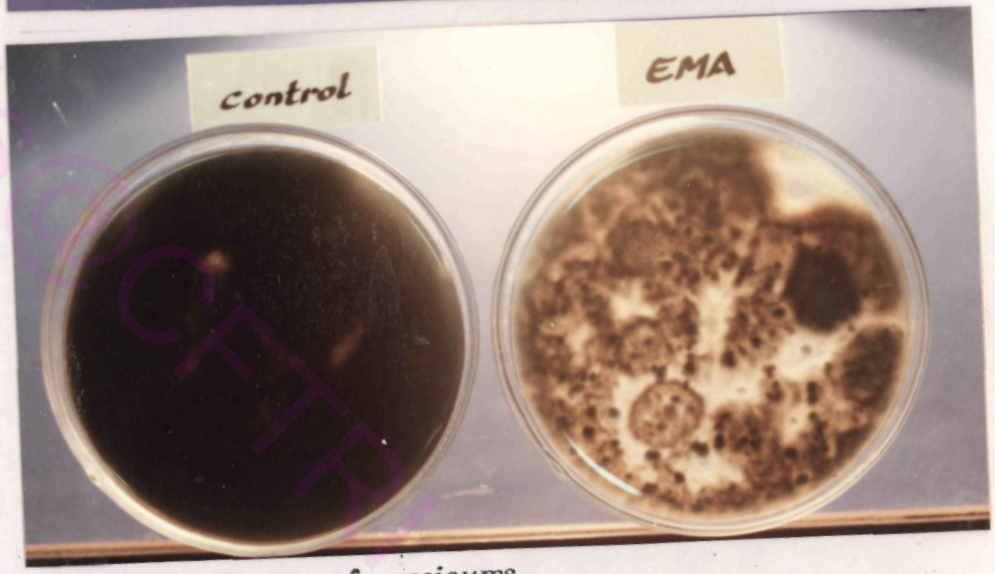


Plate 6. Invitro effect of MA on fungal pathogens of capsicums.

(a) *Fusarium oxysporum*

i) on mycelial growth

ii) Spore germination

(b) *Alternaria tenuis*

i) on mycelial growth

ii) On spore germination

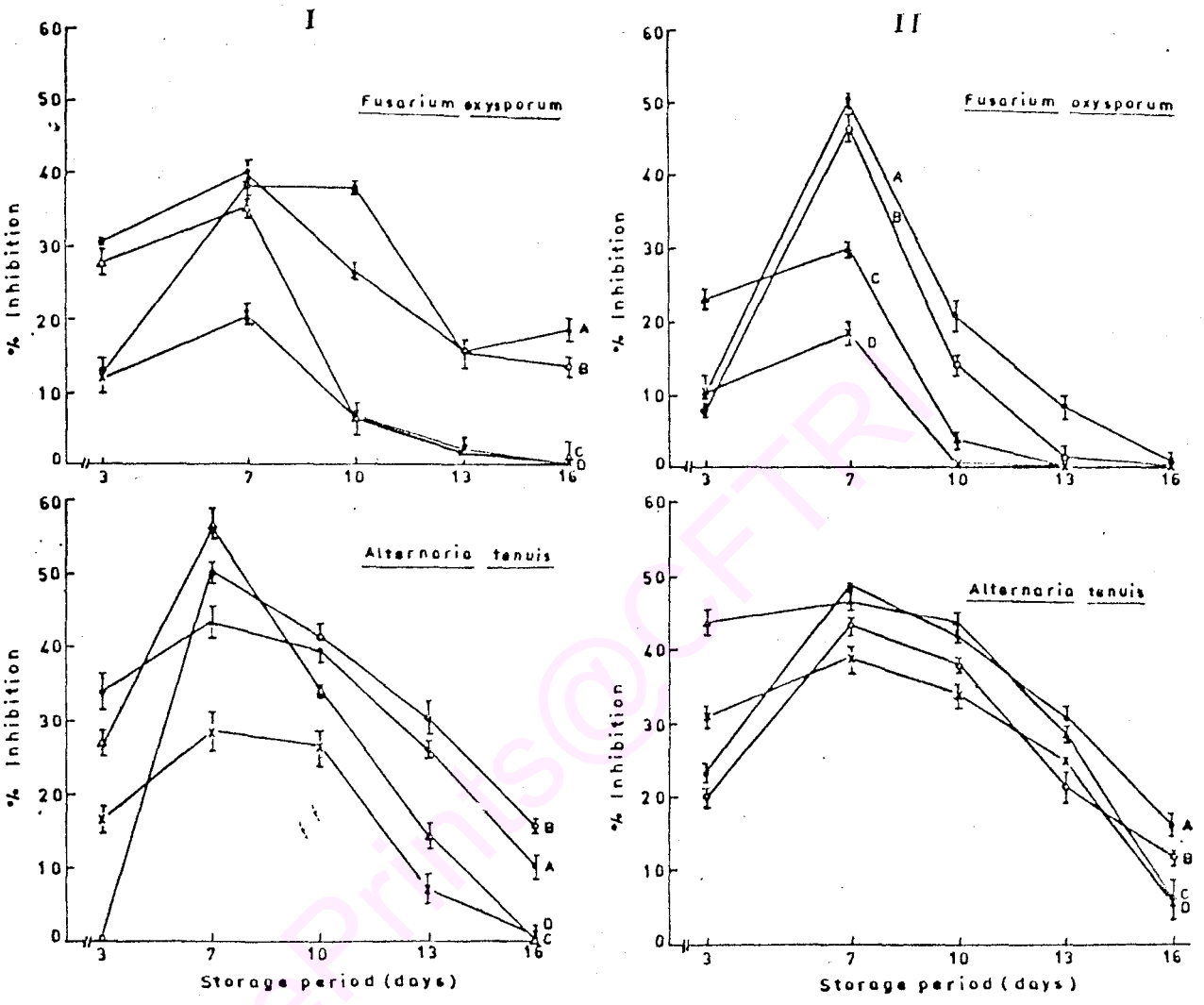


Fig 19. Invitro effect of MA on mycelial growth.

I. Effect of CO_2 (O_2 7.5%)

A - 3.5% B - 4.0%
C - 4.5% D - 5.0%

II. Effect of O_2 (CO_2 3.5%)

A - 5.5% B - 6.5%
C - 7.5% D 8.5 %

Effect on spore germination

Effect of CO_2 (with O_2 kept constant at 7.5%): In ***Fusarium****, the effect was generally inhibitory at all concentrations. The inhibition increased with increase in CO_2 concentration from 3.5% to 5.0%. With 5% CO_2, maximum inhibition (66%) occurred. However its inhibitory effects declined sharply after 13 days exposure. In* ***Alternaria*** *also CO_2 had inhibitory effects at all concentrations and it increased with increase in concentration of CO_2 (Fig. 20).*

In addition, under MA, the normal profuse growth of colonies from germinated spores were significantly restricted (Plate 6c,d).

Effect of O_2 (CO_2 kept constant at 3.5%): In ***Fusarium****, there was 56% inhibition in spore germination with O_2 concentration of 5.5 to 6.5% on 16th day. Further increase in O_2 concentration to 7.5% and 8.5% led to stimulatory effects. The behaviour of* ***Alternaria*** *was reversal of that of* ***Fusarium****. Low O_2 concentratin (5.5%) was even stimulatory. The percentage inhibition was in an increasing order with increase in concentration of O_2.*

Organoleptic qualities

Sensory qualities of capsicums (whole fruits) were studied soon after harvest, after treatment and at the end of their storage period. The results are presented in Table 41.

In colour and appearance the freshly harvested capsicums scored highest. Capsicums stored in MA maintained their appearance very well and were comparable to freshly harvested capsicums. At the end of storage period of 13 and 16 days they showed a decline but were better than 8 day stored control fruits. This trend was repeated in fruit firmness also. The calyx of the fruits which are greenish when freshly cut started turning brown and desiccated during storage. The

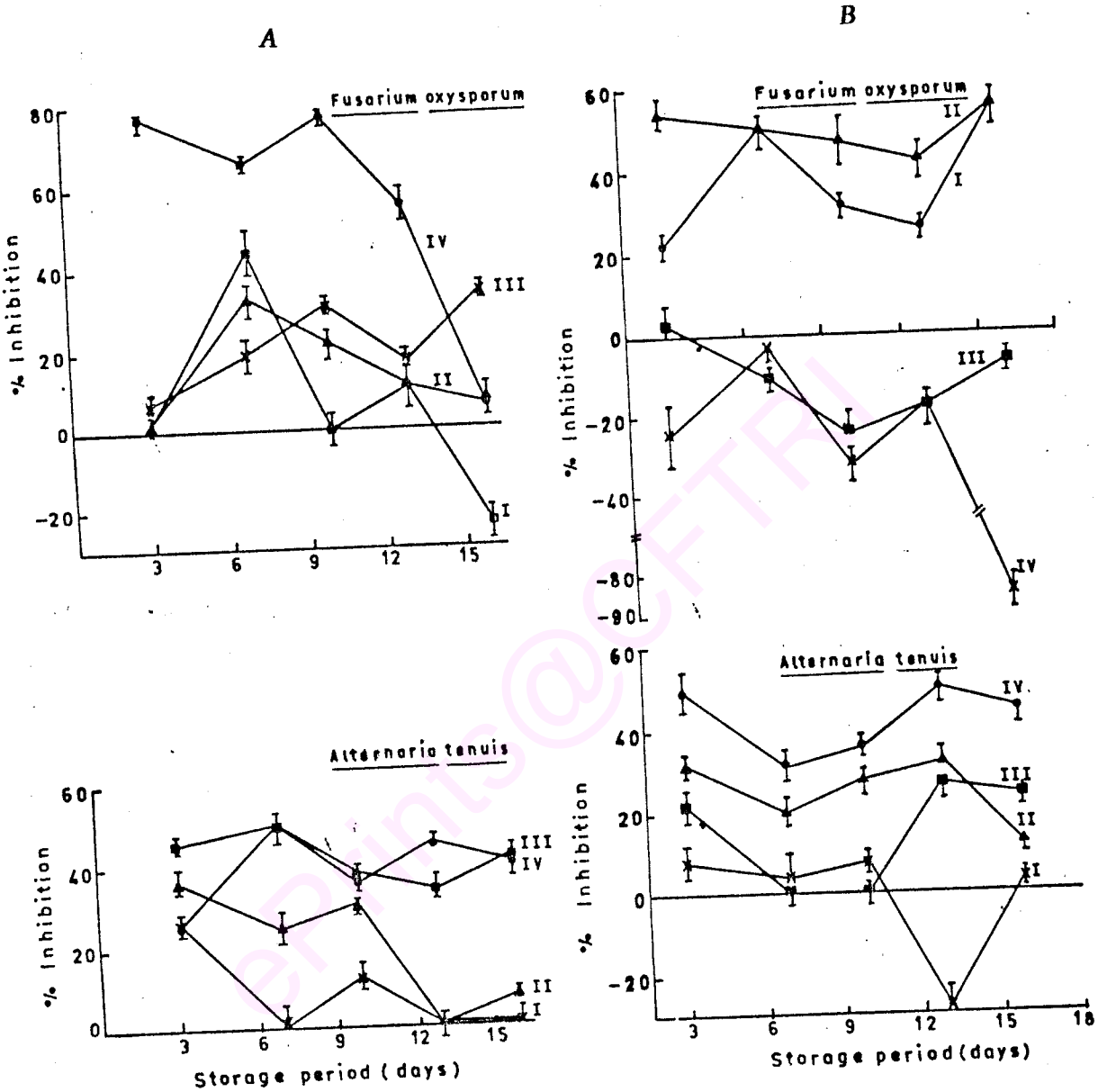


Fig 20. Invitro effect of MA on spore germination

A. *Effect of* CO_2 (O_2 7.5%)

I.- 3.5% *II* - 4.0%
III - 4.5% *IV* - 5.0%

B. *Effect of* O_2 (CO_2 3.5%)

I - 5.5% *II* - 6.5%
III - 7.5% *IV* - 8.5%

Table 42. Sensory qualities of capsicums stored in MA

Quality attributes	Control		T_1		T_2	
	Days after harvest					
	0	8	10	3	13	16
Colour and appearance	4.90	3.90	4.85	4.00	4.65	4.00
Calyx freshness	4.90	3.95	3.90	3.85	3.80	3.80
Firmness	5.00	4.30	5.00	4.50	5.00	4.40
Odour	4.95	4.80	4.90	4.85	4.90	4.80
Overall quality	4.95	4.45	4.90	4.55	4.89	4.50
Marketability	5.0	4.5	4.9	4.6	4.9	4.5

Scale 1 to 5.

