

**MANGO (*Mangifera indica* L. cv. Apple) FRUIT  
RESPONSE TO PREHARVEST BAGGING AND  
POSTHARVEST TREATMENT WITH  
1-METHYLCYCLOPROPENE**

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**Mango (*Mangifera Indica* L. cv. Apple) Fruit Response to  
Preharvest Bagging and Postharvest Treatment with  
1-Methylcyclopropene**

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**A Thesis Submitted in Partial Fulfillment for the Degree of  
Master of Science in Food Science and Postharvest Technology  
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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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## **DEDICATION**

To Eric.

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## LIST OF ABBREVIATIONS AND ACRONYMS

<b>AOAC</b>	Analysis of Official Analytical Chemistry
<b>ANOVA</b>	Analysis of variance
<b>DAB</b>	Days after bloom
<b>DAH</b>	Days after harvest
<b>FW</b>	Fresh weight
<b>HPLC</b>	High performance liquid chromatography
<b>nM</b>	Millimolar
<b>RH</b>	Relative humidity
<b>TSS</b>	Total soluble solids
<b>TTA</b>	Total titratable acidity
<b>1-MCP</b>	1- Methylcyclopropene

## ABSTRACT

This study investigated the effectiveness of preharvest fruit bagging as a control of Apple mango 'rust', and its influence on fruit growth physiology and quality at harvest and on ripening. Response of Apple mango fruit to postharvest 1-methylcyclopropene treatment was also investigated. Mango (*Mangifera indica* cv. Apple) fruit trees were randomly selected and tagged for subsequent sampling at a commercial farm in Yatta District, a semi-arid region of Eastern Province, Kenya. Sampling and analyses commenced 14 days after bloom (DAB), and fortnightly thereafter up to harvest time at 168 DAB. On-tree fruit bagging was done at 70 DAB, just before manifestation of Apple mango 'rust'. Bagged and unbagged fruits were subjected to sampling and analyses for physical, physiological and chemical parameters. After harvest, both bagged and unbagged fruits were each divided into two equal sets of fruits, with and without 1-MCP treatment at 20 ppm, and subjected to analysis for changes in physical, physiological and chemical parameters during storage at  $25\pm 1^{\circ}\text{C}$ ,  $60\pm 5\%$  RH. Sensory evaluation for fruit appearance, colour, taste, texture aroma and overall acceptance was done at harvest and on ripening on a 9-point hedonic scale using 15 untrained panelists.

Unbagged fruits had significantly ( $p < 0.05$ ) fructose, glucose and sucrose, anthocyanin,  $\beta$ -carotene and ascorbic acid contents at harvest. Bagged fruits were more green at harvest, with significantly ( $p < 0.05$ ) higher chlorophyll (b and total) contents, peel  $L^*$  value (measure of colour brightness), peel hue angle (measure of colour) and starch content. However, the difference in pulp

L values, total titratable acidity, total soluble solids, respiration rate, firmness, fruit weight, fruit equatorial diameter and minerals (Ca, Mg, K and P) content between bagged and unbagged fruits were not significantly ( $p>0.05$ ) different at harvest. No ethylene was detected up to harvest. Bagged fruits had a significantly higher sensorial score in peel colour and appearance, with no visible blemishes at harvest. The unbagged fruit had visible blackish brown blemishes due to 'rust'.

Bagging and 1-MCP treatments had a slight effect on respiration and ethylene production rates, though control unbagged fruits had higher respiration and ethylene peaks of 409.82 ml CO<sub>2</sub>/Kg/h and 378.62 nl/Kg/h, respectively. Neither bagging nor 1-MCP retarded fruit ripening. Fruits in all the treatments reached full ripeness and eating quality at 7 days after harvest (DAH). Bagged ripe fruits had higher sensorial score on peel colour, appearance and overall acceptance. The differences in taste, flavour and texture between ripe bagged and unbagged fruits were not significant ( $p>0.05$ ). Unbagged fruits had a higher postharvest weight loss, shriveled earlier and consequently, a shorter postharvest life of nine days compared to 15 days for bagged fruits. Loss of total titratable acidity, ascorbic acid and initial firmness was retarded by 1-MCP (20 ppm). Preharvest bagging was effective in controlling Apple mango 'rust'. However, postharvest 1-MCP treatment was not effective in improving the postharvest shelflife of Apple mango harvested 168 DAB.

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 Background information

Mango (*Mangifera indica* L.) fruit has an attractive colour, delicious taste and excellent nutritional properties, making it a world choice fruit (Mitra and Baldwin, 1997). The ripe mango fruit is a good source of vitamins A, C, and folate, antioxidants, calcium, magnesium, phosphorus and potassium (Kalra et al., 1995). It is consumed as a fruit and as a processed product like nectar, squash, pulp, juice, jam, jellies and canned slices (Kalra et al., 1995).

Mango tree belongs to the family *Anacardiaceae* which has 75 genera and 700 species (Lizada, 1993). The genus *Mangifera* includes 25 species (Griesbach, 2003) with edible fruits such as *Mangifera caesia*, *M. foetida*, *M. odorata* and *M. pajang*, although *M. indica*, the mango, is the only species that is grown commercially on a large scale. Mango tree is believed to have originated in the Indo-Burmese region (Lizada, 1993), but can now be found growing in more than 87 other countries throughout the tropical and subtropical world (Kalra et al., 1995). There are more than 1,100 varieties of mangoes grown around the world (Griesbach, 2003). However, they are all grouped into two races; one from India and the other from Southeast Asia (Griesbach, 2003). The Indian race is intolerant to humidity, has flushes of bright red new growth that is subject to powdery mildew and anthracnose and bears fruit with regular shape. The Southeast Asian race is tolerant of excess moisture, has pale green or red

new growth and is resistant to powdery mildew. Its polyembryonic fruit is pale green and of an elongated kidney shape. Kenyan mango varieties, Apple, Boribo and Ngowe are of Indian race (Griesbach, 2003).

## **1.2 Mango fruit industry**

Major mango producing countries in the world in descending order include India, China, Thailand, Mexico, Pakistan, Indonesia Philippines, Brazil, Nigeria, Egypt (FAO, 2003). The world mango fruit market is very competitive, with quality and price being the determinants. The leading world producers have low production costs and better market penetration (FAO, 2003). Large scale production makes these producers to benefit from economies of scale and reach high levels of productivity. Several countries, both large and small mango exporters, are geared towards increasing their exports of mangoes due to increasing world demand (FAO, 2003). Kenyan producers should aim at producing high quality mango fruits and reduce postharvest quality so as to survive the competition.

## **1.3 Preharvest and postharvest mango fruit quality**

Preharvest cultural practices and environmental conditions during fruit development profoundly influence postharvest performance and final quality (Lechaudel and Joas, 2007). Mango fruit skin, flesh and stone have specific compositions that appear to accumulate water and dry matter at different rates, depending on environmental conditions (Lechaudel and Joas, 2007). Accumulation of water results from the balance between incoming fluxes such as phloem and xylem, and outgoing fluxes such as transpiration (Ge'nard et

al., 2007). The amount of carbohydrates supplied to tree fruits depends on the amount produced by leaf photosynthesis, on sink demand and on the availability of the reserve pool (Ho, 1996). It is essential to understand how a preharvest cultural practice influence source-sink relationships involved in fruit growth before its adoption.

Mango fruit is harvested at early or late mature green stage depending of the distance to the market (Lizada, 1993). After harvest, mango fruit ripens by undergoing many physicochemical changes that determine the quality of the fruit purchased by the consumer. Appearance and eating quality are the major sensory attributes that determine consumer acceptance of mango fruit (Mtebe et al., 2006). Fruit appearance is influenced by absence/presence of physical, pathological and physiological disorders. These disorders are also the main culprits in postharvest loss of fruits and vegetables (Chege et al., 1995) due to their detrimental effect on fruit physiological and biochemical changes during postharvest handling. The disorders induce rotting and early senescence leading to poor flavor and aroma (Mtebe et al., 2006).

Eating quality is mostly determined by sugars:acid ratio together with texture and flavour (Mtebe et al., 2006). The sugars and secondary compounds content during fruit development are mainly dependent on the metabolism of unloaded sugars in the phloem and the accumulation of their metabolites. The characteristic flavour of fresh mango fruit is a complex interaction between a large number of volatile and non-volatile components (Lizada, 1993). The

non-volatile compounds, for example, sugars and acids, are responsible for the sweetness and tartness of the fruit; the volatile compounds, for example, esters and aldehydes, are central in producing the distinctive fruity flavour (Shiratake and Martinoia, 2007). Also present in the mango fruit are polyphenols or tannins, which are responsible for astringency (Lizada, 1993)

#### 1.4 Economic value of mango fruit industry in Kenya

Horticultural crops were Kenya's leading foreign exchange earner in the year 2007, raking in KSh. 62.5 billion (<http://www.hcda.co.ke>). Fruits contributed only 2.8% of total export value of horticultural produce from Kenya, whereas cut-flower and vegetables contributed 65% and 32.2% respectively. Mango fruit is in the third position after avocado and passion fruit as the most important export fruit in Kenya.

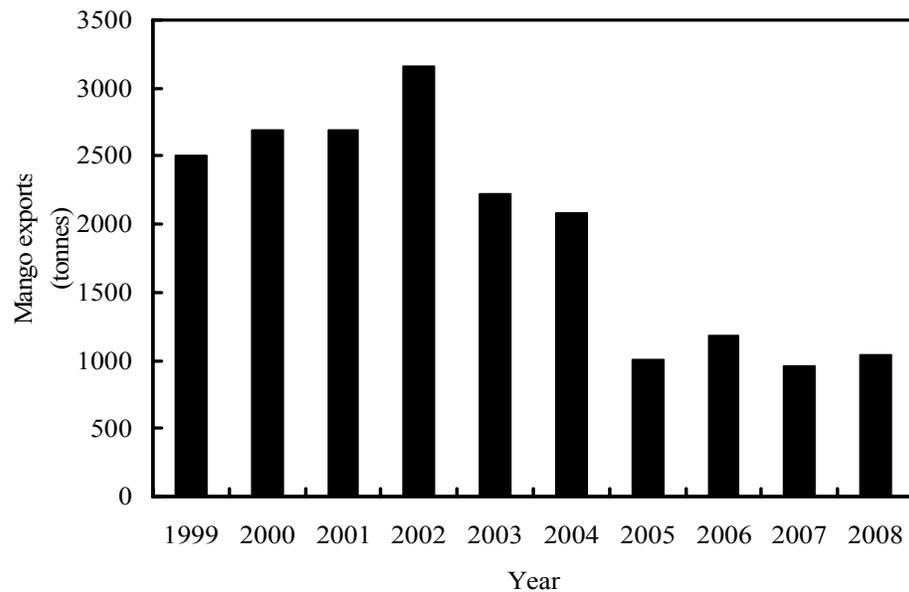


Fig 1. Amount of mango fruit exports (1999-2008). Data source: HCDA export statistics (<http://www.hcda.co.ke>).

However, exports have declined during the last decade (Fig 1) and comprise of a small proportion of national production. This has been attributed to pests, diseases and physiological disorders (Nyambo et al., 2006) and postharvest losses (FAO, 2003). However, demand for fresh mango fruits in Europe and the Middle East has been expanding (FAO, 2003).

Kenyan producers can combine selection of high quality varieties, novel production practices like organic farming, the latest postharvest handling techniques and successful image-building, including setting standards, to conquer a substantial share of profitable high-end market niches. Promoting cultivation and marketing of such high quality varieties such as Apple should be accompanied by more research on their optimum requirements during growth, harvesting and postharvest handling. Environmental concerns and minimum chemical residue requirements by the lead export markets calls for adoption of non-chemical methods, like on-tree fruit bagging to control pathological and physiological disorders in mangoes. It is against this backdrop that the cultivar Apple mango, on-tree fruit bagging and postharvest 1-methylcyclopropene (1-MCP) treatment were selected for this study. However successful adoption of these two technologies will require further research on fruit, cultivar and climate specificity. This study aims at contributing towards successful adoption of these two technologies on Apple mango fruit production in Kenya, a tropical climate.

### **1.5 Preharvest bagging and postharvest 1-MCP treatment of fruits**

Pre-harvest bagging of fruit is used commercially in countries like Japan and Australia in order to optimize fruit quality through reduced physiological and pathological disorders (Joyce et al., 1997) leading to improved appearance (Hofman et al., 1997b). On-tree bagging of individual fruits creates a microclimate within the bag for the fruit. Bagging is reported to be effective in control of insect pests (Kitagawa et al., 1992), postharvest pathogens in lychee fruits (Kooariyakul and Sardud, 1997), anthracnose and stem-end rot in mango fruit (Hofman et al., 1997b), skin blemishes (Kitagawa et al., 1992), bird damage, friction and discolouration in pears (Amarante et al., 2002b). Reduced pathological disorders by bagging in combination with postharvest 1-MCP treatment may have a multiple effect of improving mango fruit shelf life.

1-MCP binds irreversibly to ethylene receptors, delays ripening and improves postharvest quality of climacteric fruits (Sisler and Serek, 1997). Low concentration of 1-MCP slows effect of ethylene in a broad range of fruits, vegetables and floriculture crops due to very low dissociation constants for the 1-MCP-binding site complex (Blankenship and Dole, 2003). 1-MCP delayed ripening onset on fruits such as avocado (Jeong et al., 2003), banana (Pelayo et al., 2003), mango (Jiang and Joyce, 2000), nectarine (Dong et al., 2001), plum (Dong et al., 2002) and sapoddila (Quipping et al., 2006).

## 1.6 Problem statement

Apple mango is highly susceptible to russet-like physiological disorder that affects the fruit during maturation, and is severe in humid weather, rendering the fruit unfit for fresh market due to development of brown blemishes on the fruit peel (Plate 1). The resulting unattractive appearance disqualifies the fruit from the export market due to stringent quality requirements. Producers get low returns from such blemished fruits as the fruits are sold in the local low-end fresh market and to local juice processors. Due to a lack of locally validated information on best control options for Apple mango russet (commonly known as ‘rust’ by Kenyan mango growers), agribusiness oriented mango growers have tried use of broad-spectrum synthetic pesticides for its control, without success. However, preharvest fruit bagging may control ‘rust’.



Plate 1: Mature green Apple mango with ‘rust’.

Mango fruit is also perishable and postharvest losses in Kenya are very high due to inadequate postharvest technologies, but use of postharvest 1-MCP treatment may improve the postharvest shelf life and reduce the loss.

### **1.7 Rationale and justification**

Agriculture is the main occupation for 85% of Kenya's population. Increase in Kenyan population has resulted in less land that is classified as arable. However, there are huge tracts of unutilized land in arid and semi-arid areas of Kenya where commercial production of drought resistant crops like mango crop can be done. It is with this realization that the Government of Kenya created the ministry of Development of Northern Kenya and other Arid Lands, accompanied by huge investments in improving road infrastructure and water for irrigation. Good returns on these investments will be realized by promoting commercial farming of high potential crops that grow well in these areas. Mango fruit, especially Apple mango, is such a high potential crop in terms of high demand in the domestic and export markets (Nyambo et al., 2006).

### **1.8 Objectives**

The main objective of this study was to investigate the influence of pre-harvest bagging on fruit quality at harvest and response to 1-MCP. The specific objectives were to investigate the:

1. Effectiveness of on-tree fruit bagging in control of Apple mango fruit 'rust'.
2. Influence of on-tree bagging on changes in physical, physiological and biochemical parameters and sensory quality during fruit growth and postharvest period.

3. Effect of 1-MCP on postharvest fruit quality.

### **1.9 Hypothesis**

1. On-tree bagging of Apple mango fruit (*Mangifera indica* L.) will control ‘rust’ and result in improved fruit quality at harvest and on ripening without affecting fruit response to postharvest 1-MCP treatment.

2. Postharvest 1-MCP treatment will improve mango fruit postharvest shelflife.

## **CHAPTER TWO**

### **2.0 REVIEW OF LITERATURE**

#### **2.1 Mango fruit production in Kenya**

During the last 20-30 years, commercial mango production in Kenya has been developed based on locally adapted and imported cultivars (Griesbach, 2003). Depending on cultivars and environmental conditions, it takes 90 to 160 days after flowering for Kenyan mango to reach maturity (Griesbach, 2003). Mango production in Kenya is predominantly a smallholder crop in the semi-arid areas, often produced at subsistence level with minimum inputs in terms of crop management (Nyambo et al., 2006). Mango orchards are normally small, not exceeding five hectares of land (Nyambo et al., 2006). The main production areas are Coast, Eastern and Central provinces, though minimal production is also done in Rift Valley and Western Provinces.

Apple mango has emerged as a preferred cultivar in Kenya for both domestic and export market, followed by Ngowe and Tommy Atkins (Nyambo et al., 2006). Apple mango fruits are medium to large in size, nearly round in shape and have a rich yellow/orange colour when ripe. The average longitudinal and equatorial diameters measure 9.7 cm and 11 cm respectively, and the mean weight is 397 gm (Griesbach, 1992). Normally, if not diseased, the skin is smooth and thin, and the juicy yellow flesh is of excellent flavour and melting texture virtually free from fiber. The seed is relatively small. This attribute has made Apple mango the preferred cultivar in Kenya. The trees are

large/vigorous and of pyriform growth habit. However, the cultivar is susceptible to ‘rust’, anthracnose, powdery mildew and alternate bearing (Griesbach, 1992).

## **2.2 Problems in Kenya’s mango fruit production**

### **2.2.1 Poor agronomical practices**

Poor agronomical practices characterize the industry (Nyambo et al., 2006). Mango trees require intensive care during flowering to harvesting, but smallholder mango fruit farmers in Kenya allocate minimum inputs in the crop management (Nyambo et al., 2006), resulting in low quality and quantity. The chemical sprays used in conventional growing are expensive for the smallholder farmers. Organic mango fruit production has not taken root in Kenya, in spite of the better prices and ever increasing demand for organically grown fruits.

### **2.2.2 Pests and diseases**

Pests, diseases and physiological disorders are a big challenge to mango growers. Mango fruit is susceptible to a wide range of preharvest and postharvest pests and diseases. Fruit flies and mango seed weevil, both quarantine pests are the most damaging pests of mangoes in Kenya (Griesbach, 1992). Other insect pests include aphids, scales, mealybugs, coconut bugs, mosquito bugs (*Helopeltis* spp.) and mango gallflies (Griesbach, 1992). Powdery mildew and anthracnose, both fungal diseases are the major mango fruit diseases in Kenya (Griesbach, 1992).

### **2.2.3 Poor postharvest handling**

The smallholder farmers and middlemen have inadequate technological knowledge and facilities for effective postharvest handling of fresh fruits and vegetables. Poor harvesting, sanitation, packing, road infrastructure and lack of cold-chain infrastructure have resulted in poor quality mango fruit due to mechanical, biological and physiological damage. Mango processing in Kenya is not expanding at an appreciable rate, with only a negligible share of total production being processed (FAO, 2003).

### **2.2.4 Poor marketing and stringent quality requirements**

Marketing of mango fruits in Kenya is not well coordinated, like in coffee and tea sectors where marketing societies and cooperatives play a key role in produce marketing. The farmers typically sell to brokers, export agents or local traders. These tend to be unreliable trading partners because they offer low unstable prices and take an unpredictable amount of produce. The requirement for compliance with the GLOBALGAP Control Points and Compliance Criteria for all fruits and vegetables destined for the European market and quarantine restrictions by many other export markets have hindered market penetration by Kenyan exporters. This is complicated by a background of poor agronomical practices in the industry (Nyambo et al., 2006).