MA stored fruits scored lower than freshly harvested capsicums in calyx freshness. However their scores were not significantly different from that of control fruits at the end of marketable period.

In overall quality, the capsicums soon after removal from MA were as fresh as freshly harvested fruits. Similarly, at the end of their storage period their quality was comparable to 8-day air stored control fruits.

The maintenance of various quality attributes in MA stored fruits was well reflected in the high scores by panelists' acceptance for marketability. The marketability of MA stored fruits immediately after storage and after 3 days post storage period were comparable to freshly harvested fruits and after 8 day storage in air respectively. However, all the samples were rated 'acceptable'.

DISCUSSION

Experiments were conducted to extend the storage life of capsicums which are highly perishable in nature. Their shelf-life varied from 4-8 days at 27 ± 4°C depending on the condition of the raw material. Eight days was the maximum storage life of freshly harvested capsicums even after receiving post-harvest measures. The method of MA storage used in our experiments is somewhat different than the conventional MA as studied and practiced in Western countries wherein, extraneous supply of CO_2 and O_2 and removal of C_2H_4 by scrubbers and the concentration of gases are strictly monitered. In the present study however, the MA employed is essentially created by the respiratory activity of the commodities stored in MA chamber (Plate 1).

Capsicums conventionally handled could not be stored even for 5 days and suffered heavy bacterial spoilage by **Erwinia carotavora.** However, these samples under MA stored better for 5 days. The high incidence of spoilage was due to bad handling practices resulting in visible and invisible physical injuries which aggravate contamination with microbes (9).

Eliminating bad handling practices practiced in normal marketing channel, shortening the duration after harvest followed by antimicrobial treatments reduced microbial spoilage enormously and extended the life of capsicums in storage (Fig.22). Importance of post-harvest handling in reducing microbial spoilage and extending the shelf-life of horticultural crops being the objective of post-harvest technology

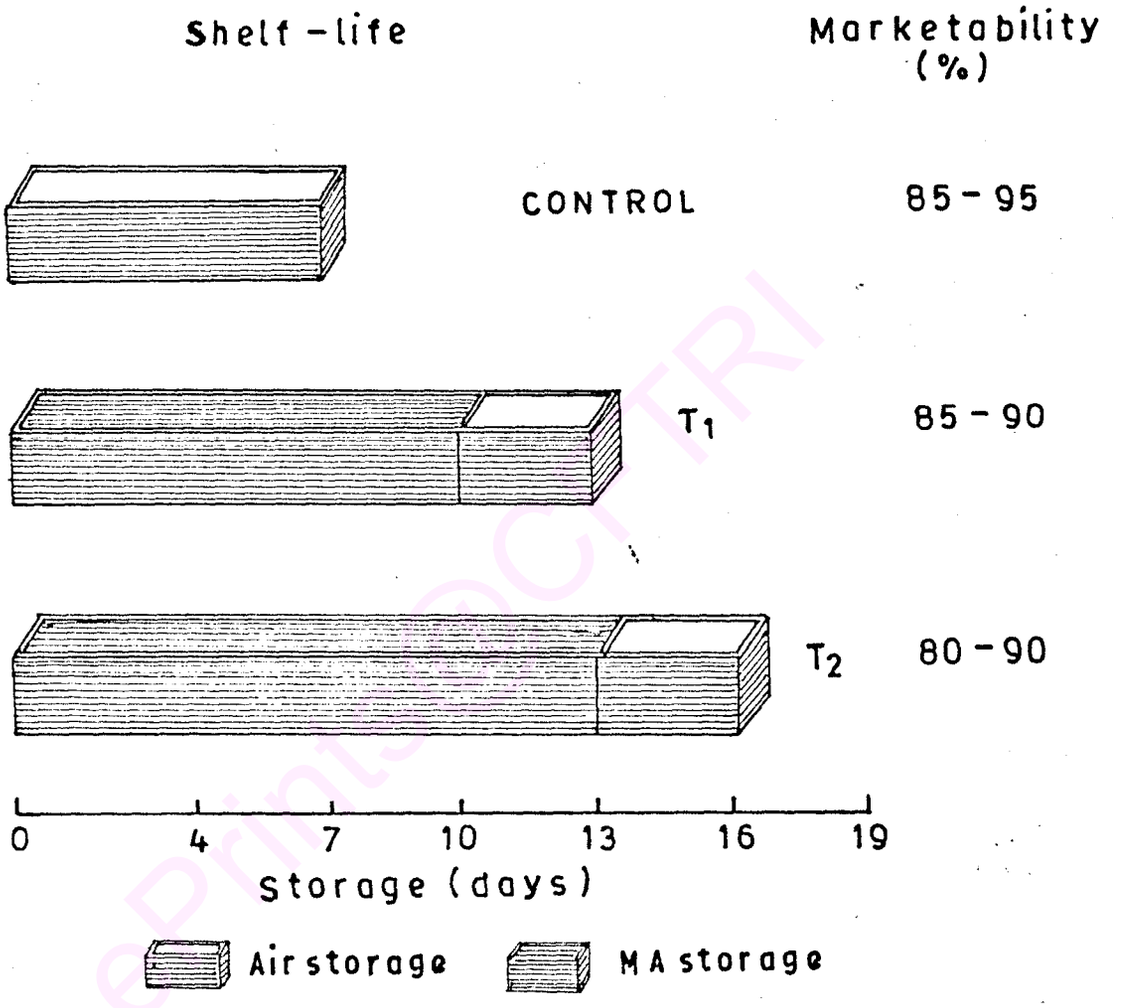


Fig. 22 Extension of shelf life of capsicums by MA storage

has been well reviewed by many workers (19,44). Further, storage under MA (4 to 5% CO_2 + 8 to 6% O_2 respectively) doubled the shelf-life of capsicums at ambient temperature (27 ± 4°C) maintaining the quality which is comparable to freshly harvested ones. Capsicums are usually stored under CA supplemented with low temperature. The recommended conditions being 4-8% O_2 and 2-8% CO_2 at 13°C (1) or 3-5% O_2 and 0% CO_2 at 8-12°C (32). However, Bussel and Keni gsberger (10) employed unit tray package of capsicums in VF films at 25°C. The levels of O_2 (14%) and CO_2 (1.5%) were not altered to the extent of having physiological effect on the commodity.

Thus, the storage environment greatly influences the extension of storage life of capsicums with quality maintenance. The interaction of the commodity with its altered environment culminating in physical, physiological and biochemical changes are discussed below.

Gaseous concentration in MA chamber: Storing capsicums in MA led to accumulation of CO_2 respired by the commodity with corresponding depletion of O_2. On 10th day of storage, the concentration was 4% CO_2 + 7% O_2 and on 13 day, 5% CO_2 + 6% O_2 at 27 ± 4°C. The build up of CO_2 and depletion of O_2 is related to the respiratory rate of the commodity and storage temperature. The concentration of CO_2 and O_2 as measured on GLC revealed that for every 1% rise in CO_2 conc. there was 3.5% depletion of O_2 inside the MA chamber. Similar rapid decline in O_2 conc. with increase in CO_2 was observed in MA package of tomatoes by Geeson **et al.**, (23). Further the increase in CO_2 conc. is dependent on PE film permeability and temperature (9,10). The O_2 was depleted to a concentration(6-8%) which could have physiological effect on the fruit (10). The CO_2 build up was 4 and 5% which is well within the recommended level of 2-8% (1,32). The build up of CO_2 and decreased O_2 could be correlated with suppressed respiration rate, PLW, ripening and fungal spoilage. This could be attributed to suppressed metabolic processes with reduction in O_2 and competitive inhibition of ethylene by CO_2 (9).

Weight loss: *Peppers which have large surface to volume ratio are highly susceptible to water loss and wilt rapidly at ambient temperature and RH. The apparent and therefore commercially objectionable, severe shrivelling symptom is 13.7% weight loss and the average % weight loss per daywith moderate shrivelling for capsicums is 2.2% (47). In our studies however, the weight loss of capsicums during the marketable period was well within this limit (12%)with % weight loss per day ranging from 1.65% in air stored and 0.72 - 0.91 % in MA stored fruits. Hence, MA significantly retarded weight loss, shrivelling and loss of freshness. However, weight loss of 5-10% and 15% are reported acceptable in bell peppers (3,46b), whereas in green beans weight loss up to 37% is tolerated (29). In general, the point at which shrivelling is visible is about half of the total figure i.e., 50% of commercially tolerable invisible weight loss (29,34).*

The reduction in weight loss in MA stored fruits is attributed to high RH inside the MA chamber which lowered the water stress resulting in reduced water loss (5). The effect of RH on water stress and quality of vegetables is well reviewed (9). In addition, altered CO_2 *and* O_2 *ratio has direct relation with reduced metabolic rate and hence water stress (46b).*

Weight loss is an essential and inevitable process that progresses unchecked at all temperature and RH. However, weight loss can be reduced by lowering the temperature (17,21). Bell peppers even at the optimal low temperature of 7°C and RH of 90%, lost about 7% of their original weight after 21 days storage. This phenomenon was also observed in bell peppers wrapped in RMF and PVF films (10).

MA storage gave protection against water loss, as a result the quality was successfully maintained. The MA stored fruits at the end of their storage period of 10 and 13 days were indistinguishable from freshly harvested capsicums in their freshness and crispness (as measured by their sensory qualities). The external appearance at the end of storage periods of both air and MA stored fruits also were comparable.

Rapid loss of weight in capsicums during post MA storage period could be attributed to relaxation from the initial suppression of metabolic activities by high CO_2 and to the changes caused by water stress (5,56).

Water stress is known to affect tissues in the same manner as senescence (12). Leopald and Coworkers (39) related water stress to membrane integrity and solute leakage in cow pea leaves. It was thus suggested that senescence occurred parallel to decline in water content but was not caused by water stress and all these changes were normal events in detached organs.

Relative humidity: *At constant temperature, weight loss has a linear correlation with RH. The upper humidity range of RH varies from 85 to 100% for different commodities (47).*

The high RH inside the MA chamber (85 to 100%) was beneficial for storing capsicums. Storing apples in 98% RH has also been found to be beneficial in maintaining the quality (6). It has been proposed that water potential regulates passive transport by influencing turgor pressure of cells (15,61). Zimmermann (61) also proposed that plant cells adjust their osmotic pressure in response to environmental stress. It is possible that maintenance of high RH inside the MA storage may be related to less loss of weight and consequently high turgor. Physical rather than physiological effects of RH (principally desiccation) predominate in capsicum as in other non-climacteric fruits such as oranges and grapes (2).

Chlorophyll breakdown: *Colour change from green to red has decisive effect on marketability of capsicums. Degradation of chlorophyll was retarded during MA storage. Similar findings were recorded in bell peppers stored under elevated CO_2 (42,56). Elevated CO_2 and reduced O_2 are known to reduce chlorophyll degradation and synthesis of carotenoids and anthocyanins in pears (35), citrus fruits (54), apples (36,40) and tomatoes (48). MA storage supplemented with cold storage*

further favoured chlorophyll retention in apples.

CA storage not only decreased breakdown of chlorophyll but also reduced or totally inhibited the synthesis of lycopenes, carotenoids and xanthophylls in tomatoes (24,48) and sweet pepper (56).

The disappearance ofchlorophyll and development of red colour however, proceded at a faster rate when capsicums were transferred to ambient conditions. The difference in colour change between air and MA stored fruits was narrowed after 16 days storage. This indicates the need for continuous presence of CO_2 to inhibit red colour development as was observed in sweet peppers stored under CA (56).