### **2.2.5 Physiological disorders of fruits peel**

Apple mango 'rust' is so far the only physiological disorder with economic significance in Kenya mango fruit industry (Nyambo et al., 2006). Fruits peel may develop some physiological disorders during maturation, leading to poor external appearance at harvest. These peel disorders are given different names descriptions depending on fruit type and mode of formation and manifestation; like 'cuticle cracking' in cherry fruit (Knoche and Peschel, 2006), russeting in citrus fruit (Albrigo and Achor, 2002) and lenticel discolouration (Bezuidenhout et al., 2005; Tamjinda et al., 1992) or 'rust' (Nyambo et al., 2006) in mango fruit. Development of these disorders is specific to climate and weather (Albrigo and Achor, 2002), and cultivar (Knoche and Grimm, 2008; Bezuidenhout et al., 2005; Tamjinda et al., 1992). Environmental factors like high relative humidity and wetness (Knoche and Grimm, 2008) and physical (Knoche and Peschel, 2006) and chemical (Bezuidenhout et al., 2005; Tamjinda et al., 1992) properties of the fruit cuticle interact to result in mango fruit peel discolouration.

The formation and development of the physiological peel disorders in different fruits is anatomically different. Russeting in apple fruit is caused by a periderm formed in response to damage of epidermal cells (Faust and Shear, 1972). Some mango fruit cultivars have been reported to develop peel disorders during maturation due to discolouration of lenticels found on the fruit peel (Bezuidenhout et al., 2005; Tamjinda et al., 1992), causing an unattractive dark brown colouration that may be wrongly associated with

pathogenic infections (Bezuidenhout et al., 2005). Apple mango in Kenya is susceptible to russet caused by periderm formation (Nyambo et al., 2006), whereas 'Keitt' and 'Tommy Atkins' in South Africa (Bezuidenhout et al., 2005) and 'Namdokmai' in Thailand are susceptible to discolouration of lenticels.

Anatomical and histochemical investigations have been done on formation, development and manifestation of lenticel discolouration in mango fruit by Bezuidenhout et al. (2005) and Tamjinda et al. (1992). The two have a similar general physiological account, but differ slightly on anatomical details, probably due to cultivar, climate and methodology differences. Mango fruit lenticels develop from ruptured stomata and enlarge as the fruit grows due to stretching of the fruit surface. Lenticels of non susceptible cultivars have been found to be insulated with a thicker phellogen containing cuticle, hence protected from the action of terpenes in fruit peel resin. The terpenes are volatile and are able to move out of the resin ducts via the sublenticellular cells to the outside of the fruit through the lenticels (Bezuidenhout et al., 2005). Action of the terpenes on the tonoplasts of the sublenticellular cells cause vacuolar bound phenols to come out and react with polyphenol oxidase present in the cell walls to form quinones. The quinones accumulate on the cell walls as brown deposits visible from the outside as dark brown rust.

Other researchers have implicated phenols in browning of plant tissues. Phenols can become oxidized to form dark coloured pigments (Tucker, 1993)

that appear as physiological disorders. Most plant cells, especially on fruit skin, undergo enzymatic browning when cell contents are exposed to atmospheric oxygen (Amarante et al., 2002b). Injured epidermal cells release phenolic compounds (mainly *o*-dihydroxy phenols) that are enzymatically oxidised by polyphenol oxidase (diphenol oxygen oxidoreductase or catechol oxidase) to form unstable quinone compounds (Mayer 1987). Susceptibility of a mango cultivar to the disorder may depend on the presence or absence of these compounds (Bezuidenhout et al., 2005), the degree of lenticel protection by endogenous wax (Amarante et al., 2002b). Russetting in Apple mango may be by a similar phenomenon. However, exact biochemistry, physical and/or physiological interactions, either singularly or in synergy, between anatomical features of susceptible mango cultivars and high humidity/low temperatures conditions is not yet clear.

Use of chemical sprays to control fruit peel russetting or cracking has not been successful (Albrigo and Achor, 2002). However, on-tree fruit bagging has produced smooth, shiny and attractive pear (Amarante et al., 2002b), grapes (Signes et al., 2007) and Keitt mango (Hofman et al., 1997b) fruits. Bagging of pears resulted in higher fruit temperatures during the day, resulting in a more uniform cuticle cover of the epidermis, less cracks in the cuticle and larger deposition of waxes on the cuticle (Amarante et al., 2002a). Therefore, bagging may control Apple mango 'rust' or lenticel discolouration by inducing deposition of lenticel protecting waxes and/or preventing cracking of the cuticle.

### **2.3 Effect of preharvest bagging on fruit quality**

Pre-harvest bagging of fruits (like most other pre-harvest practices) may affect harvest and postharvest quality of the fruit (Hofman, 1997). Bagging increase humidity and temperature (Amarante et al., 2002a) and decrease light intensity (Hofman, 1997) on the fruit. It also reduces fruit transpiration water loss (Amarante et al., 2002a; Joyce et al., 1997) during growth. Factors that reduce evapotranspiration by plant organs may reduce calcium accumulation (Hofman, 1997) on these organs. Therefore, fruit bagging may influence xylem and phloem fluxes, fruit carbon supply and eventually the fruit quality (Genard et al., 2007). Factors that reduce radiation interception by fruit or subtending leaves result in reduced soluble solids, and often higher acidity (Hofman et al., 1997a). Varying results on the effect of bagging on fruit quality have been reported. The variance may be due to fruit type, cultivar difference, timing and duration of bagging, and type of bagging material.

Bagging had no effect on fruit size of Sensation and Kensington mangoes (Joyce et al., 1997) and pears (Amarante et al., 2000a). However, Johns and Scotts (1989) reported increased fruit size in polythene bagged bananas and Estrada (2005) reported reduced weight on six mango cultivars bagged with anti-virus mesh. Litchi fruits bagged with white polyethylene bags three days before harvest had lower levels of total soluble solids and total titratable acidity at harvest, but no differences were found after six days of storage (YueMing et al., 2005). Ann et al. (1998) reported higher sugar content in mango fruit bagged with white paperbag than in fruit bagged with dark

paperbag. Joyce et al. (1997) reported reduced blush on the skin and a paler flesh for mangos bagged during fruit growth with nonperforated white opaque plastic bags than for unbagged fruit or fruit bagged with paper bags.

Effective and beneficial application of pre-harvest bagging on mango fruit for quality improvement will require an in-depth investigation on its effects on fruit physiology, quality at harvest and postharvest period. Fruit quality attributes are largely determined before harvest, while postharvest treatments, at best, maintain quality during product storage and distribution. Therefore, the relationship between production conditions and postharvest quality needs to be well understood if maximum and consistent quality for the consumer is to be achieved.

#### **2.4 Management of mango fruit pests, diseases and disorders in Kenya**

Most of the mango fruit farmers in Kenya use conventional means of cultivation, in which the principal measures in control of mango fruit pests, diseases and physiological disorders is by use of chemical sprays, orchard sanitation, chemical baits and pruning (Nyambo et al., 2006). Kenya Organic Association Network (KOAN) and Kenya Institute of Organic Farming (KIOF) have been promoting organic production for the last few years. None of the current control measures in conventional and organic mango farming in Kenya has been effective in the control of 'rust' in Apple mango. Farmers in Maragua district, Central Province of Kenya, are reported to be spraying the

fruit using a mixture of soap and white mineral oil to control the “rust” but the spray is not effective enough (Nyambo et al., 2006).

## **2.5 Mango fruit growth and ripening**

### **2.5.1 Fruit development and maturation**

Anatomically, fruits are swollen ovaries that may also contain associated flower parts. Fruit development follows fertilization, and occurs simultaneously with seed maturation. Fruits are well organized organs composed of cells from different tissues. The mango fruit is composed of one or two seeds, surrounded by a pericarp, itself composed of three tissues: the endocarp which is lignified; the mesocarp, which is composed of a large mass of cells constituting the main part of the fruit and the epicarp (peel). Complex chain of biological processes such as cell division, differentiation, size increase, metabolic transformations and vacuolar storage determine important quality traits such as fruit size, sweetness, acidity and nutritional quality (Génard et al., 2007). These biological processes are influenced by environmental factors such as light, temperature, humidity, nutrient supply and plant factors such as genetic make-up.

Fruit growth starts after bloom, with intensive cell division. Cell division is a phase of exponential cell proliferative activity, followed by a progressive decrease of this activity as cell division proceeds, and then mitosis stops and the cell population enter a stage of cell enlargement (Bertin et al., 2003). After the first phase of cell division, cell DNA endoreduplication boosts the cell sink

strength. Endoreduplication is an incomplete cell cycle that leads to DNA reduplication with no cell division, thus increasing the cell nuclear DNA content (Bertin, 2005). The amount of cytoplasm a cell can make and sustain is proportional to the amount of DNA in the nucleus (Bertin, 2005). Amplifying genome size by endoreduplication contributes to increasing cell size (Gènard et al., 2007). Cell division and endoreduplication are important components of sink strength (Ho, 1996), as they set the size limit of a cell (Ho, 1996). The rate of carbohydrate uptake by fruit cells is proportional to their level of endoreduplication (Genard et al., 2007). Cell differentiation is specialization of cells to serve distinct roles. It determines tissue appearance and the intensity of cell division and endoreduplication (Genard et al., 2007). Cell size increase involves the increase in total cytoplasmic macromolecular mass and cell expansion. Cell expansion involves irreversible increase in cell volume through vacuolization (Bertin, 2005), uptake of water and photoassimilate, and the plastic deformation of the cell wall (Gènard et al., 2007).

Fruit development is controlled by environmental, genetic, and plant factors. Environmental factors include temperature, light, and air humidity. Such factors influence important processes such as cell cycle duration, endoreduplication, photosynthesis, respiration, transpiration, phloemic transport, and metabolism (Gènard et al., 2007). The plant factors influence fruit quality through resource and hormonal controls. The complex source (leaves for carbon and roots for water) and sink (organs such as fruits) relationships in plants influence fruit growth (Ho, 1996), metabolism and

quality (Gènard et al., 2007). Carbon is allocated in plant organs according to organ demands and priority rules. Maintenance respiration costs have a first priority, with vegetative and reproductive growth being second and third in priorities respectively (Genard et al., 2007). Carbon allocated to fruit flesh is partitioned into four sugars (sucrose, sorbitol, glucose, and fructose), starch and structural carbohydrates (Genard et al., 2003).

In general, young developing fruits are extremely acidic as they accumulate organic acids such as malic, citric and tartaric acid and their pH is often below 3 (Shiratake and Martinoia, 2007). Fruit acidity is a complex process that includes metabolic processes and vacuolar transport (Lobit et al., 2002). The proton pump pumps protons into the vacuole, resulting in a drop of pH while synthesis and accumulation of organic acids within the vacuole serves as a buffer to maintain the low pH. Some fruits such as mango that accumulate high amounts of acids exhibit increasing accumulation of very high amount of sugars, mainly glucose, fructose and sucrose during growth and maturation (Ueda et al., 2000). The pH of mature fruits generally increases during maturation. Fruits do not taste acidic because large amounts of sugars accumulate during maturation (Tucker, 1993). Many mature grape berries have a pH of about 3.5, but they taste sweet because of the high amount of sugar accumulation (Shiratake and Martinoia, 2007). Citrate is the predominant acid in mango fruit (Lizada, 1993), with malate (Ueda et al., 2000) and succinate (Mitra and Baldwin, 1997) also found in significant quantities.

Compounds from secondary metabolism such as vitamins and carotenoids are essential for the nutritional and aesthetic quality of fruits (Genard et al., 2007). Secondary compounds are responsible for the typical colour and flavour of fruits. Carotenoids, chlorophyll, anthocyanin and betalain define fruit colour while in most cases, a blend between many phenolics, flavonoids, alkaloids and terpenoids define fruit flavour (Shiratake and Martinoia, 2007). Most of these secondary compounds are present in fruit tissue at concentrations in the mM range and others in even lower concentrations (Lizada, 1993). Anthocyanins are responsible for most of the red-purple colour of fruits (Lizada, 1993). Anthocyanins are water-soluble phenolic glycosides that can be found in the cell vacuoles of fruit and vegetables, but are often in the epidermal layers. They produce strong colours, which often mask carotenoids and chlorophyll (Tucker, 1993). Phenolic compounds contribute to the astringency properties associated with fruit quality (Lizada, 1993), and the defense of plant tissue against invading pathogens (Lizada, 1993). They are also substrates in a chain of biochemical reactions triggered by mechanical and/or physiological damage of cell walls to form discolouring precipitates, e.g. quinones on the fruit peel (Bezuidenhout et al., 2005)

Fruit starch is hydrolyzed during maturation and ripening (Tucker, 1993). The timing of harvest is a critical factor in determining aroma production and flavour development particularly for climacteric fruits where ripening is regulated by ethylene (Lizada, 1993). Enormous changes in production of volatile compounds that contribute to aroma and flavour changes occur as

ripening progresses. However, fruit harvested at an immature stage produce many of these compounds at rates too low to achieve a characteristic flavour (Tucker, 1993). Early harvest is advantageous commercially as softening is reduced during handling and transport but it comes at the expense of flavour development (Tucker, 1993). Mangoes accumulate their carbohydrate requirement prior to ripening and can be harvested at the mature green stage and still attain acceptable taste and flavour on ripening (Kalra et al., 1995).

### **2.5.2 Fruit ripening**

Mangoes are climacteric fruits that exhibit an autocatalytic ethylene production and increased respiratory activity after harvesting. However, maturation of mango fruit is accompanied by ripening associated changes such as decrease in starch (Kalra et al., 1995) and rupture force (Lizada, 1993), and development of yellow colour in the pulp (Mitra and Baldwin, 1997), indicating a possible ripening inducing ethylene production prior to detachment. After harvest, ripening fruit undergo many physicochemical changes that determine the quality of the fruit purchased by the consumer. Fruit ripening is a genetically programmed event that is characterized by a number of biochemical and physiological processes that alter fruit colour, aroma, flavour, texture and its nutritional value. These alterations are due to enzymatic breakdown of the cell wall, starch hydrolysis, sugar accumulation and reduction of organic acids and phenolic compounds, including tannins (Lizada, 1993). Ethylene is the major phytohormone implicated in inducement of ripening in fruits. The response of harvested fruit and vegetables to

endogenously produced and exogenously applied ethylene are numerous and varied, and they can be beneficial or detrimental (Mitra and Baldwin, 1997) depending on standing point along the market chain.

The breakdown of carbohydrate polymers is the largest quantitative change associated with ripening of mango fruits, especially the near total conversion of starch to sugars, altering both the taste and texture of the fruit (Lizada, 1993). Hydrolysis of mango fruit starch to formation of sugar is associated with the ripening process, with glucose, fructose and sucrose constituting most of the monosaccharides (Ueda et al., 2000). Total titratable acidity decreases with fruit ripening (Ueda et al., 2000), possibly due to organic acids being used as respiratory substrates (Tucker, 1993).

Colour change in ripening fruit has been associated by the consumer with the conversion of starch to sugar and the development of other desirable attributes, such that the correct skin colour is often all that is required for a decision to purchase the fruit (Li et al., 1997). The loss of green colour due to degradation of the chlorophyll structure (Lizada, 1993) is the most common visible change in climacteric fruits during ripening. The disappearance of chlorophyll is associated with the synthesis and/or unmasking of other preexisting pigments (Tucker, 1993). Most of these pigments are carotenoids that are synthesized during the development stages on the fruit but remain masked by the presence of chlorophyll (Lizada, 1993). The chloroplasts in the fruit peel are converted

into chromoplasts, which are red or yellow pigments, while others cultivars may show reddish blush because of anthocyanin.

End of fruit ripening is followed by senescence whereby anabolic reactions are suppressed by degradative changes leading to death and decay of the fruit tissue (Mitra and Baldwin, 1997). Senescence is catalyzed by postharvest disorders caused by pathogenic, physiological or mechanical damage. Mango fruit is highly susceptible to postharvest pathological disorders and extremes of temperature and thus, postharvest technologies should be designed for their control (Lizada, 1993).

Temperature is an important factor that influences the deterioration rate of harvested commodities (Burdon, 1997). Respiration generates heat as sugars and organic acids get oxidized. The higher the storage temperature, the higher the respiration rate (Crisosto and Ganer, 2001). In climacteric fruit, low temperature can be used to achieve a delay in the onset of ripening. Lowering the temperature reduces the production of ethylene and the rate of response of the tissues to ethylene (Watkins, 2006). However, exposure of mango fruit to undesirable low temperature results in chilling injury (Lizada, 1993).

Water is a major component of harvested mango fruit. Water loss is one of the main causes of physiological weight loss and deterioration that reduces the marketability of fresh fruits due to shriveling after losing only a small percentage of their original weight (Lizada, 1993). Fruit skin composition and

structure, relative humidity, fruit and surrounding atmosphere temperature and air velocity affect the rate of water loss from fresh fruit (Amarante and Banks, 2002).

## **2.6 Mango fruit quality**

Appearance and colour (Rathore et al., 2007) and eating quality (Pelayo et al., 2003) are the two most important factors influencing consumer acceptance of a fruit. Appearance is a visual assessment that can be made on the basis of size, shape, colour, wilting and shriveling, cultivar properties, mechanical damage and pathological and physiological disorders (Amarante et al., 2002b).

Texture characteristics of fruit edible tissue include juiciness and crispness (Rathore et al., 2007). The texture of fruit is affected by cellular organelles, biochemical constituents, water content or turgor and cell wall composition. Thus, any external factor affecting these traits can modify texture and can, therefore, lead to changes in final fruit quality (Genard et al., 2007). Flavour is affected by development stage at harvest, physiological abnormalities, storage conditions, microbial infections and storage with other commodities with strong aroma (Kader et al., 1978). Aroma plays an important part in the development of optimal eating quality in most fruits and results from synthesis of many volatile organic compounds during the ripening phase (Tucker, 1993). Flavour agents for most fruits contain a mixture of volatile acids, aldehydes, alcohols, esters and terpenoids (Lizada, 1993).

Mango fruit eating quality is based on the scarcity of fibers, sweetness and minimal turpentine taste (Griesbach, 1992). The flesh of most of the improved cultivars is peach-like and juicy, of a melting texture and more or less free from fiber (Griesbach, 2003). Demand is for juicy, melting fruit, but taste and aroma components are also very important. Various cultivars have characteristic taste and aroma that a producer country can capitalize on to offer superior cultivars and distinguish itself from the competition. Fruit taste and flavour are defined by the type (Tucker, 1993) and ratios (Genard et al., 2007) of organic compounds in the fruit. Cultivars with high sugar levels and well balanced sugar: acid ratios are preferred (Rathore et al., 2007).

Several minerals are known to influence quality, the most notable of these being calcium. Calcium application or high fruit calcium concentration is often associated with reduced weight loss, increased firmness, less disease, reduced susceptibility to chilling injury, physiological disorders and blemishes such as skin splitting, and slower ripening (Hofman, 1997).

### **2.7 Techniques used in mango fruit storage**

Mangoes are highly perishable tropical fruit, with a shelf life of 2 to 3 weeks at ambient temperature (Kalra et al., 1995). The trade of mango has been significantly limited due to their short shelf life and highly perishable nature (Rathore et al., 2007). Time of harvest, storage temperature and atmospheric conditions are key factors in postharvest physiology of the fruit. Harvesting mangoes prematurely will prevent fruit from reaching full ripeness. Harvesting

fruit at stages beyond mature green will reduce their shelf stability and shorten their fresh market life (Kalra et al., 1995). Postharvest treatments on mango fruit depend on the target market and are aimed at complying with quarantine restrictions, retarding ripening or initiating ripening at consumer point. Time and distance required to reach export markets necessitate subjection of mango fruit to various postharvest treatments such as cold storage, modified atmosphere, controlled atmosphere, thermal treatments and fungicidal sprays intended to lengthen their shelf life.

Thermal quarantine treatments involve heating the mango fruit at specific temperature-time regime to kill larvae and eggs of quarantine pests. Prolonged postharvest life is also achieved. Three common thermal quarantine treatments include; vapour heat treatment, forced hot-air treatment and hot water immersion treatment. Hot water treatment is the most preferred quarantine treatment because it is easily adaptable by growers and fruit distributors, uses short treatment times, is reliable and accurate in the monitoring of fruit temperatures, is efficient in killing surface decay organisms, and cleanses fruit surface during treatment. However, temperatures above 46°C have been reported to produce excessive fruit damage (Kalra et al., 1995) including skin scalding, abnormal erratic yellow patches of colour development with ripening, accelerated skin colour development (yellowing), damaged lenticels and accelerated respiration rates during pre-climacteric period (Jacobi et al., 2001). Most of the chemical quarantine treatments have been phased out due to safety and environmental concerns. Cold storage of mango is used to

prolong shelf life by slowing the metabolic rate of fruit (Kalra et al., 1995). However, cold storage of mango is limited by its susceptibility to chilling injury at storage and transport temperatures below 13 °C (Lizada, 1993). Therefore, there is need to look for cheap, convenient and reliable postharvest treatment.

Various chemical compounds that inhibit ethylene action have been found to delay onset of ripening or to retard its progression in various fruits. These include; CO<sub>2</sub> (Mathooko et al., 2001), diazocyclopentadiene (Mathooko et al., 1997), 1-methylcyclopropene, cyclopropene and 3, 3-dimethylcyclopropene (Sisler and Serek, 1997). 1-methylcyclopropene (1-MCP) is seemingly the most promising (Sisler and Serek, 1997) and of interest in this study.

## **2.8 Postharvest application of 1-methylcyclopropene in fruits**

Fruit response to 1-MCP depends on variables like cultivar differences and degree of maturity (Watkins, 2006). Its effect may also depend on tissue, organ and mode of action of ethylene biosynthesis (Sisler and Serek, 1997). In addition, application technique, the exposure environment, and the storage environment (if different from the exposure environment) influence effectiveness of 1-MCP (Watkins, 2006). Advances in fruit ripening prior to 1-MCP application may reduce its effectiveness due to elevated levels of ethylene found during ripening in some fruit cultivars (Watkins, 2006). 1-MCP may potentially improve the postharvest shelflife of Apple mango fruits.

## CHAPTER THREE

### 3.0 MATERIALS AND METHODS

#### 3.1 Experimental design, plant materials and treatment during fruit growth.

Mango (*Mangifera indica* L. cv. Apple) trees were randomly selected and tagged in a commercial farm in Yatta District, a semi-arid part of Eastern Province of Kenya during the September, 2007- February, 2008 season. Fruits of equal age were randomly selected and one set of 500 fruits bagged using “Standard” Kraft paper bags at 70 days after bloom (DAB) (Plate. 2). The bags were fastened on the fruit pedicel using a wire. The bottom edge corners of the bags were perforated for ventilation and drainage. Another equal set of fruits was not bagged (control). A separate set of bagged and unbagged fruits was tagged and used for monitoring changes in fruit equatorial diameter. The experiment was a completely randomized design (CRD).



Plate 2: Bagged and control mango fruits.

### **3.2 Preharvest sampling**

Sampling of the fruits commenced at 14 DAB and continued at 14 day intervals thereafter. Eighteen samples for weight measurement, 30 samples for measurement of fruit diameter and nine samples for analysis of the other parameters were used. Those fruit that were visually of equal size and free from defects were harvested from each of the previously tagged trees. The fruits were immediately transported, cold stored (15°C) and analyzed for changes in physical, physiological and chemical parameters in the laboratories of the Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology (JKUAT).

### **3.3 Postharvest 1-methylcyclopropene treatment and sampling**

Using fruit peel colour as a maturity index, fruits were harvested at 168 DAB. The bagged and control fruits were each divided into two equal sets. One set from bagged fruits and another one from control were each enclosed in separate 12 litre airtight containers fitted with self-sealing rubber septum. 1-MCP gas was generated from Smartfresh<sup>TM</sup> powder (Rohm and Haas Co., Japan) with 3.3% w/w 1-MCP according to manufacturer's instructions. An airtight syringe was used to inject 20 ppm of the 1-MCP gas into the containers containing the fruits. The 20 ppm 1-MCP concentration is the average effective concentration for various studies on mango fruit (Blankenship and Dole, 2003). Six samples from each treatment were used for the analysis. Fruits were arranged into two blocks of bagged and unbagged fruits in a randomized complete block design (RCBD), and then analyzed for

changes in physical, physiological and chemical parameters every three day interval during storage at ambient conditions (Temperature;  $25\pm 1^{\circ}\text{C}$  and RH  $60\pm 5\%$ ) for 15 days. A set of 1- MCP treated and untreated tomato fruits (Appendix 1) were set alongside the mango fruits to ascertain the efficacy of the 1-MCP.

### **3.4 Measurements of fruit physical parameters**

#### **3.4.1 Weight and diameter**

Fruit weight was determined using a scientific balance (Model Libror AEG-220, Shimadzu Corp. Kyoto, Japan). The fruit diameter was determined using veneer caliper (Model 530-118N 8'' P, Mitutoyo, Japan).

#### **3.4.2 Fruit firmness**

Firmness was measured along the equatorial region of the fruit using a penetrometer (Model CR-100D, Sun Scientific Co. Ltd, Japan) fitted with an 8 mm probe. Firmness is expressed as Newton (N) (Jiang et al., 1999).

#### **3.4.3 Colour**

Colour of both the pulp and peel was measured for both the red and green sides of each fruit using a Minolta color difference meter (Model CR-200, Osaka, Japan) that was calibrated with a white and black standard tile. The  $L^*$ ,  $a^*$  and  $b^*$  coordinates were recorded and,  $a^*$  and  $b^*$  values converted to hue angle ( $H^{\circ}$ ) according to McLellan et al. (1995);

$$\text{Hue angle } (H^{\circ}) = \arctan (b/a) \text{ (for } +a \text{ and } +b \text{ values)}$$

$$= \arctan (b/a) + 180 \text{ (for } -a \text{ and } +b \text{ values)}$$

$$= \arctan (b/a) + 180 \text{ (for -a and -b values)}$$

$$= \arctan (b/a) + 360 \text{ (for +a and -b values)}$$

### **3.5 Analyses of fruit physiological parameters**

#### **3.5.1 Ethylene production and respiration rates**

Depending on the size, known weights of mango fruits were placed in plastic jars ranging in volume from 500ml to 3000 ml and whose covers were fitted with a self-sealing rubber septum for gas sampling. The fruits were incubated for an hour at room temperature (22-26°C). Gas samples from the headspace gas were withdrawn using an airtight syringe and injected into gas chromatographs (Models GC-8A and GC-9A, Shimadzu Corp., Kyoto, Japan for respiration and ethylene production rates, respectively). The gas chromatograph for carbon dioxide determination was fitted with a thermal conductivity detector and a Poropak N column and that for ethylene determination was fitted with an activated alumina column and a flame ionization detector. Rate of carbon dioxide production is expressed as ml per kg per hr at standard atmospheric pressure while ethylene production is expressed as nl per kg per hr.

#### **3.5.2 Postharvest weight loss**

Weights of nine fruits from each treatment were taken on each sampling day using a scientific balance (Model Libror AEG-220, Shimadzu Corp. Kyoto, Japan). The initial weight ( $W_1$ ) of each fruit at day 0 and the weight of the

same fruit ( $W_2$ ) on each sampling day were noted. The following formula was used to calculate the % weight loss:

$$\% \text{ weight loss} = 100 \times (W_1 - W_2)/W_1$$

### **3.6 Analyses of fruit chemical parameters**

#### **3.6.1 Starch content**

This was done by the starch staining method. A slice from the equatorial region of the fruit was dipped in iodine solution (2g of  $I_2$  into 100ml of 0.01M KI solution), immediately removed and left to stand for three minutes. The percentage of dark blue colouration on the cut surface was rated as percentage fruit starch content using Cornell Starch Chart (Watkins et al., 2004).

#### **3.6.2 Sucrose, fructose and glucose contents.**

Sugars were analyzed using AOAC method (1996). Ten grams of the fruit pulp was refluxed in ethanol for one hour. The extract was then concentrated by rotary evaporation and diluted with 75% acetonitrile. Standard solutions of concentrations 0, 1, 2, 3, 4 mg of sucrose, fructose and sucrose/ml were prepared by dissolving into 75% acetonitrile. The standard solutions and the sample extracts were injected into High Performance Liquid Chromatograph (HPLC) (Model LC-10AS, Shimadzu Corp., Kyoto, Japan) fitted with a refractive index detector and having the following conditions: Oven temperature - 35°C, Flow rate: - 0.5-1.0 ml/min, Injection volume - 20  $\mu$ L, Column - NH<sub>2</sub>P-50 E. A standard curve was drawn and used to quantify the sugars content of the samples.

### **3.6.3 Total soluble solids content and total titratable acidity**

Total soluble solids (TSS) content was determined using an Atago hand refractometer (Model 500, Atago, and Tokyo, Japan) and expressed as °Brix. Total titratable acidity (TTA) was determined by titration with 0.1N NaOH in the presence of phenolphthalein indicator. The TTA is expressed as % citric acid, the predominant organic acid in mango fruit (Ueda et al., 2000).

### **3.6.4 Determination of $\beta$ -carotene content**

The  $\beta$ -carotene content of the fruit pulp was determined by a modified chromatographic procedure (Heionen, 1990). A sample of 20 gm was crushed in a pestle with a mortar. A spatula of hydroflorosupercel was added and then extraction done using 50ml acetone until the residue became white. Partitioning was done using 25ml of petroleum ether in a separating funnel. Saponification was carried out by adding an equal amount of extract in to 3ml of 10% KOH in methanol, and a few drops of 0.1% butylated hydrotoluene in petroleum ether. The mixture was kept in the dark for 16 hours followed by washing with water in a separating funnel until it became clear. Sodium sulphate (anhydrous) was added to remove water and further concentration done using a rotary evaporator. The  $\beta$ -carotene standard solutions of concentrations 10, 20, 40, 60 and 80 ppm were prepared by dissolving into petroleum ether. The  $\beta$ -carotene extracts from the samples and standard solutions were injected into the HPLC (Model LC-10AS, Shimadzu Corp., Kyoto, Japan), having the following conditions: Mobile phase – (acetonitrile: methanol: dichloromethane = 70: 10: 20), Flow rate - 1.0 ml/min, Column -

ODS 150, Injection volume - 10 $\mu$ L, Oven temperature - 35°C. The standard curve derived from the standard solutions was used to determine the  $\beta$ -carotene content of the samples.

### **3.6.5 Total anthocyanin content**

Total anthocyanin was determined in the peel using a modified pH differential method (Meyers et al., 2003). Three grams of the sample was ground and diluted with 100ml of distilled water and sediment removed by centrifugation. Two sets of the same sample were diluted with equal volume of buffers of pH 1.0 and pH 4.5 and the absorbance measured at 510nm and 700nm using a UV-Vis spectrophotometer (Model UV mini 1240, Shimadzu Corp. Kyoto, Japan). Samples diluted with the pH 1.0 buffer were left to rest for 15 minutes before measurements, whereas the samples diluted with the pH 4.5 buffer were ready for measurement after 5 minutes. The blank consisted of the corresponding pure buffer solution. To correct for turbidity (haze) the absorbance at 700nm was subtracted from the absorbance at 510nm (the wavelength of maximum absorption). To calculate the difference in absorbance between the samples the following formula was used:

$$\Delta\text{Absorbance} = (A_{510\text{nm pH } 1.0} - A_{700\text{nm pH } 1.0}) - (A_{510\text{nm pH } 4.5} - A_{700\text{nm pH } 4.5})$$

Determination of anthocyanin content was based on Lambert- Beer's Law:

$$A = \epsilon CL$$

A is the absorbance, which is measured with a spectrophotometer.

L is the path length in cm of the spectrophotometer cell. Most spectrometer cells have a path length of 1cm.

$\epsilon$  is the molar absorbance or molar extinction coefficient., a physical constant for a molecular species in a given solvent system at a given wavelength.

C is the molar concentration.

Rearranging the Lambert- Beer's Law equation and multiplying by the molecular weight (M) of the pigment, the concentration in milligrams per liter is determined as:  $C \text{ (mg/l)} = \Delta A / \epsilon L \times M \times 10^3 \times D$

Where D is the dilution factor, and  $\Delta A$  is the difference in absorbance of the sample at maximum absorption (510nm) in the pH 1.0 and pH 4.5 buffers.

### **3.6.6 Chlorophyll content**

The chlorophyll content was determined by UV-Vis spectrophotometer (Model UV mini 1240, Shimadzu Corporation, Kyoto, Japan) using the method of Arnon (1949). The chlorophyll was extracted with 80% acetone, and total chlorophyll, chlorophyll a and b contents calculated using the following MacKinney's coefficients (MacKinney, 1941) as follows:

$$\text{Total chlorophyll content } (\mu\text{g/g}) = 20.2A_{645} + 8.02A_{663}$$

$$\text{Chlorophyll a content } (\mu\text{g/g}) = 12.7A_{663} - 2.69A_{645} \text{ and}$$

$$\text{Chlorophyll b content } (\mu\text{g/g}) = 22.9A_{645} - 4.48A_{663}$$

### **3.6.7 Ascorbic acid content**

The ascorbic acid was determined using the AOAC. 967.21 (1996) method. Five grams of the pulp was ground and diluted with 10% trichloroacetic acid (TCA) to 100 mark of a 100ml volumetric flask. 2, 6-dichlophenolindophenol was titrated to 10ml of the fruit pulp filtrate. Ascorbic acid was calculated as:

Ascorbic acid, (mg/100g) = (A-B) X C X 100/S X (100/10)

Where A = volume in ml of indophenol solution used in the sample.

B = Volume in ml of indophenol solution used for the blank.

C = Mass in mg of ascorbic acid equivalent to 1 ml of standard indophenol solution.

S = Weight of the sample taken (g)

100/10 = total extraction volume/volume of titrated sample

### **3.6.8 Mineral contents**

Minerals were analyzed using AOAC (1996) method. Three grams of the pulp and peel were separately dried in the oven, ashed in the muffle furnace and diluted with 1% HCl. Mg, K and Ca were analyzed by Atomic Absorption Spectrophotometry (AOAC, 1996) method using an atomic absorption spectrophotometer (Model AA-6200, Shimadzu Corp., Kyoto, Japan). Phosphorus content was determined using ascorbic acid method with the UV-Vis spectrophotometer (Model UV mini 1240, Shimadzu Corp., Kyoto, Japan).

### **3.7 Sensory evaluation**

The sensory evaluation of the bagged and unbagged fruits at harvest and on ripening seven days after harvest (DAH) was done using a 9 point hedonic scale (Appendix 2) and a panel of 15 untrained judges. The attributes evaluated at harvest were peel colour, appearance and total acceptance. Peel appearance and colour, pulp colour and texture, aroma, taste and total acceptance were evaluated for the ripe fruits. Mango slices were at room

temperature, placed into 3-digit coded plates and presented in a random order (Mtebe et al., 2006).

### **3.8 Statistical analysis**

Data was presented as means  $\pm$  standard deviations. Comparison of means using a t-test for preharvest data and ANOVA for postharvest data, and Least Significance Difference (LSD) for mean separation was done at  $p=0.05$  using Genstat®-Third Edition.

## CHAPTER FOUR

### 4.0 RESULTS AND DISCUSSION

#### 4.1 Physiological and physico-chemical characteristics of the fruit during growth and development

##### 4.1.1 Visual appearance of the fruit

Both bagged and unbagged fruits attained maturity at the same time, and were harvested at 168 DAB. The peel of control fruits had rust lesions, visible whitish colonies of fungal infection and spots of reddish blush (Plate 3). Bagged fruits had an unblemished peel with a light green colour and no reddish blush.



Plate 3: Mature green bagged and unbagged fruits at harvest

##### 4.1.2 Changes in fruit physical parameters

###### 4.1.2.1 Fruit weight and diameter

Fruit weights and diameters for both bagged and unbagged fruits displayed growth on a single sigmoid curve (Fig. 2), with changes in fruit weight showing four distinct stages of growth.

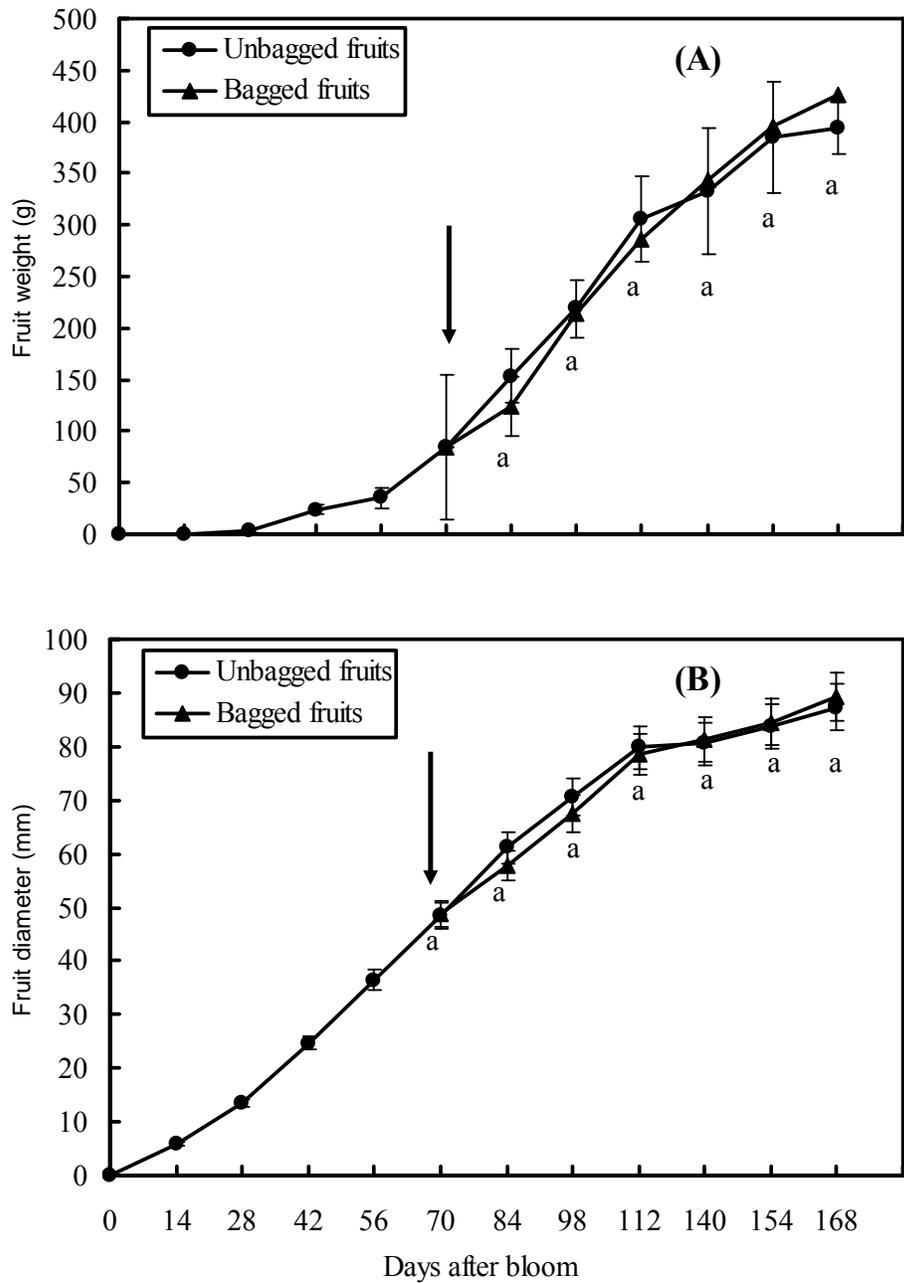


Fig. 2: Changes in fruit weight (A) and diameter (B) during growth and development of bagged and unbagged mango fruits. The vertical bars represent SE of the mean of 18 and 30 replicates for fruit weight and diameter respectively. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' denotes a period of no significant difference between bagged and unbagged fruits.