Respiration: *A combination of elevated CO_2 and lowered O_2 has additive effect in suppressing respiration (33,60). Capsicum being a non-climacteric fruit exhibited a decline in the respiratory rate from harvest till the end oftheir marketable period. In air stored fruits, CO_2 production declined gradually whereas MA stored fruits showed steep decline at the end of their marketable period. The rate of O_2 consumption by tissue slices also showed a similar trend with increase in O_2 consumed during storage. In sweet potatoes (13,51) and bananas (9) the respiration was depressed by low O_2 concentration. CO_2 when used alone reduced the respiration rate only at high concentrations (~ 20%) which result in CO_2 injury (33). However, elevated CO_2 with reduced O_2 has additive effect in suppressing respiration (33) as may be the case in our studies.*

However, control and treated capsicums stored beyond their marketable periods showed increase in respiratory rates. This may be due to increased catabolic activities leading to senescence. Pushpa and Srivastava (45) reported increased respiration rate as aresult of spoilage of bell peppers. In our studies however, the diseased fruits were not subjected to respiration studies.

Although effect of MA on ethylene production is an important factor, C_2H_4 could not be measured in our studies due to lack of necessary facilities.

Intermediary metabolism: *Studies with ^{14}C-labelled acids revealed that aspartate metabolism was accelerated while that of malate and citrate declined during storage of capsicums irrespective of storage atmosphere. Organic acids accumulated during storage by reduced conversion into other fractions. Besides this, their supply was replenished by conversion from aspartate. Intensification of these changes was observed in MA stored capsicums. Accumulation of organic acids is a common feature in CA stored fruits (33). However, in capsicums the accumulation of organic acids was not to the extent of causing any damage to the commodity as observed in apples wherein, excess accumulation of organic acids during CA storage resulted in appearance of russetting and ultimately death of the fruit (41b).*

The rate of incorporation of ^{14}C acids into CO_2 fraction was lowered during storage of capsicums irrespective of treatment. Decline in CO_2 evolution was also recorded during respiration studies both in whole fruits and slices of capsicums. This was more pronounced during post storage period of MA stored fruits.

In general, the rate of metabolism of ^{14}C-labelled aspartate, malate and citrate was suppressed during MA storage and were comparable to freshly harvested fruits. Suppression of metabolic activities by high CO_2 treatment is a common phenomenon in many commodities. During subsequent storage in air however, the MA stored fruits regained normal levels of metabolism and at the end of 16 days storage were comparable to 8 days air stored fruits.

Thus it could be concluded that the metabolism of aspartate, malate and citrate were not impaired by MA storage. This has been confirmed by histological and organoleptic qualities.

Chemical constituents: *MA storage favoured accumulation of ascorbic acid in capsicums. Wang (56) and Basiouny and Biswas (4) observed an increase in ascorbic acid content during CA storage of sweet peppers. Retention of ascorbic acid in Chinese cabbage stored under 1% O_2 was reported by Wang (57) wherein, low O_2 was more beneficial than elevated CO_2 in ascorbic acid retention. However, in the present study elevated CO_2 with lowered O_2 favoured ascorbic acid retention.*

of free fatty acids

The degree of unsaturation increased in capsicums during storage. This was effected by decrease in palmitic and significant increase in oleic acid contents. Other C_{18} unsaturated fatty acids (linoleic and linolenic acids) also increased during storage. In general, short chain, saturated fatty acids decreased while long chain, unsaturated fatty acids increased during storage. Intensification of these changes resulted in higher degree of unsaturation in MA stored fruits, especially in the polar lipid fractions. In peaches and nectarines also, the degree of unsaturation in the polar lipid fractions increased during CA storage (58). It was suggested that this was brought about by increased synthesis of unsaturated fatty acids. High CO_2 and low O_2 could also retard oxidative degradation of unsaturated fatty acids.

In healthy and undamaged commodities no loss of minerals occurred during storage. CA did not influence the mineral content. The potassium content of both air stored and MA stored capsicums remained unchanged during the storage period. Changes in other chemical constituents like TSS, pH, sugars, ash did not differ between air and MA stored capsicums.

Texture: *Texture and colour changes are the two important characters which determine the marketability other than spoilage, of bell peppers. An attempt to standardize the objective measurement of firmness by puncture test revealed 4 to 5.3 lbs to be optimum range for harvest to obtain good quality fruits. Several authors emphasised the importance of texture potential to purchaser and as a maturity index in apples, pears, peas and kiwi fruits (7,28,46a).*

The three recognised principles that are most widely used for measuring the firmness of fresh horticultural crops are puncture, deformation and extrusion (8). These methods were employed for firmness measurements of capsicums also. Our results revealed that kramer shearing of whole fruits could not be correlated with their textural properties. Capsicum being hollow inside probably change the volume drastically at initial or final rupture of the sample. Hence, the properties may be quite different from the original state because of more scope for compaction. This may also be attributed to the larger size of the fruits compared to size of the kramer shear cell due to which representative samples could not be analysed. Hence, further studies were conducted with fruit slices.

The shear stress (1/2 peak width) of MA stored bell peppers were comparable to those of freshly harvested fruits. The reason may be that peppers immediately after harvest and MA storage were fresh and turgid. Due to high turgor pressure of the cells, their cell walls are extremely stretched and are in a state of tension. Therefore, low pressure or strain is sufficient to break them since cell walls cannot stretch further. This has been observed in potatoes (18), apples (27,49). Murase and Merva (43) have shown that water potential affects rigidity of tomato epidermis. Compressive strains for both shear and torsion increased after removal from MA. This is attributed to destention of cell walls caused by loss of water. As a result, the stretchability of cells increased requiring greater compressive force to shear or rupture as in apples (49).

In the compression test, peak area reflected the sensory texture profile better than peak force. Our observations suggest that shape and width of peak indicate adhesive effects. This may be due to the fact that in many vegetables growth occurs even after harvest (55), wherein cell cohesion will be maintained. Hence, peak shape and width reflect the texture profile better than peak force which reflects the softness in texture. Peak force at specific point in the deformation

may not necessarily coincide with maximum force. Kramer (37) and Szczeniak **et al.**, *(53) also emphasized the need of force deformation curves to establish readings of an instrumental texture profile. They opined that peak force may or may not correlate with sensory scores or may be confined to only mass of the food.*

Capsicums soon after harvest exhibited typical brittleness in their texture. Their freshness and brittleness could be successfully maintained by storing them under MA. Retention of firmness in sweet peppers was reported by storing them in 5% CO_2 + 3% O_2, wherein it was observed that concentration of O_2 did not influence texture of sweet peppers (56).

Although, MA efficiently arrested fruit softening and maintained crispness during treatment period, after removal to air capsicums lost their crispness very rapidly. This clearly indicates the necessity for continuous storage of capsicums under MA. The loss in texture may be due to increased PLW, increased water stress, increased respiration, etc. (5). Similar results were obtained by Wang (56) during MA storage of sweet peppers. Isherwood (31) emphasised that no single factor is responsible for textural quality and the properties involved are interrelated. Factors considered contributory to texture of peppers include cell wall polysaccarides, cell turgor, cellular adhesion and cell wall degrading enzymes.

There was slight increase in total pectin and cellulose content and decrease in hemicelluloses during storage of capsicums both in air and in MA. This is in agreement with findings of Ben-Yehoshua **et al.**, *(5) during storage of bell peppers individually packed in HDPE. The cell wall degrading enzymes cellulase and pectinase did not show significant difference between control and MA stored fruits. Involvement of these enzymes in maintaining the texture of MA stored capsicums is not very clear.*

Spoilage: *The lowered weight loss and high RH maintained within the MA chamber had profound influence on microbial spoilage of capsicums. The former helps in maintaining the freshness of the commodity along with its inherent resistance factors. This may partly help in reducing spoilage. Fresh green vegetables are known to be resistant to microbial spoilage (19). High humidity (98-100%) also influences alleviation of fungal spoilage (9,26). However, keeping capsicums further under MA led to increased microbial spoilage which may be due to increased* CO_2 *which is known to aggravate spoilage above the critical concentration and also the response of commodities which may change from near immunity to high susceptibility within several days (52).*

Further, storage of commodities under MA is known to reduce microbial spoilage (33,52). Studies on surface mycoflora revealed the effectiveness of fungicide + bactericide in reducing significantly the viable fungal spore load. Subsequent storage in MA almost completely eliminated the expression of fungal organisms.

In vitro effect of MA: ***In vitro*** *studies carried out to assess the direct influence of MA on post-harvest pathogens of capsicums revealed that inhibition of 50% mycelial growth was achieved with highest % of* CO_2 *(5%) tried along with 7.5%* O_2 *in* ***A. tenuis*** *and* ***F. oxysporum.*** *However, Wells and Uota (59) used 20%* CO_2 *with normal* O_2 *concentration (21%) to obtain 50% inhibition of* ***A. tenuis*** *and* ***F. herbarum.*** *Our studies indicated that* CO_2 *was more effective when used along with reduced* O_2 *levels. Synergistic effect of* CO_2 *and CO and low levels of* O_2 *to reduce post-harvest pathogens is reported (20,52).*

With 3.5% CO_2 *and lowest concentration of* O_2 *tried (5.5%),* ***A. tenuis*** *was more effectively inhibited when compared to* ***F. oxysporum.*** *Well and Uota (59) achieved high % of inhibition of mycelial growth of many fungi with 4%* O_2 *in liquid media at 19°C whereas on solid media further reduction in* CO_2 *conc. below 1% was needed (22).*

The inhibition was found to be maximum at log phase and minimum at lag and deceleration phase of growth in both the organisms. The % of inhibition at log phase revealed that MA suppressed various metabolic processes of these organisms. However, the suppression seems to be reversible. The mycelial growth regained its normal growth rate after removal from MA. But those exposed to reduced CO_2 conc. regainedtheir normal growth rate slowly (41a). This phenomenon was also observed in ***Candida utilis*** *by Shikdchiko (50). He also suggested that CO_2 effect can be reversed by aeration and varying pH of the medium.*

A direct assessment of effective ***in vivo*** *composition of gases on spore germination of* ***A. tenuis*** *and* ***F. oxysporum*** *revealed that there was a linear inhibition of spore germination in both thefungi with increase in CO_2 conc. Very high conc. of CO_2 (> 32%) was required to obtain significant reduction in germination ofspores in several fungi at 19°c(59). Our results reveal CO_2 to be more effective with depelted O_2 conc. at 27°C due to synergistic action.*

Sensory qualities: *In all the sensory qualities the freshly harvested fruits and fruits at the end of MA storage were comparable. Upon 3 days storage in air, the MA stored fruits declined in quality and were comparable to 8 days air stored fruits. All the samples were acceptable.*

The market and nutritional standards go together as 'freshness' while assessing the quality attributes of capsicum. There was sufficient evidence to conclude that MA stored fruits retained their freshness and were comparable to freshly harvested capsicums at the end of 10 and 13 days storage in MA. At the end of storage period however, their freshness was comparable to 8 days air stored fruits. Firmness assessment by finger feel is more advantageous in capsicums as it involves larger surface orwhole fruit itself and hence gives better information. However, these qualities were also confirmed by instru-

mental data. Similarly, other sensory qualities like odour were also comparable in air and MA stored capsicums at the end of their marketable period. The lower scores for calyx freshness in MA stored fruits were attributed to slight desiccation. Severe calyx injury was recorded during high CO_2 (20-30%) storage of sweet peppers (56).

The other advantages of MA storage of capsicums being maintenance of freshness and green colour with minimum spoilage, preventing direct quantitative monetory loss in marketability, satisfying the qualitative sensory and nutritional needs of consumers over an extended period of storage.

CHAPTER 5

STRUCTURAL STUDIES

This chapter encompasses the light microscopic studies. However, light Microscopy rearly proves sufficient to appreciate fully the complex microstructure in fruits/ vegetables. Scanning electron Microscopy was therefore employed additionally to resolve the cellular organisation during ripening of mangoes exposed to acetylene and modified atmosphere stored capsicums. The inter-relationship of structural changes with textural & chemical changes has also been discussed.

INTRODUCTION

It is now well established that structural characteristics dictate the mechanical properties and hence the sensory response of fruits and vegetables. Consequently texture is a manifestation of its microstructure which in turn depends upon the influence of physical forces and chemical components. Though the quantification of microstructure is not yet an absolute science, it should be emphasised that with regard to fruits and vegetables it is the structural organisation than the appearence of a single component that dictate textural responses. Further it is the only conclusive method to determine the physical changes taking place inside the fruits/vegetables.

Further softening of fruits and vegetables is considered to be a senescence phenomenon. Texture assessed by subjective appraisal and mechanical force result from the interction of more than one physical characteristic. Of these, intercellular adhesion is one of the primary characteristics that influence texture. This possesses an intricate structural organisation which canbe affirmed by histological studies.

RESULTS

Structural changes in acetylene treated mangoes and MA stored capsicums were studied by light and scanning electron microscopy (SEM).

MANGO

Surface Topography

Surface of mango fruits under light microscope exhibited a homogeneous matrix with minute pin pricked-like markings distributed all over the surface (plate 7a). This gave the fruit cuticle sponge like appearance. The transverse section of peel tissue revealed that the cuticle covered the epidermis to a thickness of 7-8 μm (plate 8). When viewed under SEM, the surface showed a surprising degree of microsculpturing. It exhibited uneven surface bearing folds, knobs and plate-like epicuticular outgrowths (plate 7b). This surface microstructure did not alter very much during ripening except for clear expression of the raised, plate like or dilated structures (plate 7c).

The surface of treated fruits exhibited comparatively smoother at the ripe stage (7th day) with the basic pattern similar to control (plate 7e). Further, it was observed that there was less epicuticular knob like outgrowth with more intricate ridges or protruberances.

Anatomy :

Light Microscopy : Mango is a drupe (stony fruit) consisting of an exterior, thin, leathery percarp. Pericarp encloses fleshy, edible mesocarp which is limited interiorly by the stony endocarp which is not edible. Pericarp formed 10-15%, mesocarp 65-70% and endocarp 15-25% of the total fruit wt.

The histology of raw mango fruit is given in (plate 8i). Pericarp is made up of an outer layer of epidermis with thick walled cells varying from rectangular to oblong in shape. It formed a uneven and oftern a discontinuous layer with arch-like outline (plate 8d). Epidermis was covered by a thin, non-cellular, hyaline, homogeneous matrix, the cuticle. In a cross section, cuticle

showed many dents and depressions. The epidermis was followed by 5-6 layers of compactly arranged thick walled collencymatous cells comprising the hypodermis. Starch grains were sparse in the hypodermis (plate 8a). The cuticle, epidermis and hypodermis constitute the peel or skin of the mango fruit. The hypodermis was followed by a large cortex which occupied major portion of the fruit. The cells of the outer cortex were small, parenchymatous,spherical or ovoid in shape and compact with less intercellular spaces. The outer cortex was interspersed with lysigenous canals of oil glands (plate 8b). Oil canals were well differentiated and lined with a compact layer of rectangular cells surrounded by think walled cells. Small to large vascular bundles occured in the outer cortex may or may not be associated with oil glands (plate 8 a&b). The cortical cells increased in diameeter towards the endocarp with large intercellular spaces. The cortical cells were polygonal in shape and were elongated along the horizontal axis towards the inner cortex. The cells were thin walled and parenchymatous. Starch granules were small and sparse in outer cortical cells whereas, they are large, conspicuous and abundant in innercortical cells. The starch granules were spherical in shape (plate 8c).

Ripening Changes : At the raw green stage of fruits (mature, unripe and freshly harvested) the starch granules were abundant in the cells. Upto 7 days storage there was no marked change in their anatomy except for slight reduction in abundance of starch granules. At edible ripe stage (17th day), the starch granules degraded leaving meshy remnants inside the cells. In inner cortex, starch degradation was complete (plate 8ii). Under higher magnification, intact cells were seen separating from each other at several places. The cells retained their integrity in epi- & hypodermal and in outermost cortical cell layers. Under higher magnification, increased intercellular spaces could be seen in the hypodermal region but the cells maintained their integrity (plate 8ii,d).

Anatomy of acetylene treated fruits on 4th day resembled that of 17th day control fruits (plate 9i). The cells contained only the remnants of starch granules. The thin walled parenchymatous cells of cortex showed disorganisation at some places (plate 9ic). On 7th day, the intensity of cell disorganisation was severe and could be seen in both peel & pulp portions (plate 9ii). Epidermal and few hypodermal cells below them, were intact but their cell lumen was reduced. The lower hypodermal cells also showed disorganisation

of cell walls (plate 9ii b). Cell lysis was severe in the mesocarp region. There was complete loss of cellular organisation of the parenchymatous cells and only cell wall remnants were visible along with cell debris (plate 9ii c). There were no traces of starch granules.

SEM Studies : To study the detailed microstructure of the cell wall break down and starch granules during ripening and to confirm the results obtained by LM, SEM was undertaken. Under SEM mango fruit tissue at raw green stage (plate 10i) appeared as follows: The cuticle appeared as dark, thick, homogenous layer which was not clearly visible due to its waxy nature. The epidermal layer had thick walled cells. The starch granules were absent in epidermal and outer 2 or 3 layers of hypodermal cells and were sparse in inner hypodermal cells. They increased in abundance from outer to inner cortex (plate 10 ic & d). The periphery of cell lumen of the cortical cells were studded with numerous spherical starch granules (plate 10id).

Ripening Changes (plate 10ii) : The striking changes observed during ripening of mangoes were those of cell wall and starch granules. Three degrees of starch hydrolysis could be noticed during ripening viz.,

Slight hydrolysis	-	was deserved as slight distortion in the shape of starch granules (plate 12b).
Moderate hydrolysis	-	by disappearence of individual granules but starch had clumped reticulate appearence, some granules remained intact (plate 12c).
Severe hydrolysis	-	was characterised by the complete absence of starch granules & complete hydrolysis (plate 12d).

Normal tissues were characterised by individual, undistorted starch granules (plate 12a). With advancement in ripening the cells lost their structural integrity. The changes started from the endocarp and proceed towards exocarp. The starch granules gradually hydrolysed during ripening. In the hypodermal cells, the starch granules decreased by 7th day while on 14th day they visibly disappeared. In the mesocarp region they showed moderate hydrolysis by 14th day and severe hydrolysis by 17th day (plate 10iid).

The cell walls which are quite well defined and intact in raw fruits (plate 13a) exhibited degeneration during ripening. In control fruits on 14th and 17th days the cell walls appeared broader with a central groove, like middle lamellar region (plate 13b & c). The middle lamella appears disintegra-

ting along the cell wall axis at several places leading to separation of cells and textural breakdown. At ripe stage, the cell separation was distinct in the 'mesocarp' region (plate 13c).

Effect of Treatment (plate 11) : Changes observed on 14-17th days in case of control were visible on 4-7th days in acetylene treated fruits. The starch grains showed severe hydrolysis on 4th day itself (plate 11d). The starch grains showed complete absence on 7th day (11 iid). However, some granular depositions were seen all over the tissues. The cell wall breakdown was not regular as in control fruits. There was irregular disintegration of cell walls resulting in complete loss of cell integrity giving a meshy appearence (plate 11 iid and 13 e).

Capsicum

Surface Topography : The epicuticular wax was smooth and free from any outgrowths. The surface exhibits regular, polygonal reticulations, the margins of reticulations apeared like grooves (plate 14g iv). In cross section, it could be seen that the margins of reticulations did not correspond to individual epidermal cell margins. They represented the cuticular or epicuticular pattern. The cross section also confirmed the absence of any projections in the cuticle. The cuticle at the junction of epidermal cells was found to extend inwards to a considerable depth or to the length of anticlinal walls. They may represent the transcuticular canals (plate 15).

Viewed under SEM, the surface showed a magnified view of light microscopy. The pattern consisted of repeated polygons or hexagons with slightly depressed margins (plate 14 ia). Such a relatively flat surface under SEM typically makes the surface of the object appear glossy and lustrous. Even after removing the epicuticular waxes with chloroform the reticulations were visible suggesting that the reticulations represented the cuticular pattern (plate 14 v).

It was found that when viewed with SEM, the angle of observation can affect the interpretation of the image. A 180° rotation of the photograph can result in 'optical illusion' resulting from shadow effect. The reticulations may appear depressed with elevated margins. This phenomenon can be observed by inverting and comparing the plates (14 a & b).

Capsicums stored under MA for extension of shelf life and air stored fruits were studied. Capscium is a berry with an axial placentation. The placenta is attached to the flesh of the fruit only at the stalk end. There occurs a cavity between the placenta and fruit wall.

The epidermis was continuous, unilayered with rectangular thick walled cells arranged uniformly. Epidermis was covered by a thin layer of cuticle. Which appeared uniform in cross section (plate 15a). In the cuticle, perpendicular to the surface light coloured regions which may represent transcuticular canals were visible. Epidermis was followed by 4-5 layers of rectangular, compactly arranged hypodermal cells, without intercellular spaces. Hypodermis was followed by cortex. Towards the endocarp the cells incresed in size. These cells were irregular in shape and size. They were thin walled with few intercellular spaces. The cortex was innervated with vascular bundles [plate15 (iii) b]. The innermost layer of cortex had very large aerenchyma cells which were bounded by layers of very small cells (Plate 15b).

Changes During Storage *: Control fruits during 8 days storage in air lose considerable amount of moisture reducing the turgidity of tissues. The fruit wall thickness which measure 2.5mm at harvest decreased to 1.5mm on 8th day of storage. This resulted in shrinkage of the tissues. No other changes were visible (plate 15i, iv).*

Under MA storage, the moisture loss was significantly reduced and fruits maintained their turgidity and crisp texture. The fruit wall measured 2.5mm in thickness both during storage and post storage period in T_1 *&* T_2 *groups. Other changes were not clearly evident.*

Even under SEM, no major anatomical changes were seen in MA stored fruits (15 v & vi), indicating that MA had no adverse effect on capsicum fruit anatomy.