Mango fruit growth follows a single sigmoid pattern (Kalra et al., 1995). Minimal increases in fruit weight characterized stage I, 0 to 28 DAB. The gain in fruit weight and diameter at this stage is due to cell division and increased cell mass (Bertin et al., 2003). A slow rate of gain in weight and diameter at stage I was observed in breadfruit (Worrel et al., 1998). Increased rate of weight gain characterized stage II, 28 to 56 DAB, possibly due to cell enlargement and formation of intercellular spaces (Bertin et al., 2003). This stage represents a period of mesocarp cell enlargement (Worrell et al., 1998). After cell division ceases, fruit growth continues mostly by cell expansion (Bertin et al., 2003). Fruit growth starts after bloom, with intensive cell division, but the proliferative activity of the cells slows as development proceeds (Ho, 1996). Then mitosis stops and the cell population enter a stage of cell enlargement (Bertin et al., 2003). Stage III, 56 to 112 DAB, was characterized by rapid gain in fruit weight and diameter. This may have been due to massive starch deposition (Ueda et al., 2000). Increased starch storage was responsible for rapid weight gain of breadfruit during development (Worrell et al., 1998). Stage IV started 112 DAB and represents cessation of growth and start of maturation (Kalra et al., 1995).

Bagging of fruit had no significant effect on both fruit weight and diameter. This could be due to bagging having been done after sink strength had already been determined during the early stages of fruit growth. Bagging had no effect also on weight and of 'Kensington' mango (Joyce et al., 1997) and pear (Amarante et al., 2002a) fruits.

#### 4.1.2.2 Changes in fruit firmness

Fruit firmness is an important quality index during loading and shipping.

Bagged and control fruits had a similar pattern of changes in firmness (Fig. 3).

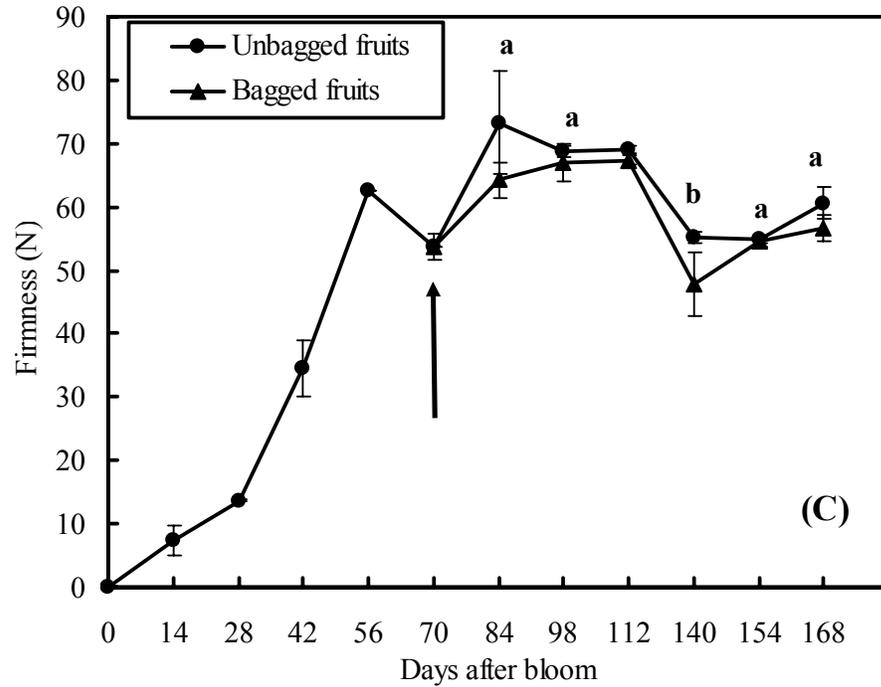


Fig. 3: Changes in fruit firmness during growth and development of bagged and unbagged mango fruits. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

There was an increase in fruit firmness during growth and development up to 112 DAB, followed by a decline in the early stages of maturation up to 140 DAB, and then an increase prior to harvest. Increase in fruit firmness is due to synthesis and deposition of structural carbohydrates such as pectin and hemicelluloses in the cell walls of the fruit epicarp (Genard et al., 2003), while

decline in fruit firmness during maturation is due to synergistic degradation action of cell wall hydrolases (Chin et al., 1999) in concert with expansins (Sane et al., 2005) on cell wall structural carbohydrates. Total pectin content in Irwin mango decreased during maturation with a concomitant decrease in its flesh firmness (Ueda et al., 2000).

Increase in fruit firmness immediately before harvest was contrary to observations on many mango cultivars. Tommy Atkins (Madigu, 2007) and 'Chiin Hwang No. 1' (Ueda et al., 2001) mango fruits firmness remained almost constant during maturation up to harvest. This may be due to varietal differences in cell wall ultrastructure, pericarp structure and cuticular layer formation (Lizada, 1993). Massive wax deposits occur in some cultivars during development of cuticle layer on the peel as mango fruit matures (Petti et al., 2007). The cuticular layer in the fully mature Carabao mango is well defined and its ultrastructure cannot be altered even by overnight soaking or rubbing with methanol or chloroform (Lizada, 1993). 'Chiin Hwang No. 1' mangoes were found to decline in pulp rupture force during maturation (Ueda et al., 2001).

Bagged mango fruit had a non-significantly ( $p>0.05$ ) lower firmness at harvest. This reflected possible differences in peel composition and/or structure between the treatments. These results are at variance with those found on pears (Amarante et al., 2002a). This indicates that bagged mango

fruits may be more susceptible to mechanical damage during loading and shipping, and lead to adverse effects on marketability.

#### **4.1.2.3 Changes in fruit pulp and peel colour**

Fruit colour is an important quality attribute. Colour may influence flavour recognition and consumer perception. Changes in fruit pulp and peel brightness, as indicated by L\* values, are shown in Fig. 4. Pulp L\* values increased up to a maximum of 85.53 and 84.7 for bagged and control fruits, respectively at 140 DAB, and then declined until harvest at 168 DAB. This decline coincided with colour change from white to whitish-yellow. A decline in pulp L\* value from maturation to ripening was observed in 'Chiin Hwang No. 1' mango fruit (Ueda et al., 2001). There were no significant difference ( $p>0.05$ ) in pulp L\* value between bagged and unbagged fruits. Peel L\* value for bagged fruit remained almost constant between 140 DAB and 168 DAB while those for control declined to significant ( $p<0.05$ ) lower values at 154 and 168 DAB.

The a\* and b\* values were reported as hue angle ( $H^\circ$ ) as per the Hunter's colour system (Appendix III), and not alone as they are not independent variables (McGuire, 1992). A  $90^\circ$  hue angle represents yellow,  $180^\circ$  represents bluish/green,  $270^\circ$  represents blue, and  $0^\circ$  ( $360^\circ$ ) represents red/purple (McGuire, 1992). The pulp hue angle for both bagged and unbagged fruits declined throughout growth, development and maturation (Fig. 5 A), with no

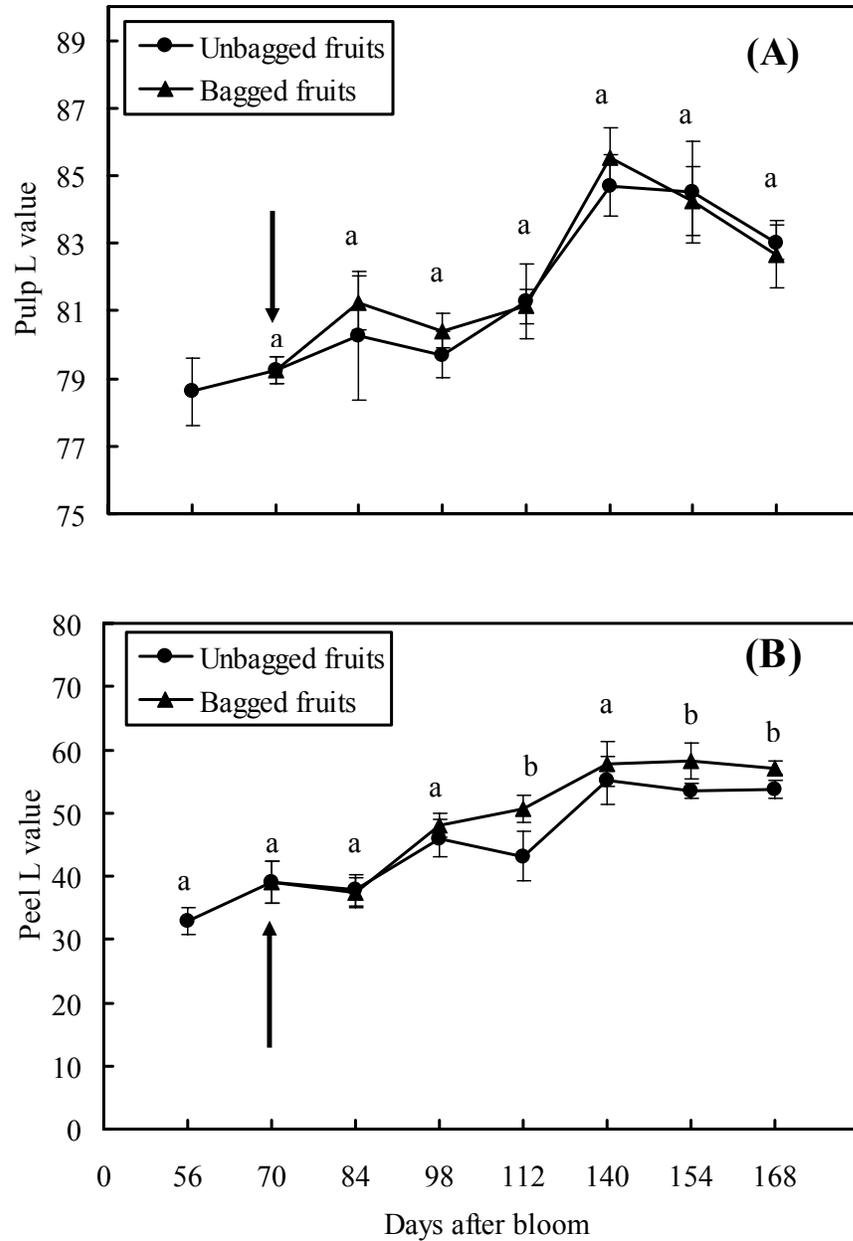


Fig. 4: Changes in pulp (A) and peel (B) L value during growth and development of bagged and unbagged mango fruits. The vertical bars represent SE of the mean of 9 replicates. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes periods of non-significant and significant differences, respectively between bagged and unbagged fruits ( $p=0.05$ ).

significant differences ( $p>0.05$ ) in all the sampling days. The declining hue angle values during maturation corresponded with a colour shift from white to yellow. The pulp hue angle value at 168 DAB for bagged and control fruits were  $99.8^\circ$  and  $98.8^\circ$  respectively, with visible whitish-yellow due to synthesis and accumulation of  $\beta$ -carotenes.

Peel hue angle for bagged fruits increased in a nearly linear manner up to 140 DAB, and then declined slightly up to harvest (Fig. 5 B). Peel hue angle for control fruit increased up to 140 DAB, but in an intermittent manner with periods of slight decline at 84 and 112 DAB. Results on fruit anthocyanin content showed 112 DAB with the highest anthocyanin content (Fig. 7B). Therefore, the decline in peel hue angle in unbagged fruits (towards or more red) at 112 DAB may have been due to high levels of anthocyanins (Signes et al., 2007) and increased redness ( $<$  hue angle). Reduced redness ( $>$ hue angle) in bagged grapes may have been due to modification of the internal atmosphere (Signes et al., 2007) and/or elevated temperature inside the bag (Amarante et al., 2002a), which retarded maturity and reduced anthocyanin accumulation (Hofman et al., 1997b). Results on fruit chlorophyll content indicate 84 DAB with reduced chlorophyll levels (Fig. 6) that may have been effectively masked by the accumulating anthocyanins to cause a colour shift toward red ( $<$  hue angle). A colour shift from green ( $-a^*$ ) towards red ( $+a^*$ ) in grape fruit lead to a decline in hue angle (Signes et al., 2007). Loss of green colour ( $-a^*$  towards  $+a^*$ ) caused decrease in peel hue angle during maturation of 'Chiin Hwang No. 1' mango fruit (Ueda et al., 2001).

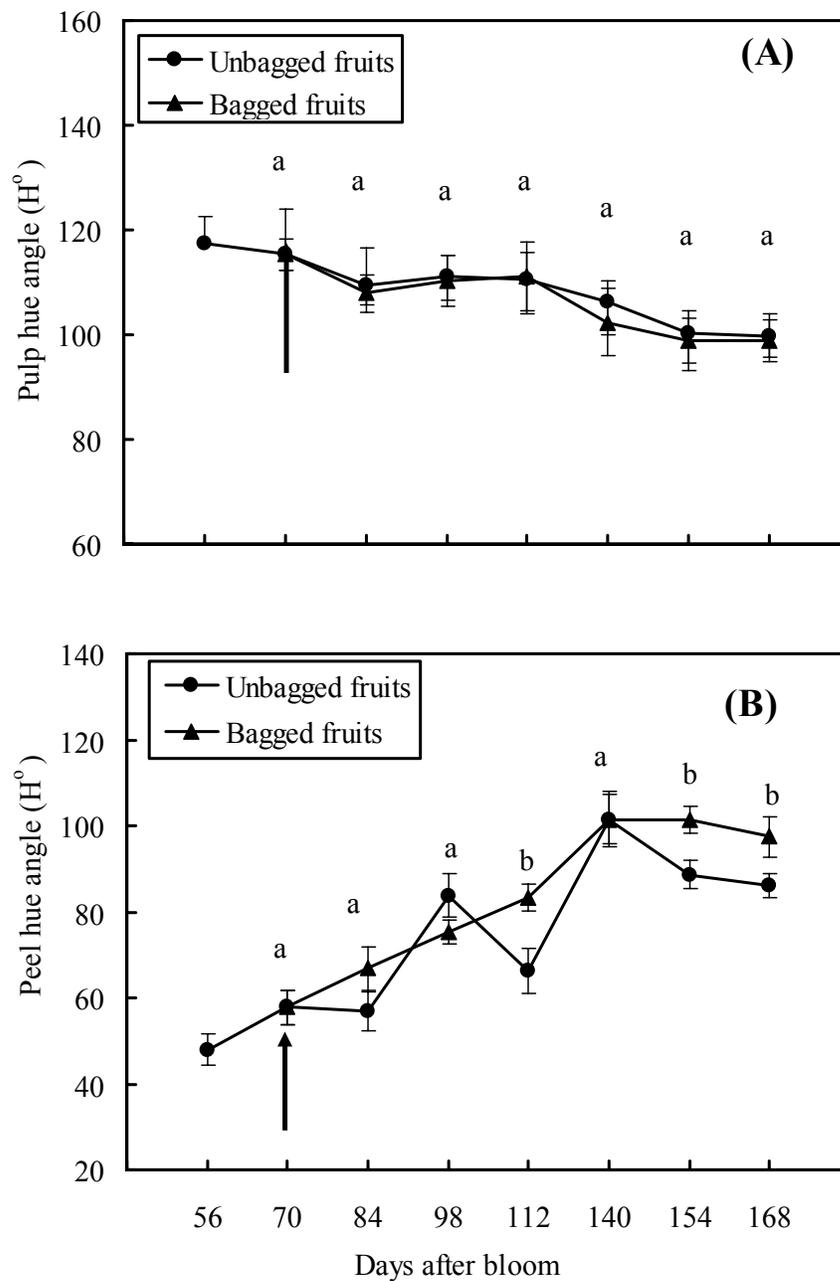


Fig. 5: Changes in pulp (A) and peel (B) hue angle during growth and development of bagged and unbagged mango fruits. The vertical bars represent SE of the mean of 9 replicates. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

Unbagged fruit exhibited a bigger decline in peel hue angle (degreening) during maturation resulting in significant ( $p < 0.05$ ) lower values at 154 and 168 DAB, probably due to more exposure to catalyst of oxidative degreening. These results conform to the results on chlorophyll content. Therefore, bagging (reduced light) increased  $L^*$  value and hue angle of mango fruit peel at harvest. Similar results were reported on peach fruit (Lewallen, 2000) who reported that as one moves from the outside to the inside of the canopy (reduced light),  $L^*$  values, chromaticity, and hue angles of peach fruit peel increased. Cellulose bagged grapes presented higher values of lightness and hue angle, but lower values of redness, yellowness and chroma, while the non-bagged grapes were slightly darker ( $<L^*$ ), more red ( $>a^*$ ), with a higher intensity of color ( $>C^*$ ) but were less blue ( $<b^*$ ) than bagged samples (Signes et al., 2007).