Plate 7. Surface view of mango fruits.

a - under light microscope
b - de- under SEM

Control fruits	***Acetylene treated fruits***
b - at raw stage	*d - after treatment (4th day)*
c - at ripe stage (17th day)	*e - at ripe stage (7th day)*

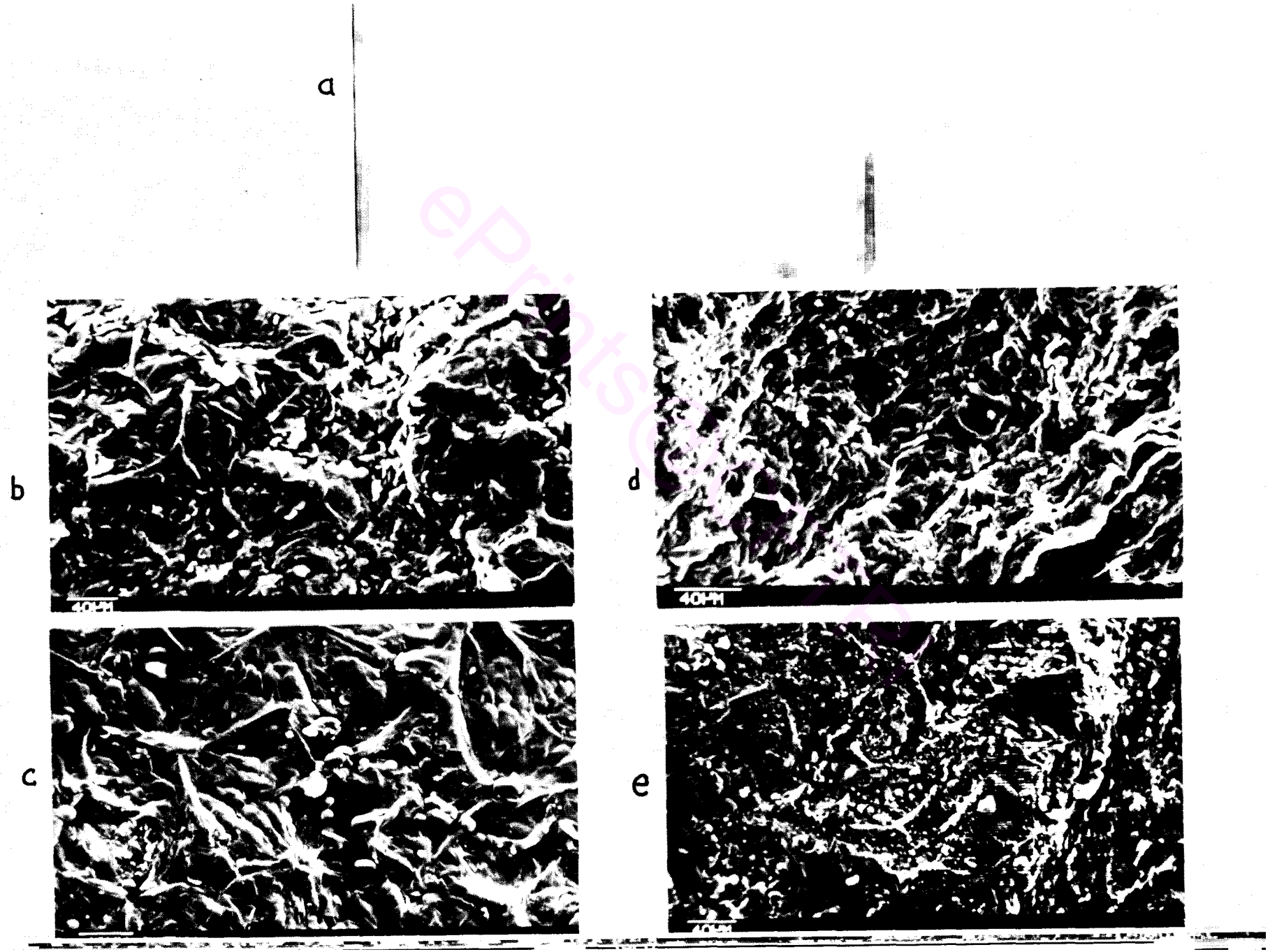

a
b
40μm
d
40μm
c
e

Plate 8. Anatomy of control mangoes under light microscope

i) at raw stage *ii) at ripe stage (17th day)*

a *- Epidermis, hypodermis with outer cortex*

b *- Outer cortex*

c *- Inner cortex*

d *- Enlarged portion of epi-and hypodermis*

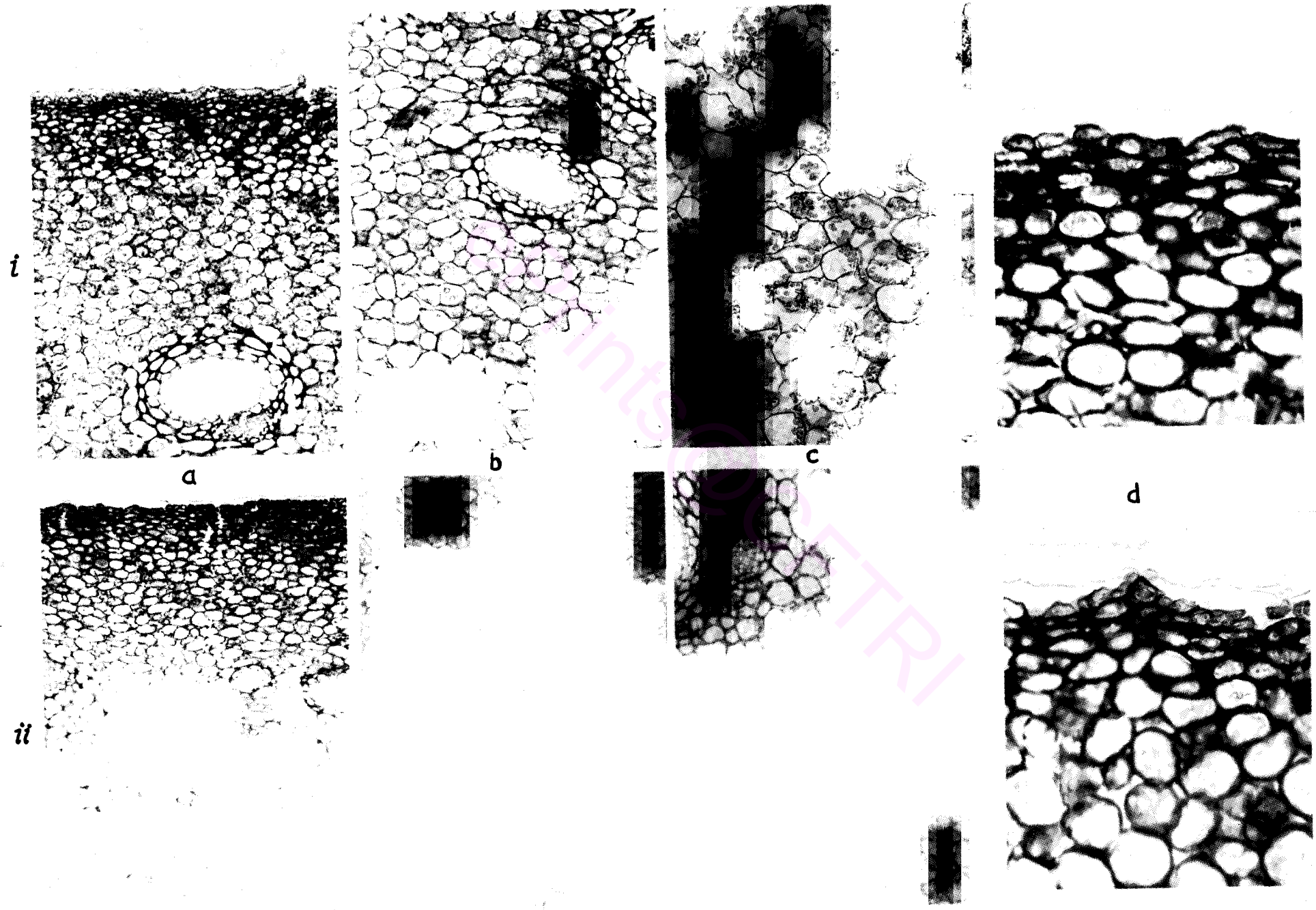

i
a
b
c
d
ii

Plate 9. Anatomy of acetylene treated mangoes under light microscope

i) after treatment (4th day) ***ii) at ripe stage (7th day)***

a - *Epidermis, hypodermis with outer cortex*

b - *Outer cortex*

c - *Inner cortex*

d - *Enlarged portion of epi-and hypodermis.*

Plate 10. Ripening changes in control mangoes under SEM

i) at raw stage ***ii) at ripe stage (17th day)***

a - *Epidermis with hypodermis*

b - *Hypodermis*

c - *Outer cortex*

d - *Inner cortex*

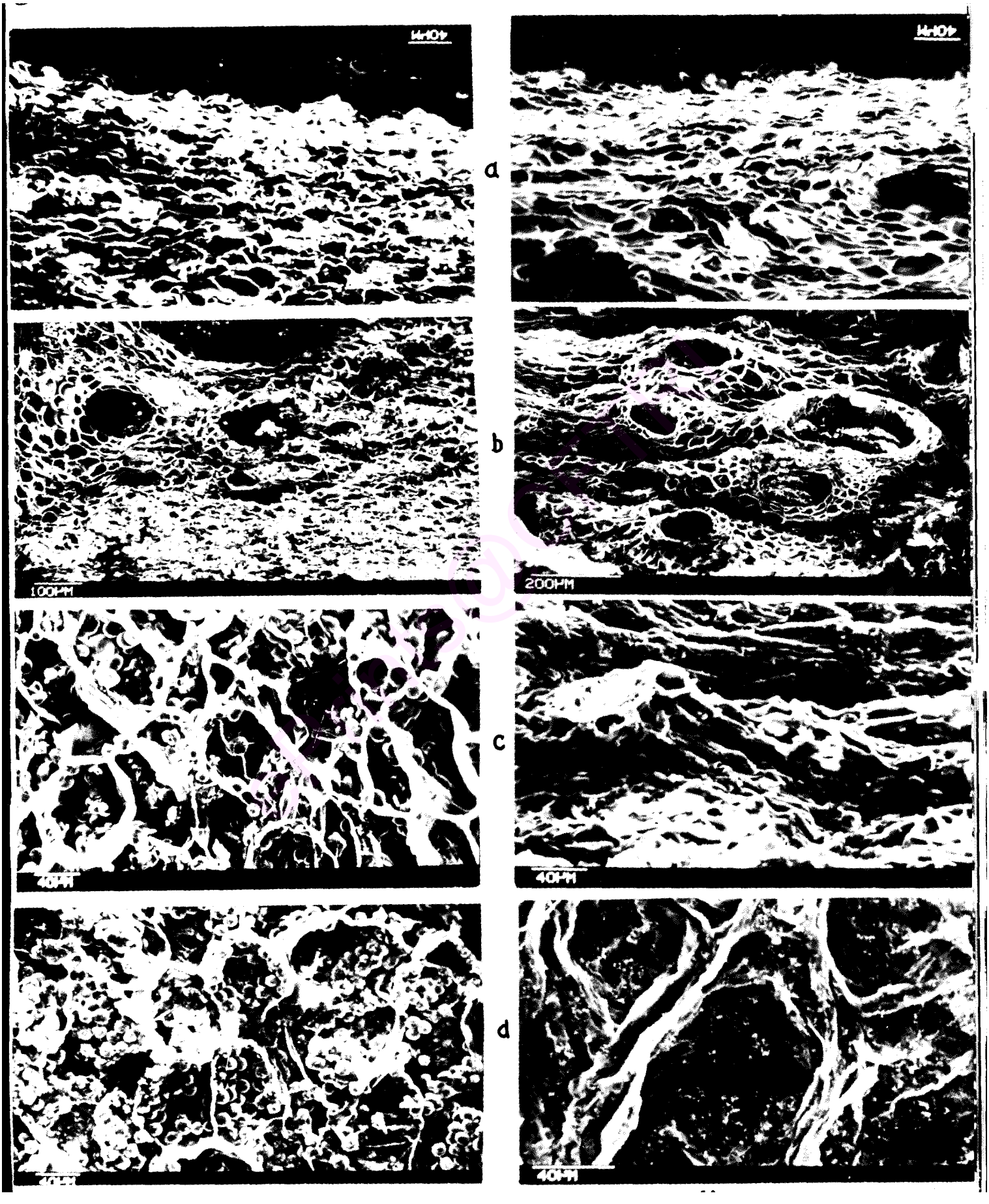

a
b
c
d
100μm