#### **4.1.3 Changes in fruit biochemical composition**

##### **4.1.3.1 Fruit peel chlorophyll content**

Changes in chlorophylls a and b fruit contents are illustrated in Fig. 6. Bagged and control fruits had a similar pattern of changes in chlorophyll a content with significant differences ( $p < 0.05$ ) only at 98, 112 and 140 DAB. A higher content of chlorophyll b was observed in bagged fruits, with significant differences ( $p < 0.05$ ) at 98, 112, 154 and 168 DAB. Chlorophyll a content was higher than chlorophyll b in the early days of growth in both bagged and control fruits, whereas chlorophyll b content was higher than chlorophyll a at harvest. This implied that chlorophyll a degraded faster than chlorophyll b

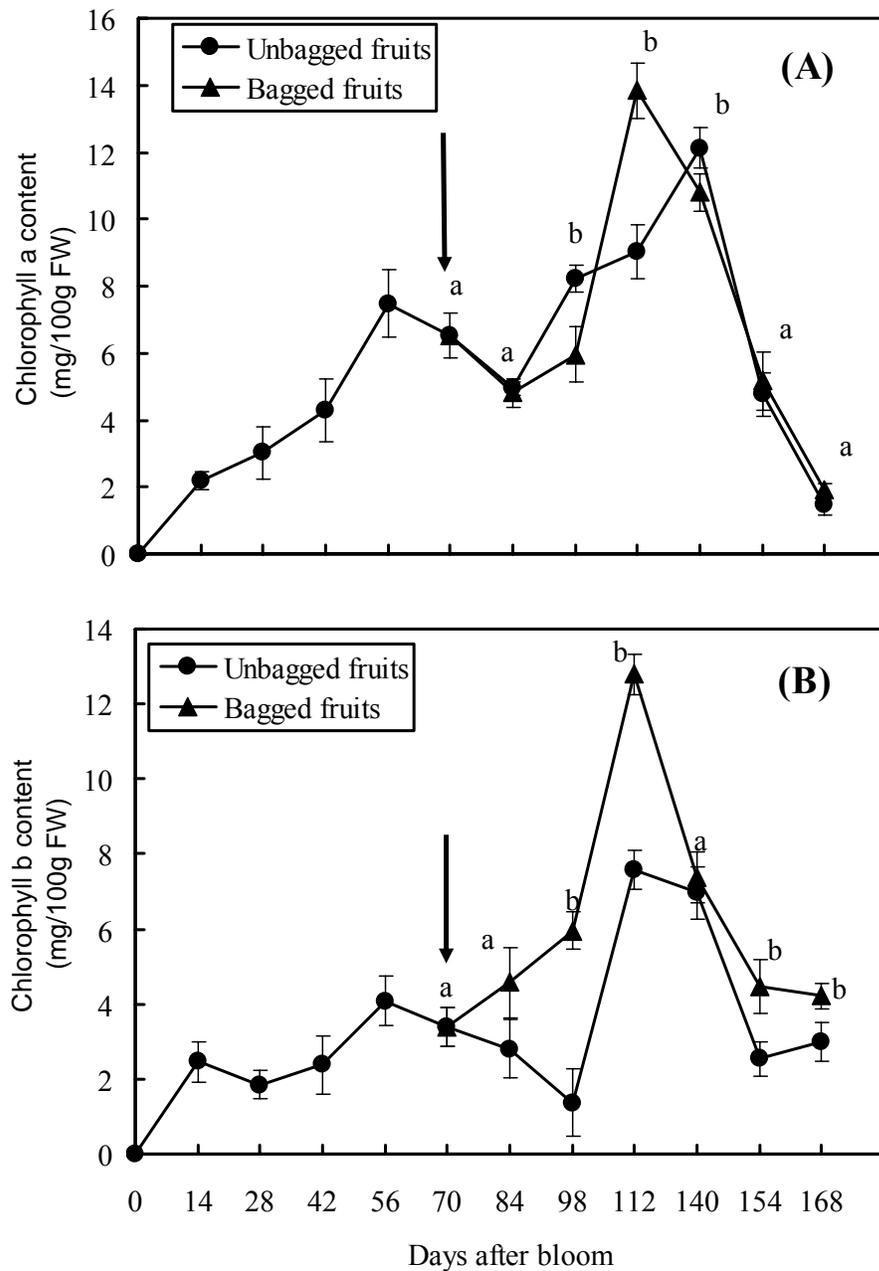


Fig. 6: Changes in chlorophyll a (A) and chlorophyll b (B) content of bagged and control mango fruits during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

during maturation. Chlorophyll a was preferentially degraded relative to chlorophyll b in Tommy Atkins mango (Medlicott et al., 1986).

Bagged fruits had significantly ( $p < 0.05$ ) higher total chlorophyll content at harvest (Fig 7). Indeed, bagged fruits had a higher peel hue angle (Fig. 5 B) and visually greener (Plate 3) at harvest. Decrease in chlorophyll content prior to harvest is due to enzymatic degradation, resulting in unmasking of beta-carotenes and anthocyanins (Tucker, 1993). This implies that reduced radiation by bagging reduced the rate of chlorophyll degradation, and limited the unmasking of the anthocyanins leading to lack of the characteristic reddish-purple colouration of Apple mango. This may complicate use of bagged mango fruit peel colour as a maturity index and consumer perception. However, improved appearance by bagging (Plate 3) may counter the negative effect on peel colour. Once solubilised, chlorophyll can be oxidized to the colourless purin and chlorin products (Lizada, 1993). Bagging resulted in greener fruit also in 'BC-2 Fuji' apples (Fallahi et al., 2001), pears (Amarante et al., 2002a) and grapes (Signes et al., 2007).

#### **4.1.3.2 Pulp carotenoids and peel anthocyanins content**

The  $\beta$ -carotene content was initially low, and then increased sharply from 98 DAB up to harvest for both bagged and unbagged fruits (Fig. 8 A). Anthocyanin content increased up to maturity (112 DAB), then decreased up to harvest (Fig. 8 B). Carotenoids are terpenoid compounds derived from acetyl via the mevalonic acid pathway. The  $\beta$ -carotene is synthesized in green and

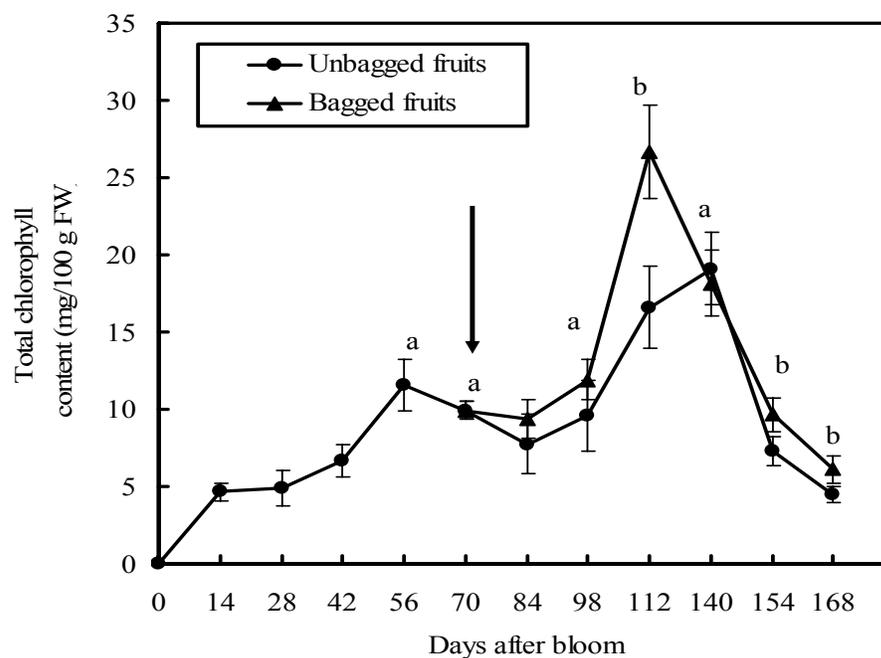


Fig. 7: Changes in total chlorophyll content of bagged and unbagged mango fruits during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

ripening fruit (Lizada, 1993). The  $\beta$ -carotene content increased from the mature green to the ripe stage for 'Tommy Atkins' mangoes, (Madigu, 2007). Unbagged fruits had significantly ( $p<0.001$ ) higher  $\beta$ -carotene levels than bagged fruits. Reduced light exposure in bagged fruits may have reduced  $\beta$ -carotene biosynthesis. Elevated temperature and greater exposure to sunlight increase carotenogenesis (Dutta et al., 2005).

Preharvest bagging reduced anthocyanins in Apple mango, as also reported in Keitt mango (Hofman et al., 1997b) and 'Fuji' apple (Fan and Mattheis, 1998)

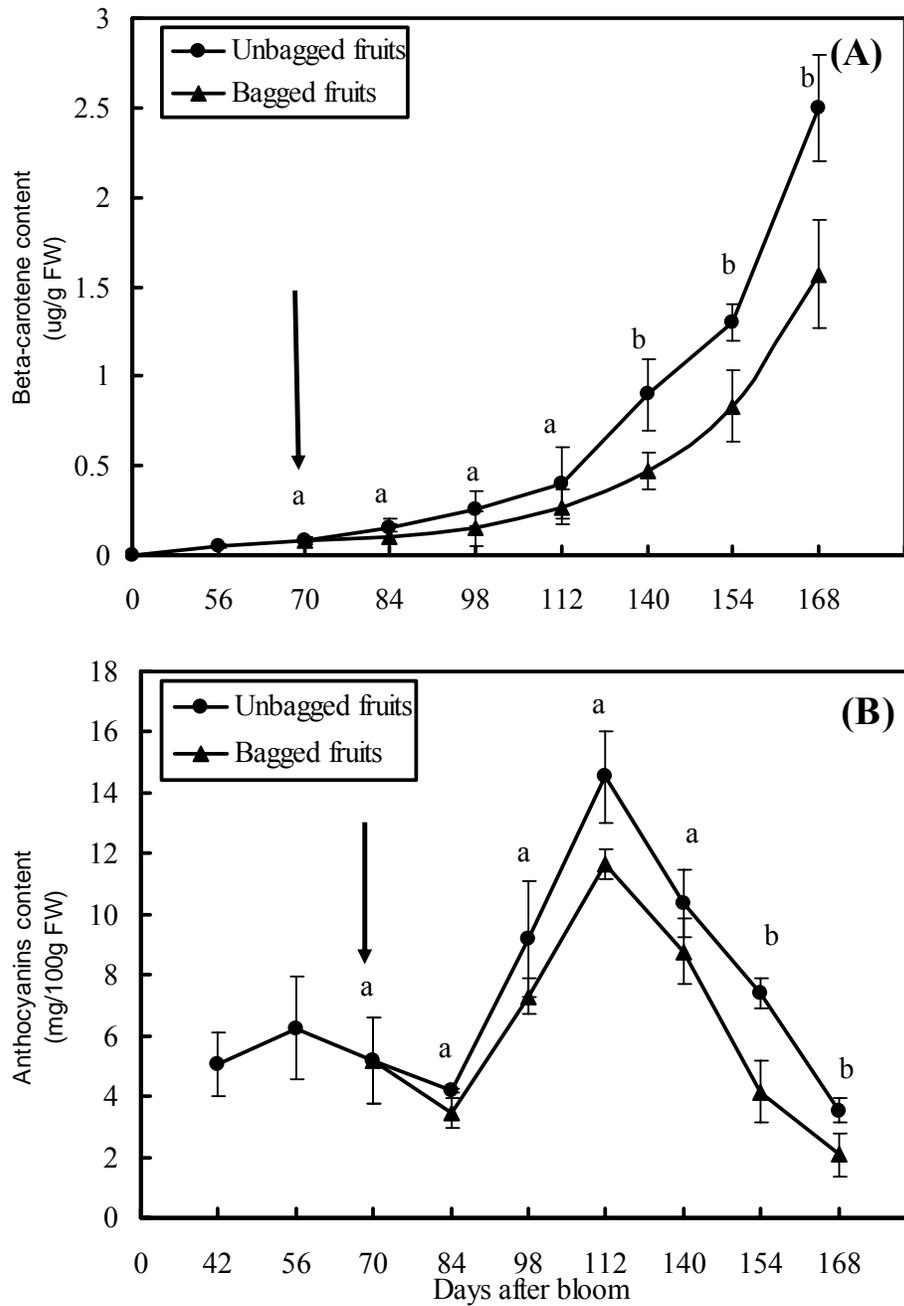


Fig. 8: Changes in pulp  $\beta$ -carotene (A) and peel anthocyanins (B) content of bagged and unbagged mango fruit during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

fruits. Reduced red blush in bagged fruits may be due to modification of the internal atmosphere (Hofman et al., 1997b) and/or elevated temperature inside the bag (Amarante et al., 2002a), which retards maturity and reduces anthocyanin accumulation (Fan and Mattheis, 1998). Preharvest bagging of mango fruits with perforated Kraft paper bags in this study might have caused substantial modification of light absorption by the fruit to cause a reduction in anthocyanin synthesis and/or accumulation.

#### **4.1.3.3 Starch and sugar contents**

Fruit starch content increased during growth and development, but decreased during maturation (Fig. 9 A). Sucrose content increased up to harvest in both bagged and unbagged fruits (Fig. 9 B). There was a rapid increase in starch content between 42 and 56 DAB, indicating a rapid starch deposition, probably due to high activity of sucrose synthase (Marini et al., 1991). Starch accumulation is slow initially, but increases remarkably during growth and then decreases strikingly during maturation of 'Chiin Hwang No. 1' (Ueda et al., 2001) and Irwin (Ueda et al., 2000) mango fruits. The decrease in starch levels during maturation is due to hydrolysis by amylase (Ueda et al., 2000). A similar pattern of changes in starch content was found on Tommy Atkins (Madigu, 2007). At 140 DAB, bagged and unbagged mango fruits had similar starch contents. However, at 168 DAB when fruits were harvested, bagged mangoes had significantly higher levels of starch ( $p < 0.05$ ). This implied that the rate of starch hydrolysis was lower in bagged fruits.

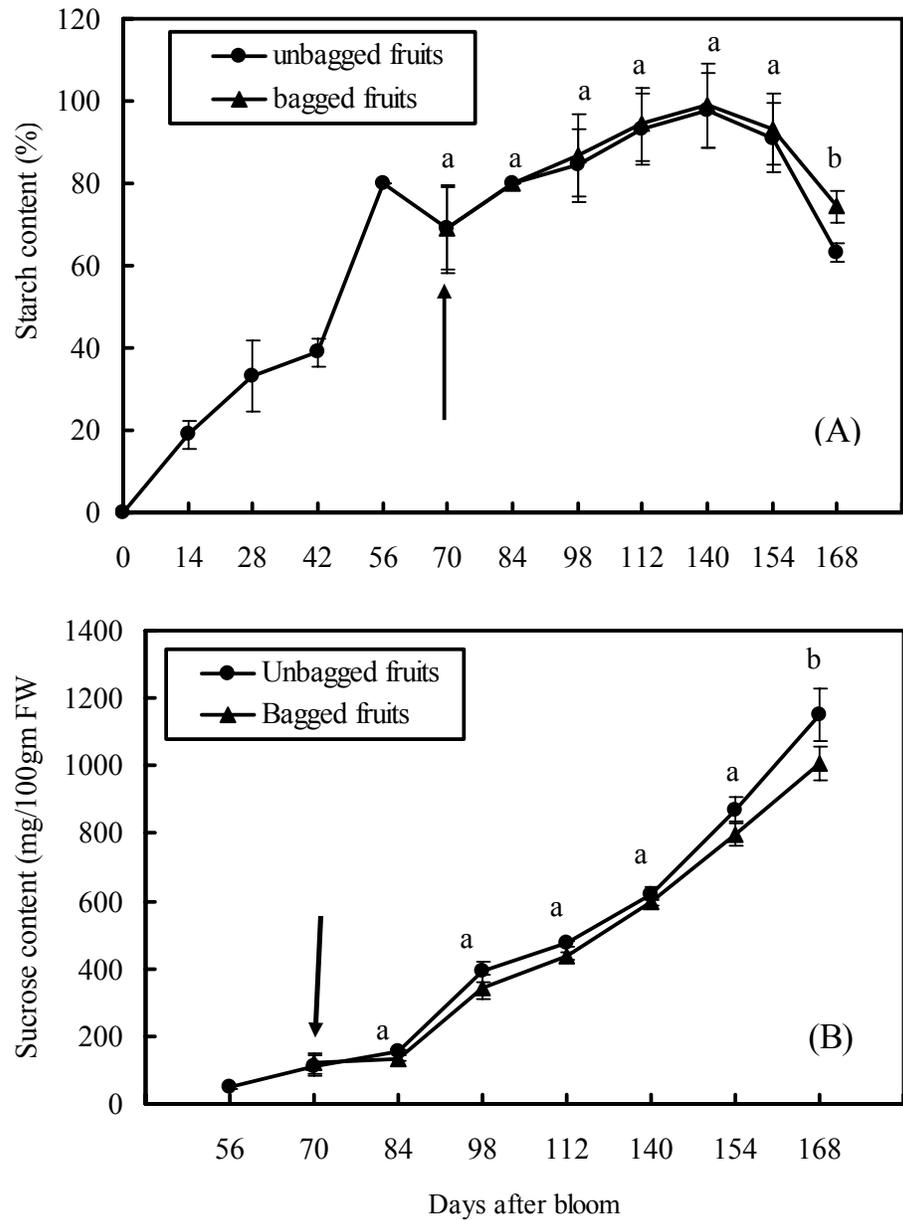


Fig. 9: Changes in starch (A) and sucrose (B) content of bagged and unbagged mango fruits during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

Sucrose content for both bagged and unbagged fruit increased steadily initially, then rapidly after 84 DAB to become the predominant sugar up to harvest. Sucrose accumulation prior to maturation of fruits is due to direct phloem unloading (Genard et al., 2007) whereas starch hydrolysis was responsible for rapid increase in sucrose during maturation (Lechaudel et al., 2005; Ueda et al., 2000; Lizada, 1993). Bagged fruits had consistently lower sucrose levels that were significant ( $p < 0.05$ ) only at harvest, possibly due to lower rate of starch hydrolysis.

Fruit glucose levels declined up to the lowest level at 84 DAB, but increased thereafter up to 168 DAB (Fig. 10 A), whereas fructose content increased continually 168 up to harvest (Fig. 10 B). The initial predominance of glucose may reflect its preferential synthesis from assimilate due to its high requirement for metabolism in the young fruit (Worell et al., 1998). The lower levels of glucose in the latter phase of fruit growth may be attributed to reduced metabolism and preferential channeling of assimilate to sucrose (Genard et al., 2007) and starch (Worrell et al., 1998). Similar trends were observed in Tommy Atkins (Madigu, 2007), Irwin (Ueda et al., 2000) and Dashehari (Kalra et al., 1995) mango fruits. The differences in fruit glucose levels were significant ( $p < 0.001$ ) during maturation and at harvest whereas fructose content was significant only at harvest.