200μm
40μm
40μm
40μm

Plate 11. Ripening changes in acetylene treated mangoes under SEM

i) after treatment (4th day) *ii) at ripe stage (7th day)*

a *- Epidermis with hypodermis*

b *- Hypodermis*

c *- Outer cortex*

d *- Inner cortex*

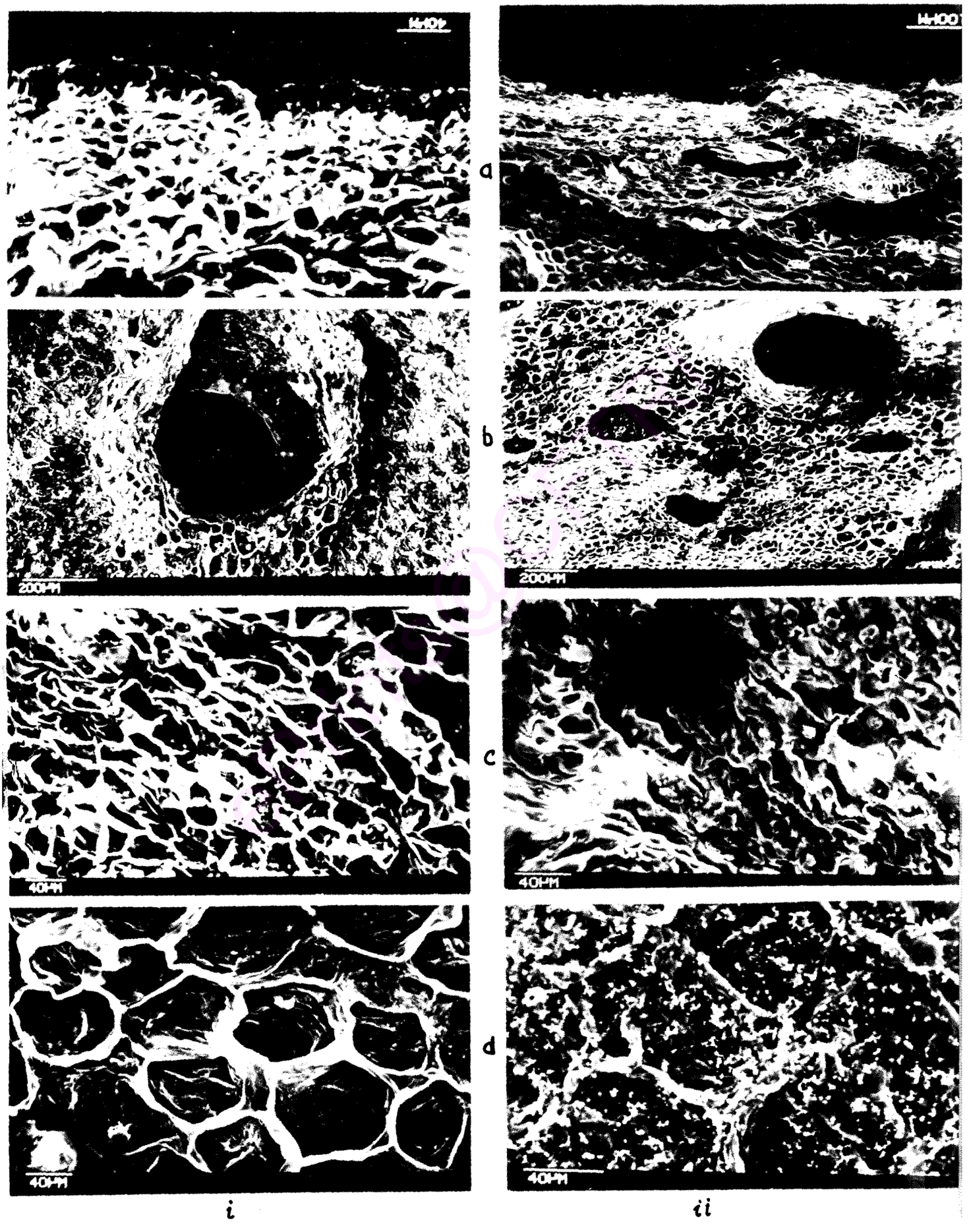

a
b
c
d
200μm
40μm
i
ii

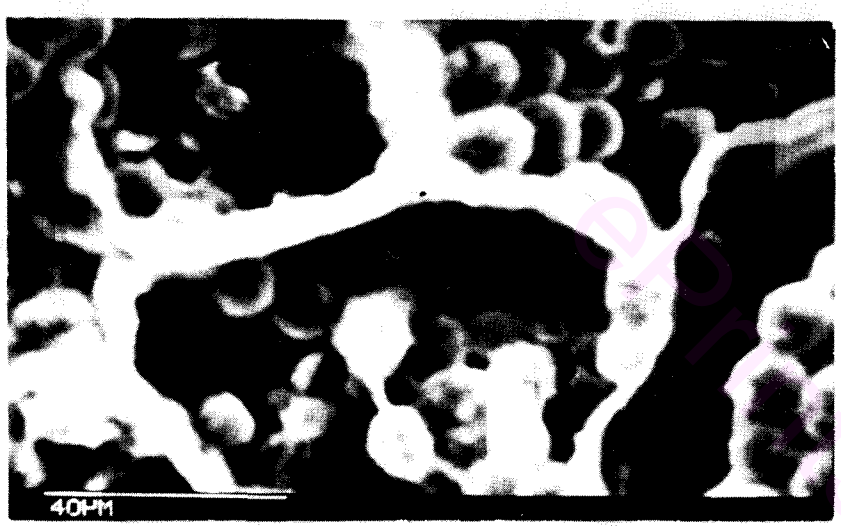
Plate 12. Hydrolysis of starch during mango ripening under SEM

a *- Unhydrolysed*

b *- Slight hydrolysis*

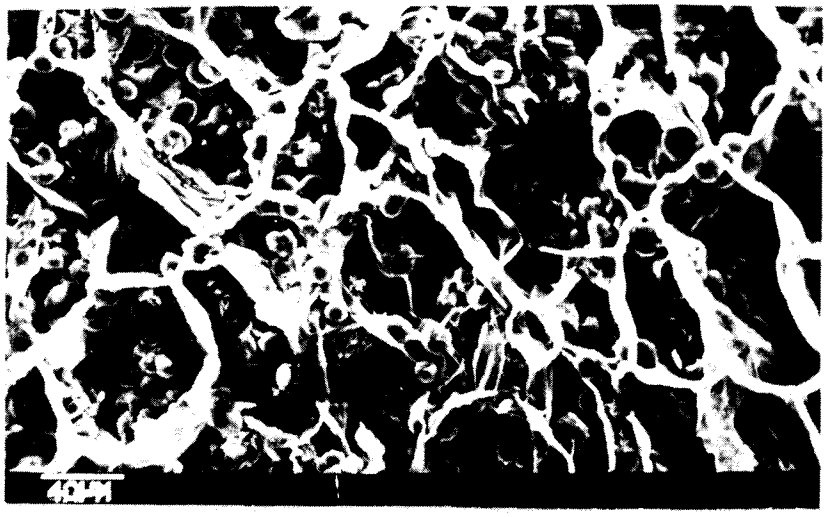
c *- Moderate hydrolysis*

d *- Severe hydrolysis*

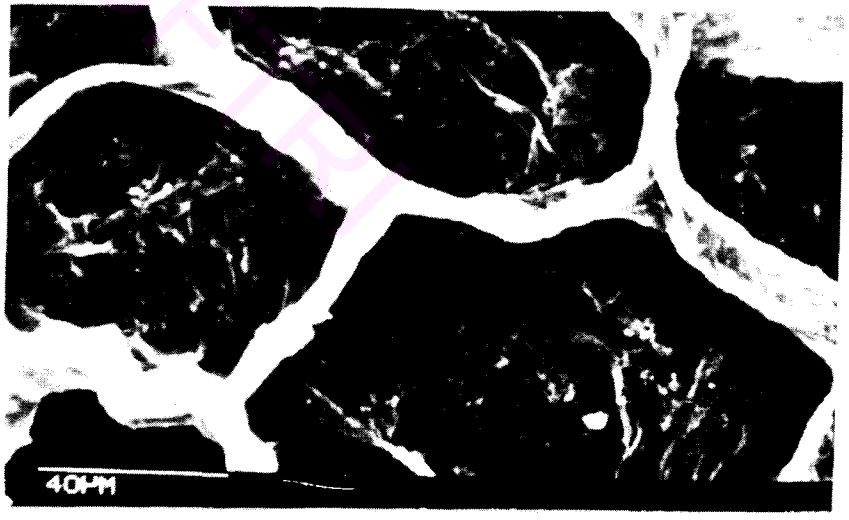


a

b



c



d

Plate 13. Cell wall changes during mango ripening

Control fruits

a *- at raw stage*

b *- on 14th day*

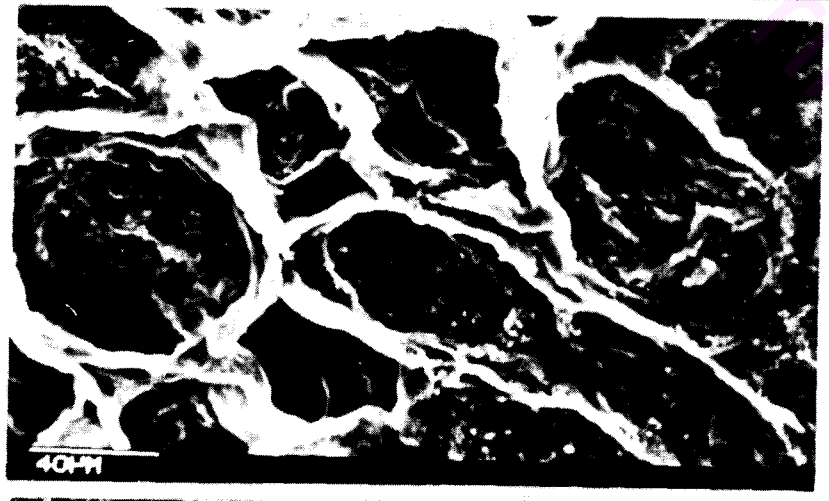
c *- on 17th day (ripe stage)*

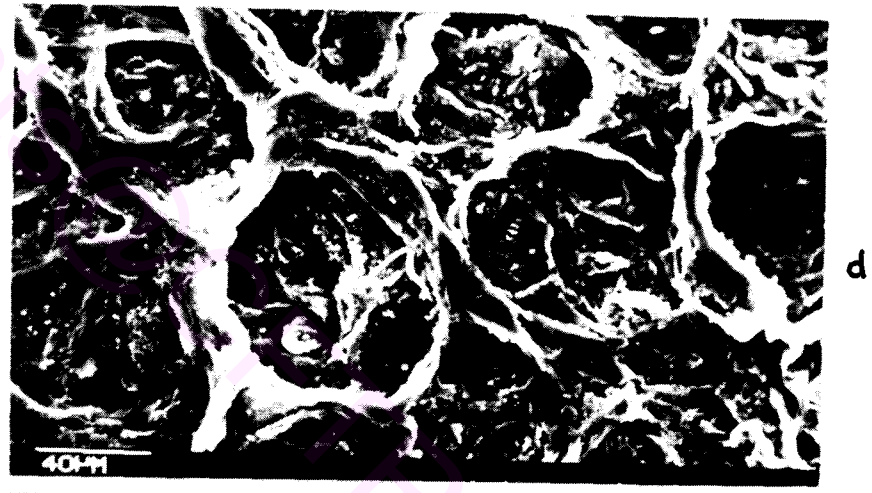
Acetylene treated fruits

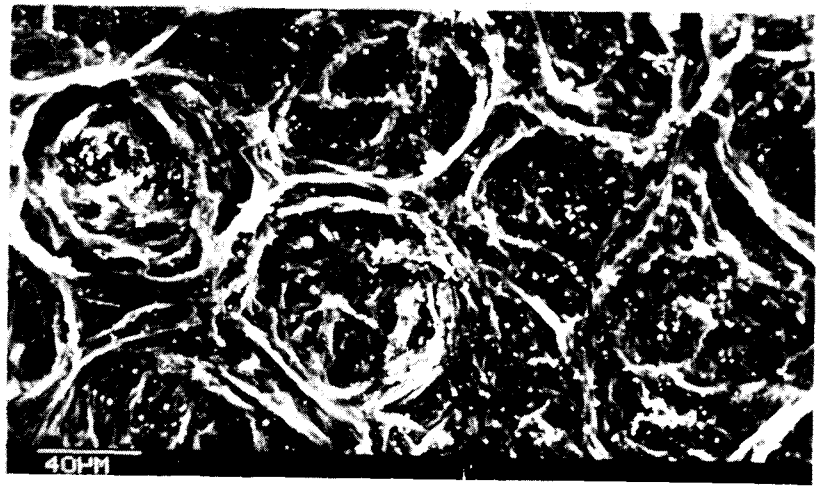
d *- on 4th day (after treatment)*

e *- on 7th day (at ripe stage)*


a
40μM


b
40μM


d
40μM


c
40μM

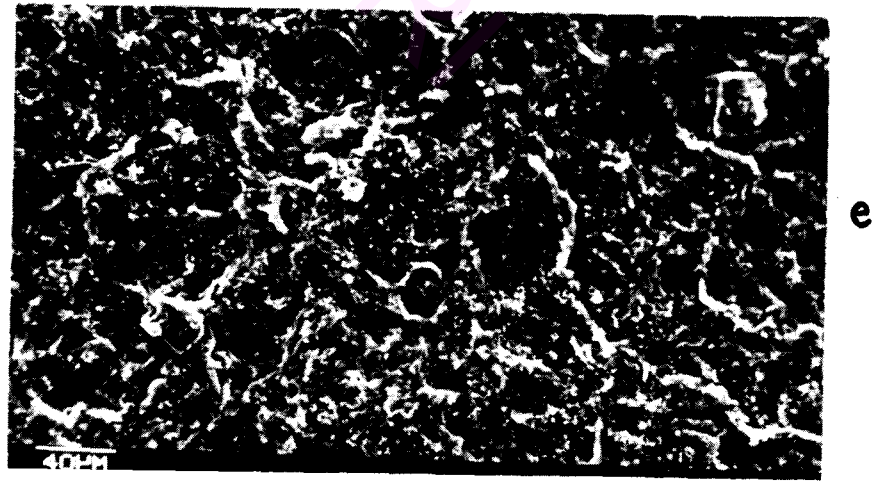

e
40μM

Plate 14. Surface view of capsicums under SEM

i) Air stored fruits :

a - at harvest b - on 8th day

ii) MA stored fruits (T_1) :

a - on 10th day b - on 13th day

iii) MA stored fruits (T_2) :

a - on 13th day b - on 16th day

iv) *Capsicum surface under light microscope*

v) *Capsicum surface after chloroform wash*

vi) *Capsicum surface after extended storage in MA.*

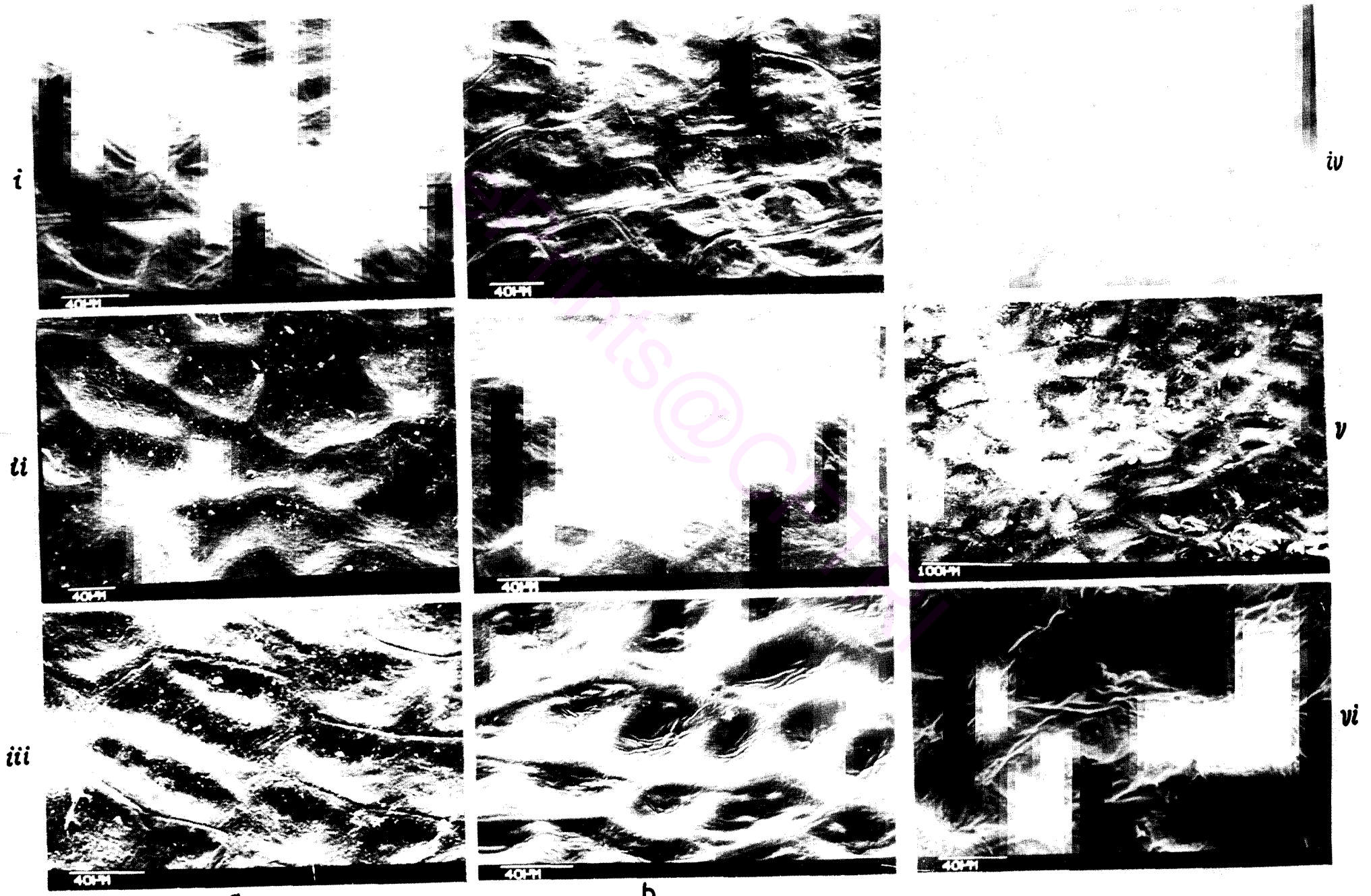

i
ii
iii
iv
v
vi
40μm
40μm
40μm
40μm
100μm
40μm
a
b

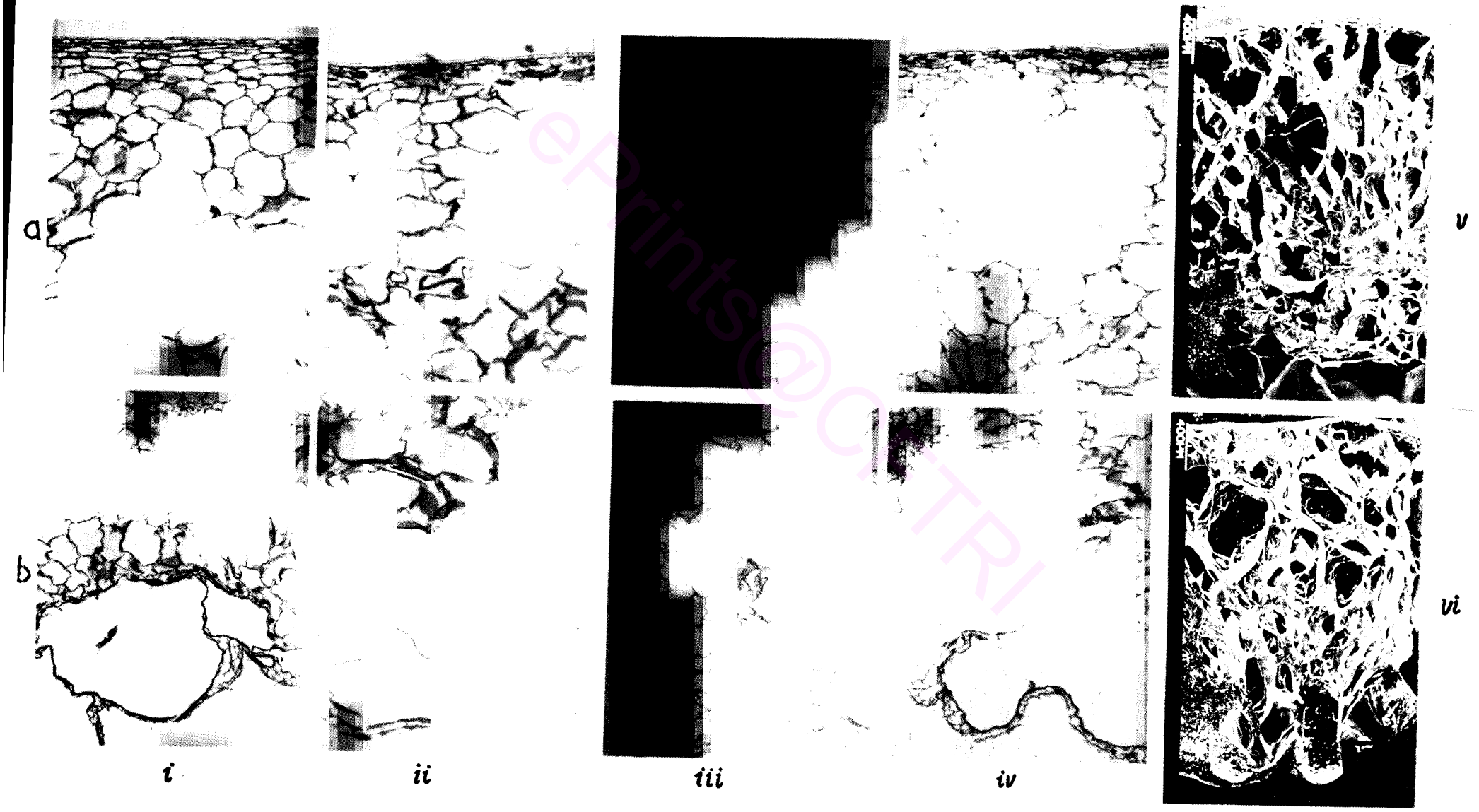
Plate 15. Anatomy of capsicums during storage in MA under light microscope

Air stored — ***MA stored (T_2)*** — **Air stored** *(under SEM)*

i) *at harvest* ***ii)*** *after 8 days* ***iii)*** *on 13th day* ***iv)*** *on 16th day* ***v)*** *at harvest* ***vi)*** *after 8 days*

a - *Epicarp with a portion of mesocarp*

b - *Mesocarp with endocarp*



DISCUSSION

MANGO

The surface and cross sections of mango fruits exposed to acetylene were observed under light and scanning electron microscopes at different ripening stages.

Surface topography. *The surface of mango fruit is not lustrous as in capsicum which is due to its cuticular microstructure. The considerably thick cuticular layer of mangoes is sculptured into crystalline and knob like outgrowths interspersed with lenticels. The uneven surface of these projections produce diffused reflection of light which gives the fruit a dull appearance. This is a common feature in several fruit and leaf cuticles (12,15).*

During ripening there were no distinct changes in the structure and configuration of cuticle except for clear expression of the crystalline plate like structures. This may be possibly due to the shrivelling of the fruits by PLW of 12-13% over the ripening period of 17 days.

The comparatively smoother surface of acetylene treated mangoes at ripe stages may be correlated to slight increase in temperature in the storage chamber (during CaC_2 treatment). Leece (15) observed cuticular ridges to be more widely placed reducing the surface roughness

at higher temperatures. This reduction in surface roughness along with reduced PLW (8-10%) due to reduction in ripening period might be the reasons for better external appearance of acetylene treated fruits. In addition, increased carotene syntheses that imparts uniform colourtion also enhances fruit appearance. This smoothening of cuticular surface might also be due to the fruit senescence resulting from increased metabolism (like respiration and other ripening changes) in acetylene treated fruits. A reduction in epicuticular waxes and erosion of fine structure was reported in leaves due to withering process or senescence (6).

Anatomical changes. *Light microscopic studies revealed only apparent structural changes predominantly in the fleshy mesocarp but not in the peel which sustains more resistance. SEM revealed these changes more clearly.*

During ripening there was slight collapsing of the peel tissue but with intact cell boundaries. In the treated fruits at ripe stage (7th day), some of the hypodermal cells also exhibit cell wall disintegration. However, not much noticeable change occurred in the peel portion. The thick cell wall matrix interspersed with more cellulose and lignin in the epidermal and hypodermal cells might have resisted cell separation. However, the thin walled parenchymatous cells of the cortex readily lose intercellular adhesion leading to cell separation. The microstructure of intercellular cement of fruit tissues is known to be rich in pectic substances (1). Thus loss of cell adhesion is a consequence of dissolution of pectic substances in the middle lamella. The cell separation can be correlated with decrease in pectic substances in our studies. The solubilisation of middle lamella during ripening (20) and its correlation with fruit softening has been well documented in many fruits and vegetables (7,21). The role of cell wall degrading enzymes such as pectinases, cellulase, etc. in degrading the cell walls have been correlated with fruit softening (3,13).