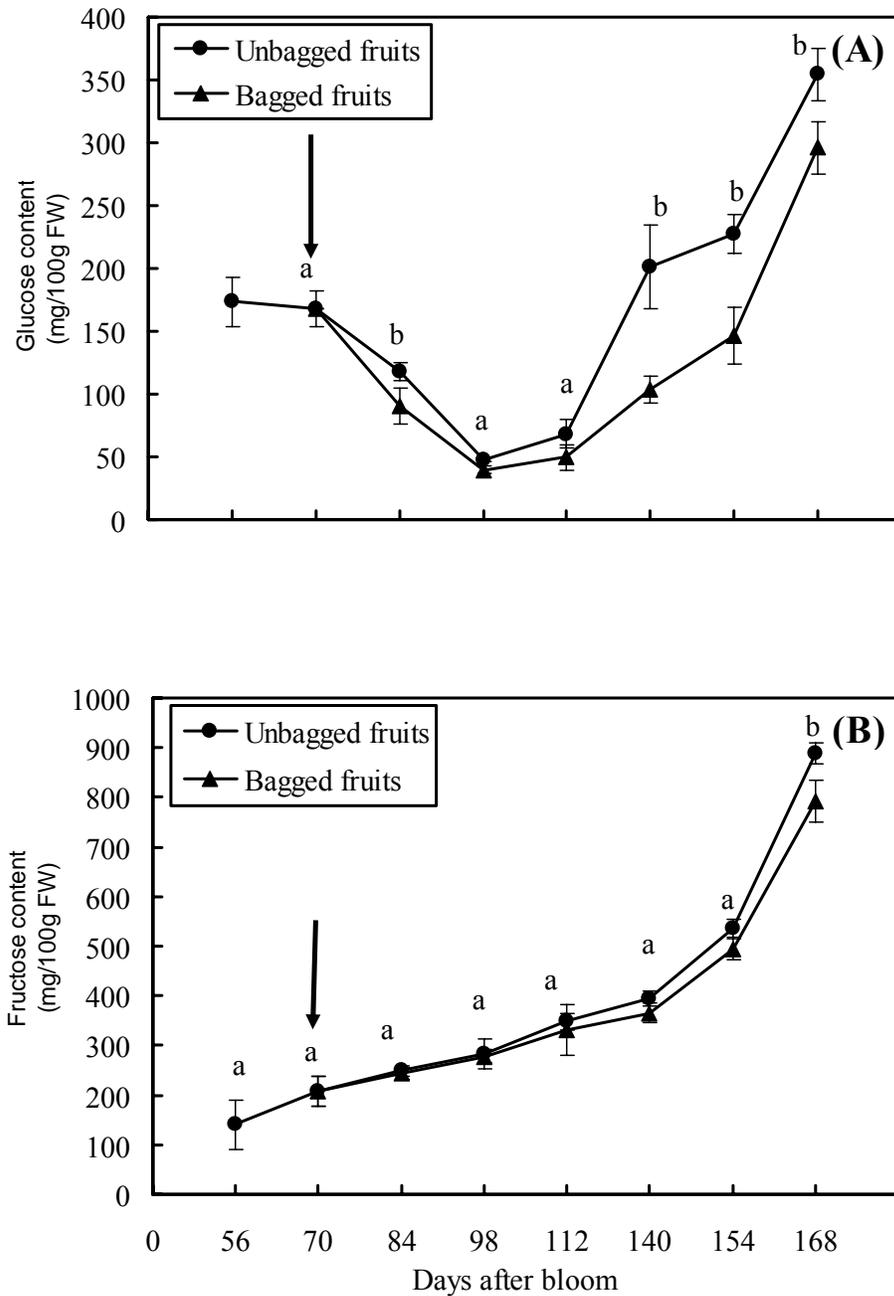


Fig. 10: Changes in glucose (A) and fructose (B) content of bagged and control mango fruits during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

The sum total of each of the three sugars at harvest were 2394.92 mg/100g FW (2.4%) and 2092.21 mg/100g FW (2.1%) for unbagged and bagged fruits, respectively. Large variations in mango fruit sugars content have been reported, probably due to varietal and climatic differences. Mature green 'Lirfa' mango fruit had a low total sugar level of about 85mg/100g FW (0.085%) (Lechaudel et al., 2005) whereas 'Chiin Hwang No. 1' (Ueda et al., 2001), Irwin (Ueda et al., 2000) and Dashehari (Kalra et al., 1995) mango fruits had far much higher levels of 6.3, 14 and 9%, respectively.

#### **4.1.3.4 Titratable acidity and total soluble solids**

Total titratable acidity (TTA) decreased gradually from 70 to 168 DAB in both bagged and control fruits (Fig. 11). This could have been due to decrease in citric acid (Lechaudel et al., 2005; Ueda et al., 2000) as it got used up as a respiratory substrate (Lizada, 1993) or transformed to other metabolites (Lobit et al., 2002). Total titratable acidity declined during maturation of 'Irwin' (Ueda et al., 2000) and 'Chiin Hwang No. 1' (Ueda et al., 2001) mango fruits, probably due to an increase in citrate lyase activity (Ueda et al., 2001). Citrate concentration increased at the beginning of peach fruit growth mainly due to its metabolism (Lobit et al., 2002). However, the concentration decreased after mid-growth due to dilution effect of increase in fruit size (Lobit et al., 2002). Citrate metabolism, fruit growth and hence citrate dilution slows during maturation (Wu et al., 2007). Therefore, decrease in citrate content during maturation may indicate degradation (Lobit et al., 2003). There was no significant difference ( $p>0.05$ ) in TTA between bagged and unbagged fruits

up to harvest. Likewise, bagging had no effect on total titratable acidity in pears (Amarante et al., 2002a) and Keitt mango fruits (Hofman et al., 1997b), but caused significant reduction of TTA in litchi fruit (Yueming et al., 2005).

There was a steady increase in total soluble solids (TSS) for both bagged and unbagged fruits, although a slight decline was observed between 84 and 98 DAB. The decline could have been caused by the dilution effect of high rainfall in the field around the time. Increased water supply reduced 'Braeburn' apple fruit sugars by dilution effect (Mills et al., 1996). Continuous increase in TSS was observed during growth of Irwin mango fruit (Ueda et al., 2000). Total soluble solids in Tommy Atkins mango fruits decreased during mid-growth, but increased prior to harvest (Madigu, 2007). Increase in TSS is due to increased accumulation of reducing sugars and partial breakdown of pectin and celluloses (Worrell et al., 1998). Bagged fruits had consistently lower TSS levels. Bagged apples had lower TSS levels (Signes et al., 2007). However, studies have shown that bagging has no effect on TSS in lychee (Tyas et al., 1998), pears (Amarante et al., 2002a) and Keitt mango (Hofman et al., 1997b).

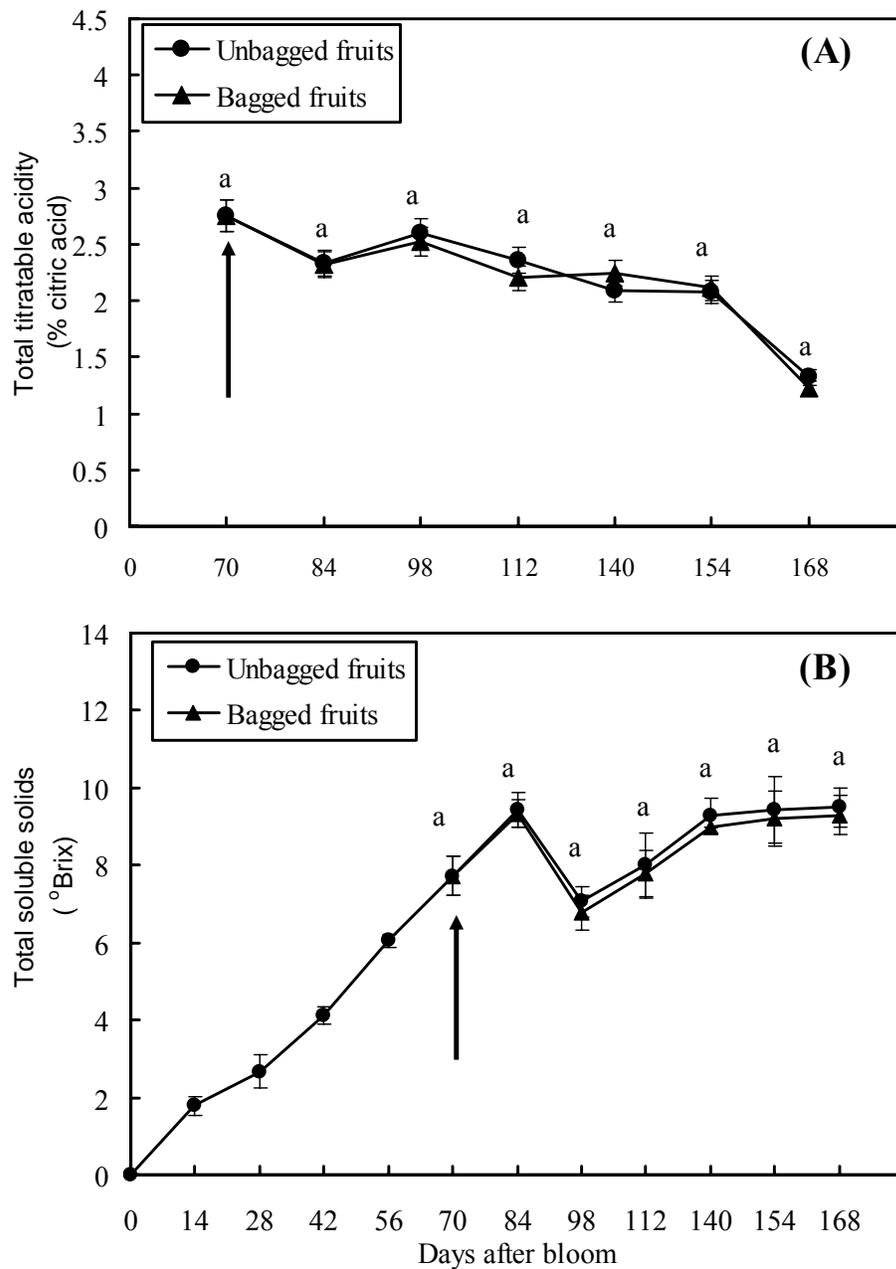


Fig. 11: Changes in total titratable acidity (A) and total soluble solids (B) of bagged and control mango fruits during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' denotes a period of no significant difference between bagged and control fruits.

#### **4.1.3.5 Fruit ascorbic acid content**

Ascorbic acid content of both bagged and control fruits decreased rapidly prior to maturation (Fig. 12). Bagged fruit had a steady decrease in ascorbic acid content during maturation whereas that of control fruit increased slightly before a final decrease after 154 DAB. Decrease in ascorbic acid content may be due to oxidative processes (Lee and Kader, 2000). Indeed, unbagged fruits that were more prone to oxidative processes due to greater exposure had more rapid decline in ascorbic contents prior to maturation and harvest. Ascorbic acid decreased in ripening apples and mangoes but increased in ripening apricots, peaches, and papayas (Lee and Kader, 2000). Bagged fruits had significantly ( $p < 0.001$ ) higher levels of ascorbic acid prior to maturation and significantly ( $p < 0.001$ ) lower levels prior to harvest. The amount and intensity of light during the growing season has a definite influence on the amount of ascorbic acid formed in fruits (Lee and Kader, 2000). Ascorbic acid is synthesized from sugars supplied through photosynthesis in plants. Outside fruit exposed to maximum sunlight contain higher amount of vitamin C than inside and shaded fruit on the same plant (Lee and Kader, 2000). In general, the higher the light intensity during growth, the higher the ascorbic acid content of plant tissues (Lee and Kader, 2000). Slight increases in ascorbic content during maturation of unbagged fruits may have been due to net accumulation resulting from increased synthesis, possibly favoured by increasing sugars accumulation and maximum light exposure.

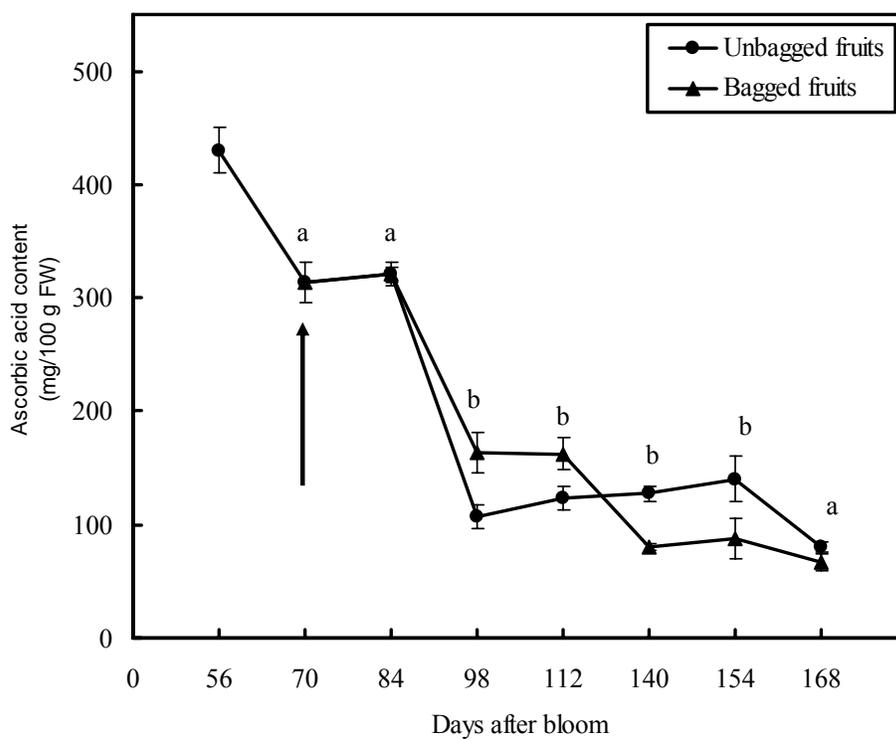


Fig. 12: Changes in ascorbic content of bagged and control mango fruits during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. 'a' and 'b' denotes a period of non-significant and significant difference, respectively between bagged and unbagged fruits ( $p=0.05$ ).

#### 4.1.3.6 Changes in selected minerals content

Calcium, magnesium, potassium and phosphorus contents of the pulp and peel for both bagged and non-bagged fruits during growth and development are shown in Table 1. Calcium content in the peel and pulp of both bagged and non-bagged fruits decreased during growth and development. However, the peel had higher calcium content than the pulp. Declining levels may be due to dilution effect of increase in fruit size (Ding et al., 1995). Calcium content of

Table 1: Mineral content in the peel and pulp during growth and development of bagged and control mango fruits.

DAB	Mineral	Mineral content (mg/100 g FW)			
		Peel		Pulp	
		Bagged <sup>a</sup>	Control	Bagged	Control
56	Calcium	ND <sup>b</sup>	11.9 ±0.10	ND	12±0.19
	Magnesium	ND	9.4±0.33	ND	8.9±1.09
	Potassium	ND	95.6±10.11	ND	89.6±9.64
	Phosphorus	ND	13.5±2.15	ND	16.7±0.11
70	Calcium	ND	10.2 ±0.29	ND	2.2±0.50
	Magnesium	ND	9.3±0.27	ND	8.3±0.50
	Potassium	ND	85.3±5.25	ND	76.5±10.5
	Phosphorus	ND	16.8±2.21	ND	18.1±0.54
84	Calcium	4.7±0.07 <sup>c</sup>	4.7 ±0.05 NS <sup>g</sup>	2.2±0.05	2.1±0.54 NS
	Magnesium	9.0±0.07	8.9±0.05 NS	8.4±0.05	8.8±0.61 NS
	Potassium	65.3±6.82	71.1±2.05 NS	80.4±6.48	75.4±6.58 NS
	Phosphorus	15.7±0.77	12.8±0.25 NS	18.0±0.87	21.9±1.51 NS
98	Calcium	5.7 ±0.07	7.7±0.03 *** <sup>f</sup>	1.3±0.05	2.2±0.52***
	Magnesium	9.0±0.05	8.4±0.03 NS	6.3±0.06	7.0±0.44NS
	Potassium	50.4±5.14	55.7±2.08 NS	264.6±15.49	289.4±6.87 * <sup>d</sup>
	Phosphorus	18.2 ±0.57	22.5±0.90 NS	23.3±0.65	23.1±1.55 NS
112	Calcium	4.4 ±0.04	4.5±0.07 NS <sup>f</sup>	1.9±0.01	2.3±0.59*
	Magnesium	14.3±0.04	11.6±0.07***	9.2±0.01	10.0±0.07 NS
	Potassium	70.6±4.04	60.2±7.07 NS	462.6±7.38	430.4±9.14***
	Phosphorus	20.9±0.89	21.0±1.16 NS	28.6±0.71	28.3±2.05 NS
140	Calcium	2.2 ±0.03	2.2±0.07 NS	1±0.01	1.2±0.01 NS
	Magnesium	14.3±0.05	11.6±0.07***	8.1±0.05	6.3±0.01** <sup>c</sup>
	Potassium	60.7±2.86	59.3±5.14 NS	110.0±8.60	125.9±9.54 NS
	Phosphorus	19.1±0.53	18.3±0.97 NS	23.5±0.51	26.6±1.74 NS
154	Calcium	2.2±0.02	2.5 ±0.03* <sup>d</sup>	1±0.02	1±0.02 NS
	Magnesium	18.7±0.02	15.5±0.03***	4.6±0.02	3.8±0.01 NS
	Potassium	59.5±2.12	69.0±4.46*	91.6±3.02	86.4±6.43 NS
	Phosphorus	17.7±1.07	20.6±1.03 NS	32.7±1.00	28.5±1.38 NS
168	Calcium	2.2±0.01	2.3 ±0.04 NS	1±0.02	1.1±0.01 NS
	Magnesium	12.2±0.02	10.3±0.04*	2.1±0.01	2.4±0.02 NS
	Potassium	65.0±7.10	63.3±5.40 NS	79.3±2.02	63.4±4.18 NS
	Phosphorus	22.2±1.41	22.4±1.04 NS	26.0±1.88	25.4±1.45 NS

<sup>a</sup>Bagging treatment was done at 70 DAB

<sup>b</sup>ND denotes not determined.

<sup>c</sup>Data values are means ± SE of nine replicates

<sup>d</sup>\* denotes significantly different, p<0.05.

<sup>e</sup>\*\* denotes significantly different, p<0.01

<sup>f</sup>\*\*\* denotes significantly different, p<0.001.

<sup>g</sup>NS denotes not significant, p >0.05.

Lirfa (Lechaudel et al., 2005) and Tommy Atkins (Madigu, 2007) mangoes decreased during fruit development. Calcium is considered an immobile nutrient that is supplied to the fruit mainly during the period of cell division and early days of growth (Salisbury and Ross, 1992). The differences in calcium contents between bagged and unbagged fruits were not significant ( $p>0.05$ ) for both peel and pulp, probably because bagging was done after the critical period of calcium supply to the fruit (Joyce et al., 1997). The calcium content of fruit is of general importance in terms of postharvest quality (Hofman, 1997). Fruit with higher calcium contents tend to have higher mechanical strength, longer shelf life, enhanced resistance to disease and lower incidence of physiological disorders (Joyce et al., 1997).

Magnesium content was higher in the peel than in pulp for both bagged and control fruits. The differences in magnesium content of the peel, but not that of the pulp, between bagged and unbagged fruits were significant ( $p<0.05$ ) during maturation. Potassium is an important component of the buffer system in the vacuole (Lechaudel et al., 2005). The potassium content of the peels decreased prior to maturation, but remained nearly constant from maturation to harvest in both bagged and unbagged fruits. Potassium content in peel and pulp was higher than magnesium and calcium contents. Lechaudel et al (2005) reported that  $K^+$  represented more than 80% of the cation pool in 'Lirfa' mango fruit flesh, followed by  $Ca^{2+}$  and  $Mg^{2+}$ . Potassium contents of bagged and unbagged fruits were significantly different ( $p<0.05$ ) only at 154 and 112 DAB for peel and pulp, respectively.

Phosphorous is an important component of many plant compounds, including DNA, cell membranes, and energy-yielding intermediates of photosynthesis and respiration. Peel and pulp phosphorous content increased prior to maturation of both bagged and control fruits, but remained nearly constant during maturation. Phosphorous content of green house cucumber increased slowly during early stages of growth but increased rapidly with increasing maturity (Ding et al., 1995). The phosphorous contents of both peel and pulp between bagged and unbagged fruits were not significant ( $p>0.05$ ).

The potassium, magnesium and phosphorous are readily re-distributed within the plant canopy (Salisbury and Ross 1992), and their fruit contents may have not been affected by bagging. Generally, bagging had no effect on Apple mango fruit mineral content. Similarly, bagging of pear fruit had no effect on minerals content (Amarante et al., 2002a). Fruit bagging is likely to offer resistance to water vapour movement, reduce the transpirational flux of water and, therefore, of minerals to developing fruit (Amarante et al., 2002a).

#### **4.1.4 Changes in fruit physiological parameters**

##### **4.1.4.1 Respiration rate**

As shown in Fig. 13, the respiration rate was very high immediately after full bloom with a value of 469.31 ml CO<sub>2</sub>/Kg/hr at 14 DAB, and then declined sharply to 133.55 ml CO<sub>2</sub>/Kg/hr at 28 DAB. The differences in respiration rate between bagged and unbagged mango fruits were not significant ( $p>0.05$ ). This compares well with studies on pears (Amarante et al., 2002a) that gave

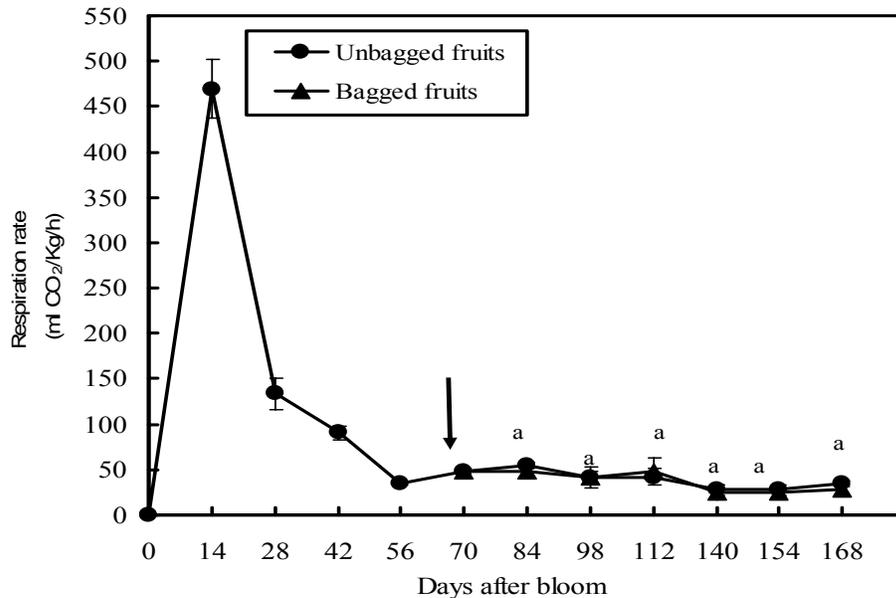


Fig. 13: Changes in respiration rate of bagged and unbagged mango fruits during growth and development. The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. The arrow indicates when bagging treatment commenced. ‘a’ denotes a period of no significant difference between bagged and unbagged fruits.

similar results. The initial high respiration rate was probably due to high cellular activity as result of cell division and differentiation. The rate declined gradually to 35.16 ml CO<sub>2</sub>/Kg/hr at 56 DAB, possibly due to endoreduplication, and then remained almost steady thereafter for both bagged and control fruits. Similar observations were found on Irwin mango fruits (Ueda et al., 2000). This implied a reduction of the high energy needs of the fruit after mitosis had stopped.

#### 4.1.4.2 Ethylene production rate

No ethylene was detected in both bagged and control fruits up to harvest. It is possible that the ethylene produced was too low for detection or production of

detectable levels was for a short period that coincided with none of the sampling days. Varying observations on ethylene production by growing mango fruit have been reported. Ethylene was not detected in Tommy Atkins mango fruits (Madigu, 2007). Carabao mangoes had detectable levels prior to full maturity but not at full maturity (Lizada and Cua, 1990). Ethylene production may decrease as mango fruit matures, become undetectable prior to maturity and reappear upon ripening (Lizada, 1993). Mango fruits produce very low levels of ethylene, though it is among the most ethylene sensitive fruits (Mitra and Baldwin, 1997).

#### **4.1.5 Sensory quality of fruits at harvest**

Sensory scores and visual appearance of bagged and unbagged fruits are shown in Fig. 14 and Plate 3, respectively. The bagged mangoes were rated significantly ( $P < 0.05$ ) superior in terms of appearance, colour and overall acceptability. The peel of bagged fruits had a smooth texture and a blemish free light green colour, and could have been the reason for its higher rating on colour and appearance by the panelists. Similar results were reported on pears (Amarante et al., 2002a). However, portions of unbagged fruit peel had a characteristic orange colouration that probably, could have resulted in higher colour and appearance scores were it not for the presence of dark brown ‘rust’ lesions.

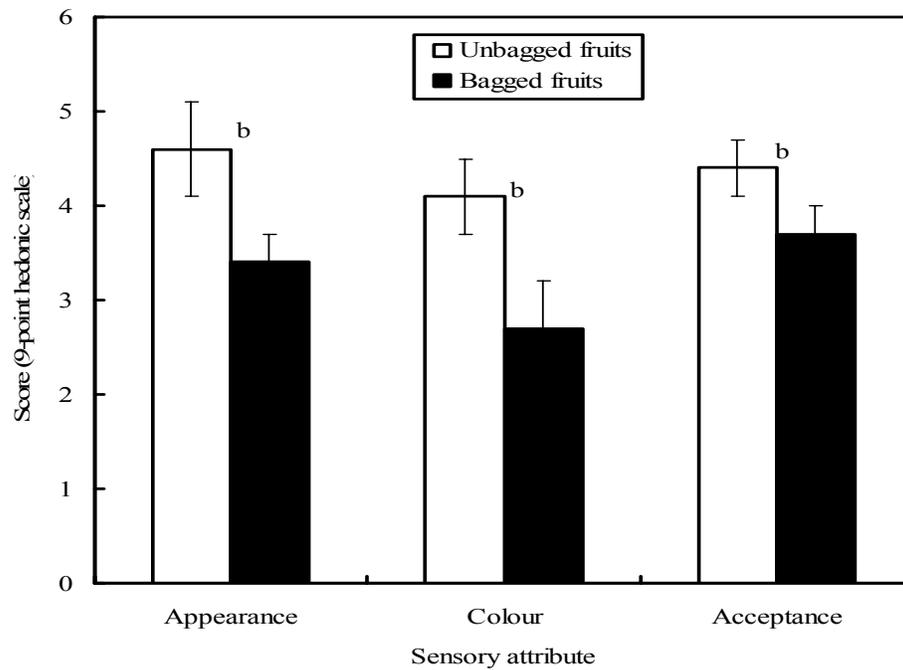


Fig. 14: Sensory quality scores on peel appearance and colour, and overall acceptance for bagged and control mango fruits at harvest. Rating was done on a 9-point hedonic scale (1-like extremely and 9-dislike extremely). The vertical bars represent SE of the mean of 15 replicates of panelists. 'b' denotes a period of significant difference ( $p=0.05$ ) between bagged and unbagged fruits.

## 4.2 Fruit Postharvest Behaviour and Response to 1-Methylcyclopropene Treatment

Ripening mango fruits exhibited profound physicochemical changes that are characteristic of climacteric fruits, though these changes occurred before the climacteric peak and peak ethylene production. Senescence of unbagged and bagged fruits started at 9 and 15 DAH, respectively (Plate 4). Therefore, bagging improved postharvest shelflife of Apple mango fruit.

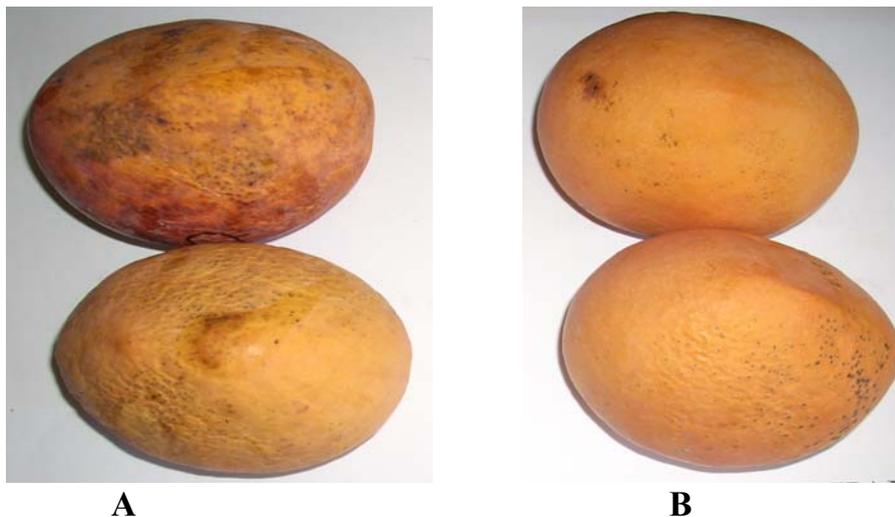


Plate 4: Visual appearance of unbagged (A) and bagged (B) fruits at 9 and 15 DAH, respectively. (Storage:  $25\pm 1^{\circ}\text{C}$  and  $60\pm 5\%$  RH. DAH = Days after harvest).

### 4.2.1 Changes in fruit physical parameters

#### 4.2.1.1 Fruit pulp and peel colour

Intense colour changes occurred immediately after harvest in all the treatments for both pulp and the peel. The peel colour changed from light green to full yellow by 8 DAH. However, bagged fruits had more uniform yellowing (Plate 5). Therefore, bagging and 1-MCP treatments did not have any visible effect



0 DAH  
Bagged fruits  
Without 1-MCP

0 DAH  
Bagged fruits  
with 1-MCP



4 DAH  
Bagged fruit  
without 1-MCP

4 DAB  
Bagged fruit  
with 1-MCP



6 DAH  
Bagged fruits  
Without 1-MCP

6 DAH  
Bagged fruits  
with 1-MCP



8 DAH  
Bagged fruits  
without 1-MCP

8DAH  
Bagged fruits  
with 1-MCP

Plate 5: Visible colour changes of bagged mango fruits, with and without postharvest 1-MCP (20 ppm) treatment and storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . DAH = Days after harvest.

on fruit degreening. Nevertheless, 1-MCP treated fruit had a more uniform ripening. However, 1-MCP retards degreening in guavas (Azzolini et al., 2005), bananas (Jiang et al., 1999) and sapodilla (Quiping et al., 2006) fruits.

Pulp L values declined rapidly after harvest in all the treatments, then followed by inconsistent changes from 3 DAH (Fig. 15 A). Therefore, both bagging and 1-MCP did not have clear effects on the changes in pulp L value during ripening of Apple mango fruit. However, Azzolini et al., (2005) reported less intense pulp colour and retarded degreening in ‘Pedro Sato’ guava fruits treated with 1-MCP (300 nl/L). The fruit peel brightness (L\* values) changed slightly in all treatments during storage (Fig. 15 B). The peel L value of ‘Chiin Hwang No. 1’ mango fruit remained nearly constant during ripening (Ueda et al., 2001). However, bagged fruits with and without 1-MCP treatment maintained their significantly ( $p < 0.05$ ) higher values, except at 9 and 12 DAH.

Pulp and peel hue angle decreased with storage time in all the treatments (Fig. 16), and colour changed from whitish yellow to intense yellow, indicating increased synthesis and accumulation of beta-carotene (Azzolini et al., 2005). There were no significant differences ( $p > 0.05$ ) in pulp hue angle values between all the treatments.

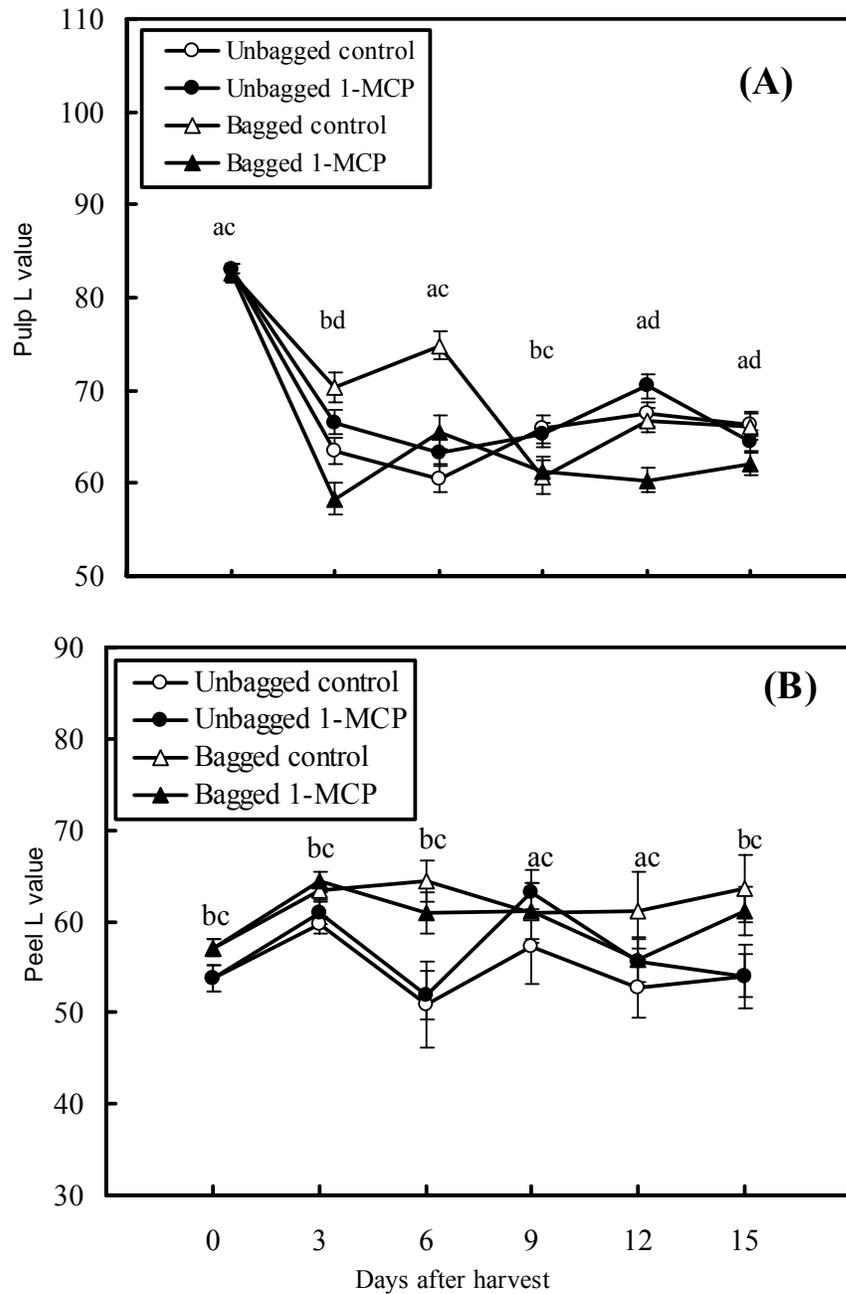


Fig. 15: Changes in pulp (A) and peel (B) L values of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. ‘a’ and ‘c’ denotes a period of no significant difference for bagging and 1-MCP treatments, respectively, while ‘b’ and ‘d’ denotes a period of significant difference for bagging and 1-MCP treatments, respectively .

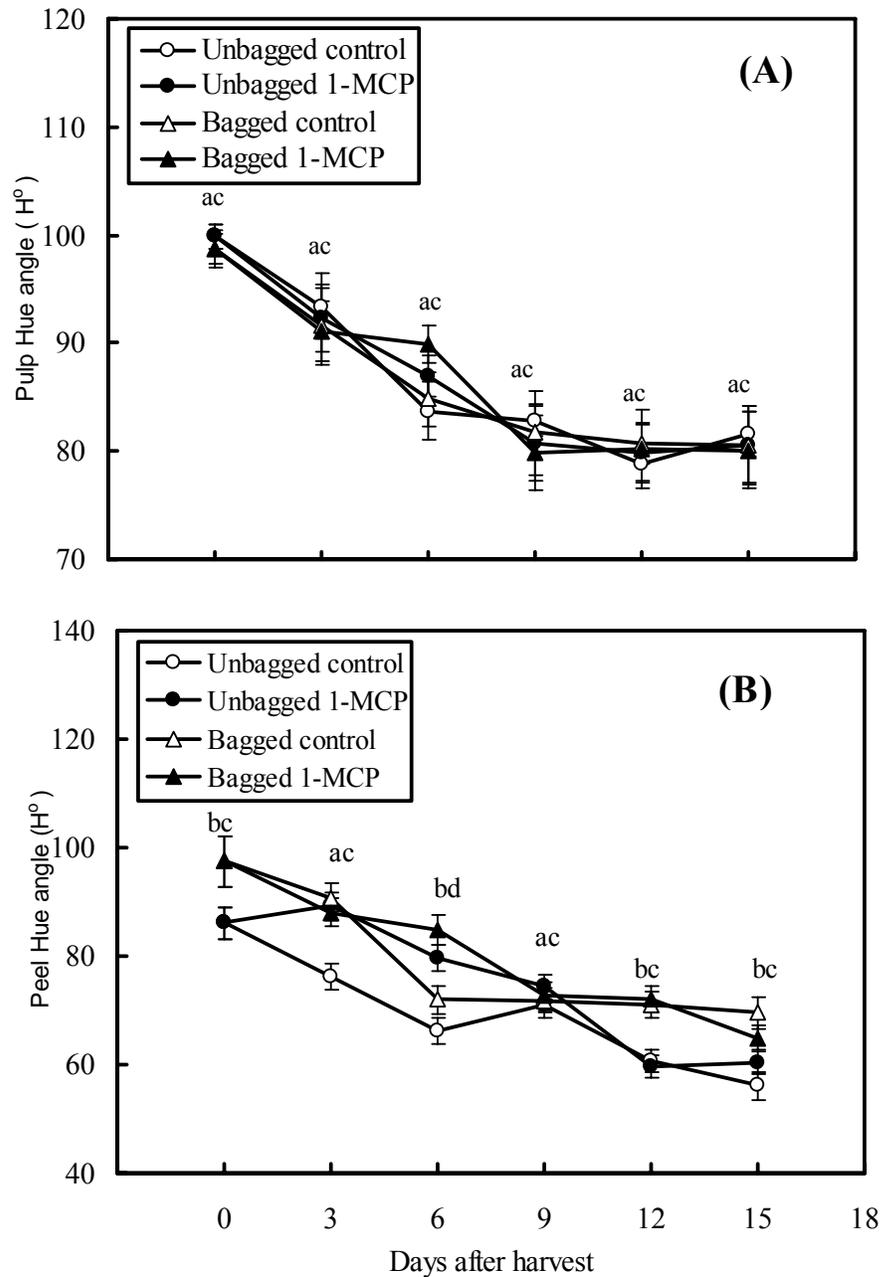


Fig. 16: Changes in pulp (A) and peel (B) hue angle of both bagged and unbagged mango fruit, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

#### **4.2.1.2 Fruit firmness**

There was a rapid decrease in fruit firmness immediately after harvest, but with significantly ( $p < 0.05$ ) more decrease in both bagged and unbagged fruits without 1-MCP treatment (Fig. 17). Firmness of the fruits decreased gradually thereafter. Fruits in all the treatments reached eating softness on 6 DAH. Decrease in fruit firmness is due to the action of cell wall hydrolases (Lohani et al., 2004) and expansins (Sane et al., 2005). Intense loss of firmness indicated high sensitivity of fruit softening to ethylene and limited inhibition of this loss by 1-MCP.

Bagging and 1-MCP treatment significantly ( $p < 0.05$ ) reduced fruit softening up to 3 DAH, but not thereafter. Failure by 1-MCP to inhibit firmness loss after 3 DAH could be attributed to rapid regeneration of more ethylene receptors. Plotto et al. (2003) reported inhibition of firmness loss in 1-MCP treated Tommy Atkins but not in Kent mango fruits, and suggested that this may be attributed to a variety difference or that mangoes were subjected to treatment at an advanced stage of maturity. 1-MCP reduced softening of Dashehari mango (Sane et al., 2005), peach (Mathooko et al., 2001) and bananas (Lohani et al., 2004) during the first 2-3 days of storage, but not thereafter. Bagging had an effect on fruit response to 1-MCP at 3 DAH. The 1-MCP treated unbagged fruits had significantly ( $p < 0.001$ ) higher firmness than 1-MCP treated bagged fruits.