In control fruits the cell separation in the mesocarp region appears to follow the normal pattern i.e. along the middle lamella, similar to

that described during ripening of apples, pears and tomatoes (4,9). Similar pattern of wall disorganisation was also exhibited by tomato cell wall material treated in vitro with purified PG enzyme (9). However, treatment with acetylene favoured the disruption of cell walls along many axes, highly reducing the cell integrity. This may be correlated to increased activity of cell wall degrading enzymes at the ripe stage of most fruits resulting in over softness. The loss of cell integrity may partly explain increased softness recorded by instrumental textural studies. However, this decreased firmness favoured the sensory textural quality of the treated fruits recording higher scores for texture attributes. This softening is also preferred in marketing and processing of mangoes.

High potassium leakage in treated fruit slices reflects the loss of membrane integrity which is obvious with disruption of cell walls. This is also responsible for loss of fruit firmness as observed in many fruits and vegetables (5). It has also been reported that changes in permeability and cellular organisation may be due to osmotic damage to the delicate cells of ripe fruits .

The most striking histological change recorded during ripening of mangoes was breakdown (hydrolysis) of starch granules which occur abundantly in the mesocarp cells at raw stages. Starch hydrolysis progressed rapidly with ripening manifesting the change from hard, crisp, raw and sour pulp into soft, sweet and juicy at ripe condition. The decrease in starch granules was associated with increase in free sugar content of fruits. The raw fruits wherein, the mesophyll cells were compact and studded with starch granules, were significantly firm than ripe fruits in which starch granules were completely absent. This hydrolysis of starch might cause a slight distention of the cells which may 'round off' leading to cell separation and increase of intercellular spaces. No such correlations were reported in mango but a detailed investigation in this regard is reported in potatoes (22).

CAPSICUM

The surface and sections of air stored & MA stored capsicums were studied by light and scanning electron microscopy at various periods of storage.

Surface topography. *In capsicums the surface structure seems to be unaffected by MA storage (at the concentration of CO_2 and O_2 and duration of exposure employed). Minor changes were however expressed after their removal from MA. The smooth, cuticular surface and absence of lenticels make the surface of capsicum appear lustrous or shiny, which bears resemblence to tomato fruit surface morphology (6). During storge the reticulations became more apparent which may be correlated to PLW and the resulting collapse of underlying epidermal and other tissues. Grout and Asten (11) correlated excessive water loss to reduced quantities of epicuticular wax in Brassica. However, these changes could not be differentiated with the help of light microscope and PLW below12% is not detectable visually . The pronounced manifestation of reticulations during storage could also be correlated to the thinning of epicuticular waxes, exposing the underlying cuticular pattern of reticulations. This was also evident by the decrease in the quantity of epicuticular waxes both in air and MA stored fruits during prolonged storage. Thinning of epicuticular waxes may be attributed to senescence as reported in leaves (12,15). Miller (17) observed in apples that deposition of epicuticular wax continues during leaf expansion or fruit maturation at a rate that accomodates increase in area. At full maturity the deposition rate decreased or new wax deposition ceased. But growth seems to continue even after harvest in many vegetables (2) . This could as well be the case in capsicums resulting in thinning of epicuticular waxes. In air-stored control fruits however, epicuticular waxes increased in content till the end of storage period. Accumulation of waxes throughout the life of the fruits has been observed (10,16). Further, in both air-stored and MA stored capsicums, the proportion of soft waxes decreased with concomitant increase in hard waxes during storage. The increased synthesis of hard waxes observed by Morozova (19) during in storage of apples has been hypothesised to be due to the biosynthesis of fatty acids of soft waxes prior to*

those of hard waxes (16). Relatively higher proportion of soft waxes in MA stored capsicums may signify that their cuticular membrane is less permeable to gases, especially O_2. This low permeability could be related to fall in respiration in MA stored capsicums as was observed in apples stored in CA (16).

Under prolonged storage of capsicums in high CO_2 and low O_2 (7% CO_2 + 3% O_2) however, the epicuticular wax appears to be highly disturbed at several places (Plate 14,vi). It formed intricate eruptions with ridges. This may be due to increased CO_2 and/or lowered O_2 concentration. Changes in surface cuticular composition have been reported during CA storage of apples (16). Cassagnne and Lessire (8) established a dynamic relation between the cuticle and epidermal cells of Allium porum leaves. They believed that constitution of cuticle and internal lipids of epidermal cells attain a new steady state with changes in environment, altering the composition of the wax in cuticle. Such changes in capsicums are yet to be elucidated. This uneven distribution of surface waxes under extended MA storage may be responsible for increased invasion by pathogenic organisms there by increasing the spoilage and limiting further extension in storage life under MA. It is very well known that cuticle along with waxes play an important role in defence mechanism in plants (14).

The cuticles of capsicums under light microscope revealed the presence of transcuticular canals perpendicular to the surface (Plate 15). This has been observed in many fruits by Miller (18) . Such canals however could not be observed in mangoes although reported earlier by the same author (17). The possibility of their absence due to varietal or environmental differences cannot be ruled out.

No critical data are available on the relative amounts of epicuticular and cuticular waxes in mangoes and capsicums and their behaviour under altered storage conditions to draw meaningful conclusions with the results obtained. Vast scope for further research seems probable in this direction.

Anatomical changes. *Histological studies of capsicum under LM did not show changes in cellular organisation except for significant reduction in thickness of tissue during air storage. The reduction in thickness is mainly attributed to increased water loss due to transpiration.*

Viewed under SEM no marked changes were seen except for decrease in cell size in parenchyma and arenchyma tissues (Plate 15). This further confirms that the MA storage of capsicums as used in the present study had no deleterious effects on structural integrity of capsicums resulting in retention of freshness comparable to freshly harvested capsicums.

The reticulations observed on the surface of capsicums did not correspond with epidermal cell margins indicating that they were cuticular patterns and not just epidermal cell impressions. They were visible even after removing the epicuticular waxes.

There was no visible cell wall breakage and/or cell separation as in a mangoes however, capsicums were not studied at red ripe stage.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

This chapter gives concise resume of the research findings on the accelerated ripening of mangoes by acetylene and extension of shelf life of capsicums at ambient temperature. Based on the results obtained, the possible future lines of research work have been posed. The chapter is followed by a list of literature cited in the present dissertation.

SUMMARY & CONCLUSIONS

Mangoes :

In an attempt to provide a better understanding of the performance of acetylene in accelerating ripening and to resolve the confusion regarding the quality of treated Alphonso mangoes, the research undertaken, revealed that acetylene induced :

- *early ripening*
- *uniform and attractive peel colour development*
- *optimum softening*
- *rapid breakdown of starch and cell wall*
- *normal chemical changes*
- *less synthesis of aroma & pulp carotenoids*
- *acceptable overall organoleptic qualities*

The histological studies regarding the textural quality during ripening in mangoes is first of its kind. Few reports of this kind are available in other fruits. The study provided a substantial visual evidence to conclude that C_2H_2 has profound influence in rapid breakdown of cell wall along with severe hydrolysis of starch. The severe breakdown of cell walls was not a common phenomenon during normal ripening of mangoes. This may be an advantage to processing purposes for quick and high yield of juice.

The less synthesis of aroma of treated mangoes is attributed to the less synthesis of lipids, less conversion of unsaturated fatty acids (linoleic acid) or less synthesis of carotenoids in the pulp. Other sources of aroma development and factors being affecting are worth investigating.

The preponement of respiratory climacteric by acetylene treatment was accompanied by other physical, physiological, biochemical & structural changes. Thus acetylene may mediate an interaction of various ripening changes. It may act directly in initiating or catalysing the endogenous production of ethylene. It may act simultaneously or sequentially with the internal ethylene of the fruits. The above study revealed that acetylene has differential effects on various quality attributes of mangoes. Some of them are desirable while others are not. The mechanism by which it triggers or regulates these quality attributes is not very clear. However, the properties of acetylene to favour marketable quality of 'Alphonso' mangoes in a short time along with its simplicity and low cost of the treatment have favoured commercial usage and wide application.

Capsicum :

The present investigation undertaken to evaluate the potential application of commodity - MA to extend the shelf life of capsicums revealed that the technique was sufficiently simple and effective in doubling storage life of capsicums at ambient temperature (27±4°C). The quality of capsicums under MA storage exhibited :

- *maintenance of freshness and firmness*
- *overcoming objectionable shrivelling*
- *low spoilage rate*
- *no adverse effect on:*

  Physical, chemical , histological, sensory and market qualities.

The mode of action of the modified storage environment may be either by the effect of elevated CO_2 and depleted O_2 concentration in reducing spoilage and postponement of ripening and senescence. The increased relative humidity inside the chamber leads to reduction in PLW and hence maintenance of freshness and turgidity of capsicums. As it appears, altered storage environment and relative humidity could have synergistic effects in extending the shelf life of capsicums.

The reduced rate of spoilage of capsicums may be attributed to the direct inhibitory effect of MA on mycelial growth and restricting the colony development after spore germination. The latter restricts the spread of pathogens to neighbouring fruits. The indirect effect of MA is

by maintenance of fresh conditions which offers sufficient resistance to invasion by potential pathogens. Despite this, spoilage by fungi and bacteria formed a limiting factor for further storage of capsicums beyond 13 days in MA. The dynamic relationship of the fungi with its storage environment appears to be much more complicated and it is worthwhile to unravel their mechanism of infection which may help in preventing infection and to further extend the storage life of the commodity.

The present study fulfills the consumer's requirements i.e., maintenance of quality without hike in cost of capsicums. The additional cost of MA storage can be compensated by low rate of spoilage and extended marketable period.

This type of MA storage may be of particular benefit to the third world countries with storage and transportation problems. Since the technique employed is sufficiently simple and effective in extending storage life of capsicums at ambient temperature, this can be readily adopted for short term storage and transport. This can be used even at a farmer level. Validity of this storage treatment under different atmospheric conditions and optimisation of MA for different cultivars of capsicums need to be established. The possibilities of adopting this method for other perishables may fulfill the need to extend for their short term storage at ambient temperature.

To conclude, the foregoing discussion to some extent answers the action of acetylene in inducing early ripening in mangoes. It also accomplishes the successful extension of shelf life of capsicums under ambient temperature. In addition it raises several questions. Advances in this direction would make possible rationalization of these treatments either by supplemental or complimental treatments or to potentiate the treatment to regulate ripening or senescence in mango and capsicums.

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ABBREVIATIONS USED

CA	-	*Controlled atmosphere*
°C	-	*Degree celsius*
CO_2	-	*Carbon - di-oxide*
CaC_2	-	*Calcium carbide*
C_2H_2	-	*Acetylene*
C_2H_4	-	*Ethylene*
DF	-	*Degrees of freedom*
Dia	-	*Diameter*
D.Wt	-	*Dry weight*
F.Wt	-	*Fresh weight*
G	-	*Grams*
Hr	-	*Hour*
Kg	-	*Kilograms*
M	-	*Molar*
µ	-	*Microns*
MA	-	*Modified Atmosphere*
Mg	-	*Milligrams*
µg	-	*Micrograms*
Min	-	*Minute*
mM	-	*Milli Mole*
N	-	*Normal (Normality)*
NL	-	*Neutral Lipids*
O_2	-	*Oxygen*
O.D.	-	*Opitical Density*
%	-	*percent*
PH	-	*Post Harvest*
PL	-	*Polar Lipids*
ppm	-	*Parts per million*
RH	-	*Relative Humidity*
SE	-	*Standard Error*
Sec	-	*Seconds*
V	-	*Volt*