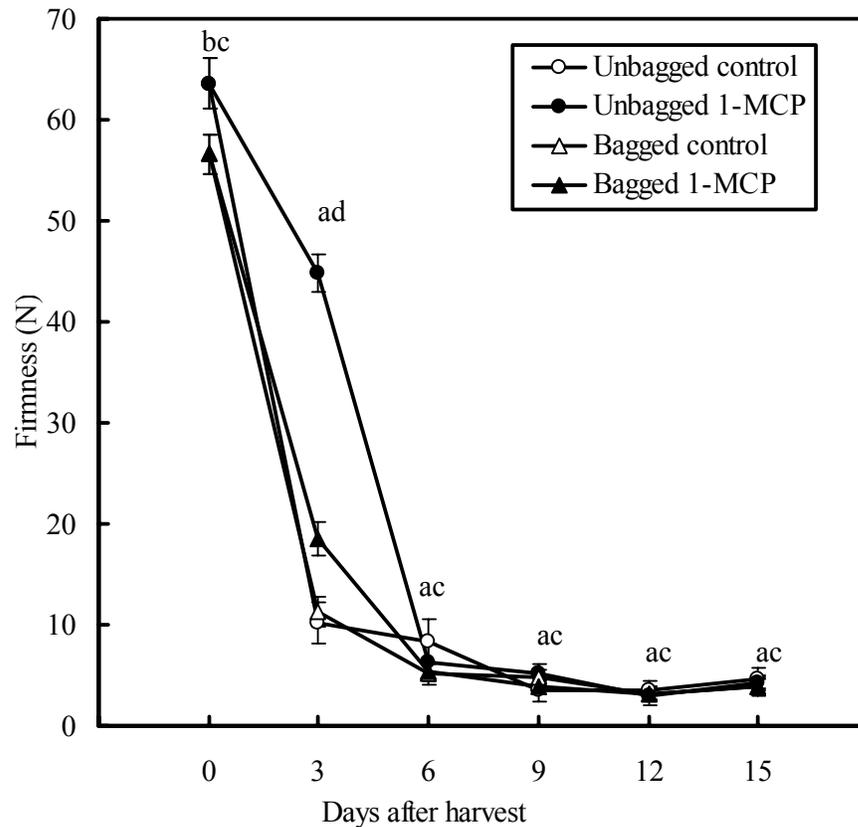


Fig. 17: Changes in firmness of both bagged and unbagged fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

Unbagged fruits could have had better skin penetration by 1-MCP. Bagged pear fruits stored at  $20^{\circ}\text{C}$  also had a larger decrease in firmness (Amarante et al., 2002a). There was no correlation between firmness and weight loss. Thus, fruit softening was due to cell wall disassembly and not loss of turgor pressure, a view expressed by Lizada (1993).

## **4.2.2 Fruit chemical changes**

### **4.2.2.1 Fruits starch and sucrose content**

There was a rapid decrease in starch content and a rapid increase in sucrose content in all the treatments during the first nine days of storage (Fig. 18). Starch content reduced to zero percent at 9 and 12 DAH for unbagged and bagged fruits, respectively. This decrease in starch content is due to its hydrolysis by amylases leading to complete disappearance of starch granules (Lizada, 1993). The decrease in fruit percent starch was matched by a rapid increase in sucrose content, which was the predominant sugar.

Sucrose contributed 57% of total sugars in ripe 'Keitt' mangoes, with fructose and glucose making up 28 and 15%, respectively (Medlicott and Thompson, 1985). The sucrose content of Haden, Irwin, Kent and Keitt mango fruits increased spectacularly during ripening, and contributed to most to the increase in sweetness (Vazquez-salinas and Lakshminarayana, 1985). Sucrose metabolism during mango ripening is accompanied by increase in sucrose synthetase and invertase activities (Lima et al., 1999). The difference in percentage starch and sucrose contents between bagged and unbagged fruits, and between 1-MCP treated and untreated fruits were not significant ( $p < 0.05$ ) during storage. However, 1-MCP slowed starch hydrolysis in Tommy Atkins mango fruit (Plotto et al., 2003) and Sapodilla fruits (Quipping et al., 2006).

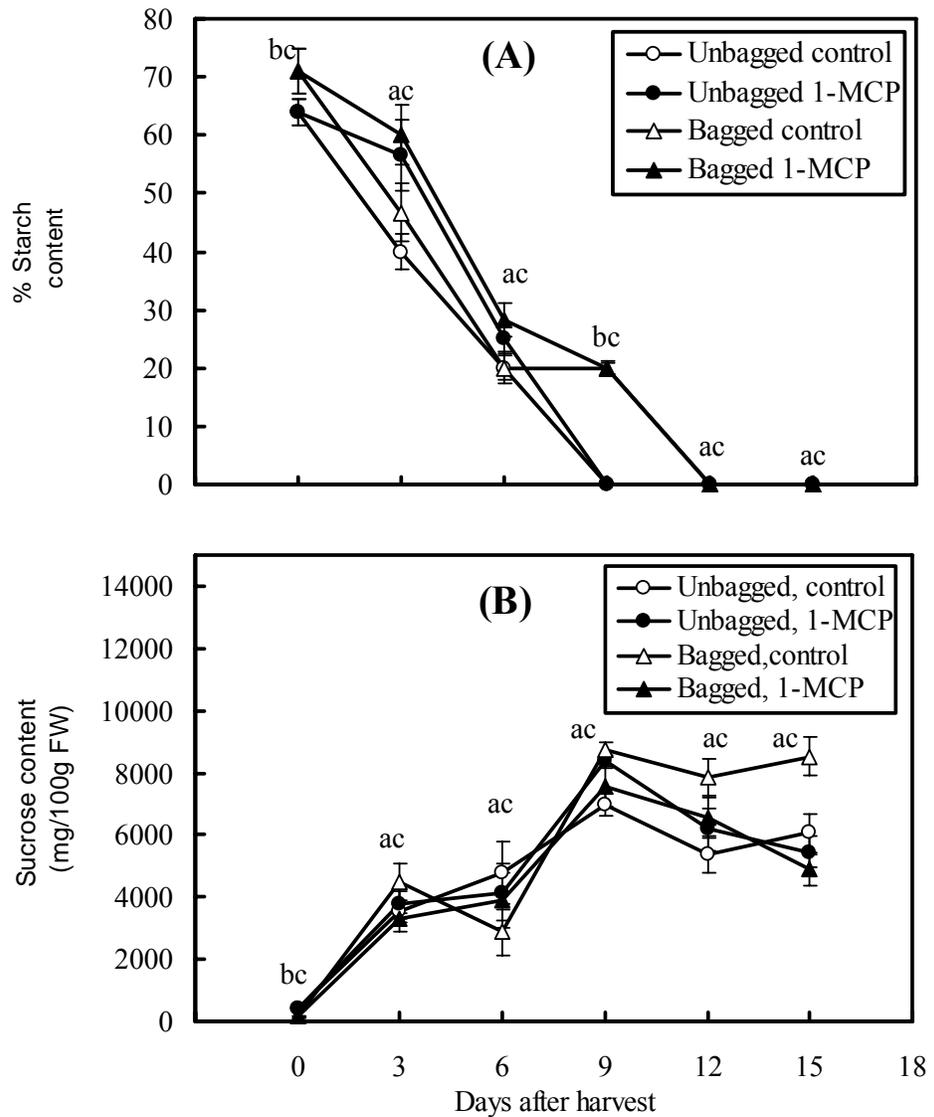


Fig. 18: Changes in starch (A) and sucrose (B) content of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

#### **4.2.2.2 Fruit glucose and fructose contents**

Glucose and fructose contents increased with increase in DAH in all the treatments, but decreased during senescence (12 and 15 DAH) (Fig.19). Depending on cultivar, glucose and fructose contents may increase simultaneously with the increase in sucrose content or may have a gradual decline during mango fruit ripening (Mitra and Baldwin, 1997). ‘Chiin Hwang No. 1’ mango fruit sucrose, fructose and glucose contents increased on ripening (Ueda et al., 2001). The differences in fruit sugars content between bagged and unbagged fruits, and also between 1-MCP treated and untreated fruits were not significant ( $p < 0.05$ ) during ripening.

The postharvest fruit sugars content levels (Fig. 18 B and 19) were higher than the preharvest levels (Fig 8 B and 9), with the four treatments reaching levels of between 11.0 and 12.5% at 9 DAH. Higher sugar levels have been reported in ripe Amrapadi, Mallika and Bishawanath mango fruits (Hossain et al., 2001) with 26.85, 23.13 and 18.70% total sugars, respectively. The average of total free sugar content in ‘Chiin Hwang No. 1’ mango fruit (Ueda et al., 2001) was about 6.3% at harvest and 16.5% on ripening. The big variations could be attributed to varietal difference. Signes et al. (2007) also reported such high total sugar levels in Black Table grapes, with 17.94 and 18.54% in bagged and unbagged fruits, respectively.

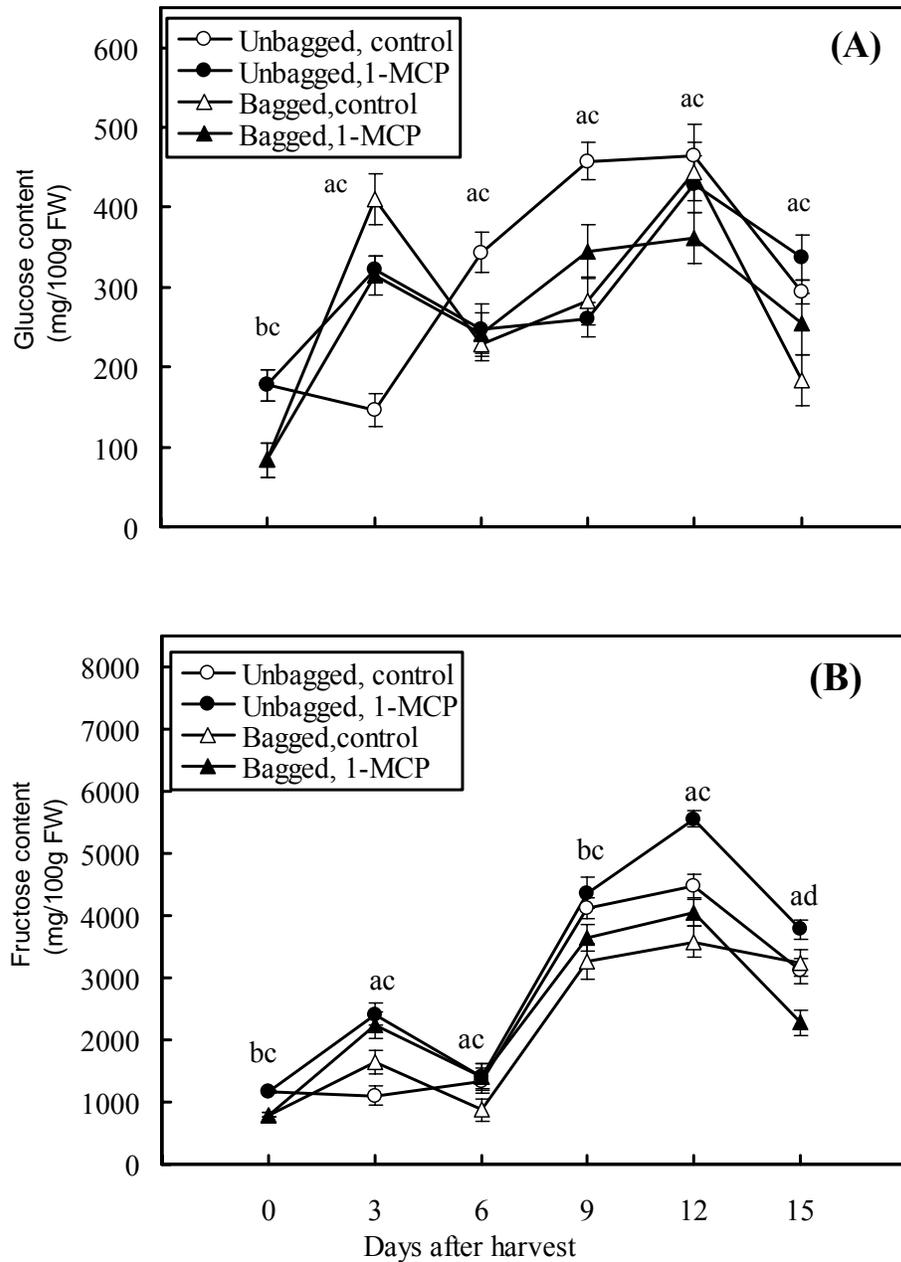


Fig. 19: Changes in glucose (A) and fructose (B) content of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

#### **4.2.2.3 Total titratable acidity and total soluble solids content**

The percent TTA for all the treatments decreased rapidly up to 6 DAH, then gradually thereafter (Fig. 20 A). Total soluble solids content increased up to 12 DAH in all the treatments (Fig. 20 B). A rapid decrease in TSS content was observed at 12 DAH except for the 1-MCP treated bagged fruits. Increase in TSS in mango fruit is due to breakdown of complex carbohydrates into simple sugars (Saranwong et al., 2003) and solubilisation of cell wall pectins (Rathore et al., 2007). Bagging and 1-MCP treatment did not have any effect on fruits TSS during ripening. Bagging did not affect TSS content of Keitt mango fruit (Hofman et al., 1997b) but 1-MCP treated Tommy Atkin mango fruits showed lower TSS due to reduced starch degradation (Plotto et al., 2003).

Mangoes exhibit a continuous decrease in acidity during ripening (Lizada, 1993) due citric acid, the predominant acid being used as respiratory substrate (Rathore et al., 2007). Effect of preharvest bagging on total titratable acidity during mango fruit ripening was not significant ( $p>0.05$ ). Similarly, bagging lychee fruit (Tyas et al., 1998) did no affect TTA during ripening. Bagged and unbagged fruits treated with 1-MCP had significantly ( $p<0.05$ ) higher TTA compared to fruits not treated with 1-MCP (Fig. 19 A). 1-MCP treatment prevented ethylene-induced acidity loss in sapodilla (Quipping et al., 2006), tomatoes (Wills and Ku, 2001) and plums (Dong et al., 2002). However, Dong et al. (2002) found that application of 1-MCP did not affect the content of TTA in apricot. Effects of 1-MCP on TTA may depend on fruit cultivar and storage conditions (Blankenship and Dole, 2003).

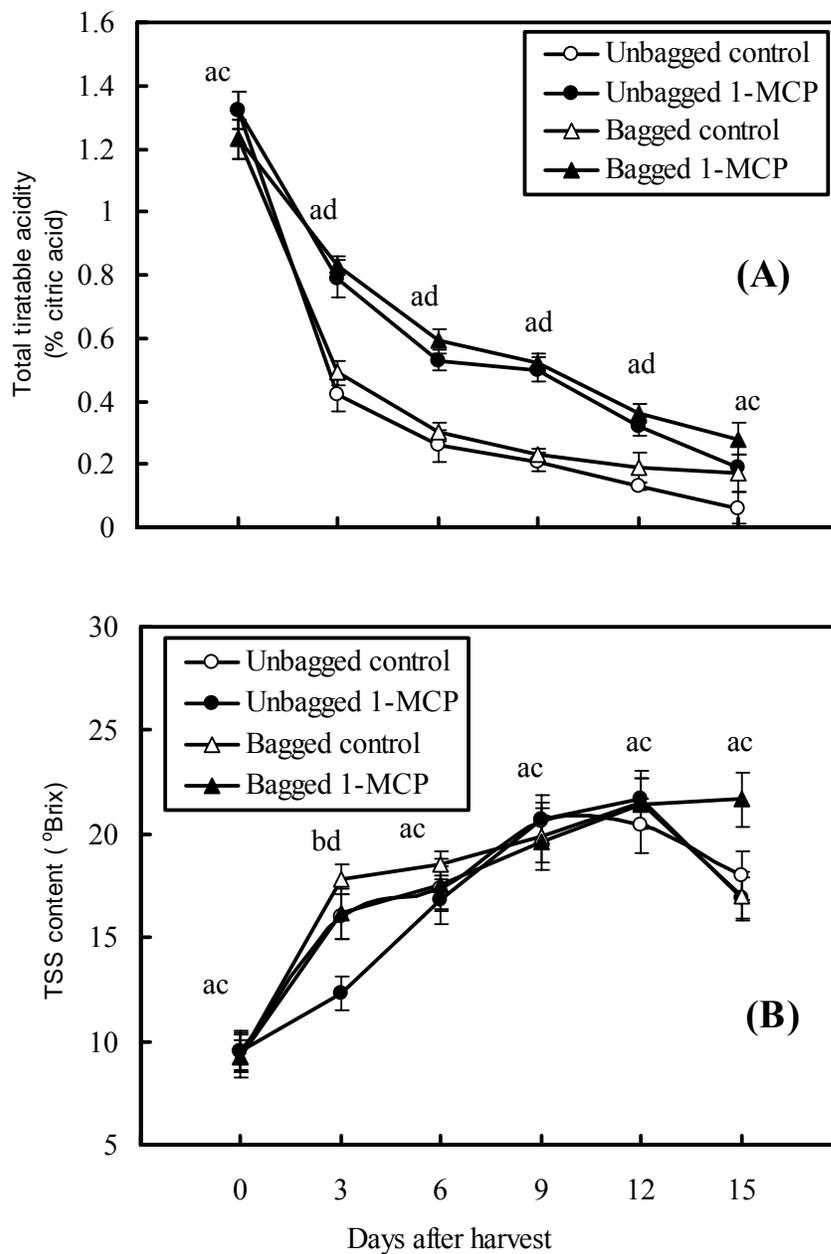


Fig. 20: Changes in total titratable acidity (A) and total soluble solids (B) content of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

#### **4.2.2.4 Chlorophyll content of fruit peel**

The chlorophylls a and b (Fig. 21 A and B) and total chlorophyll (Fig. 22) contents of fruit peel decreased with storage time in all the treatments. The decrease in chlorophyll content of ripening fruits is due to physico-chemical degradation of chloroplasts in which the granal membrane is lost (Lizada, 1993). A rapid loss of chlorophylls was observed between 3 and 6 DAH for chlorophyll a and during the first 3 DAH for chlorophyll b. Bagging and 1-MCP treatment had no significant ( $p>0.05$ ) effect on degradation of chlorophyll a. However, their effects on chlorophyll b were significant ( $p<0.05$ ), with bagged and 1-MCP-treated fruits having higher contents up to 9 and 6 DAH, respectively. Bagging and 1-MCP treatment resulted in significantly ( $p<0.05$ ) higher total chlorophyll content up to 6 DAH (Fig. 21). However, these differences were not expressed physically as there were no visual differences in the yellowing process in all the treatments (Plate 5). Contrary to these results, 1-MCP was found to be an excellent inhibitor of degreening in bananas (Jiang et al., 1999; Li et al., 1997), sapodilla (Quiping et al., 2006), avocado (Herskovitz et al., 2005; Jeong et al., 2003) and tomatoes (Mostofi et al., 2003).

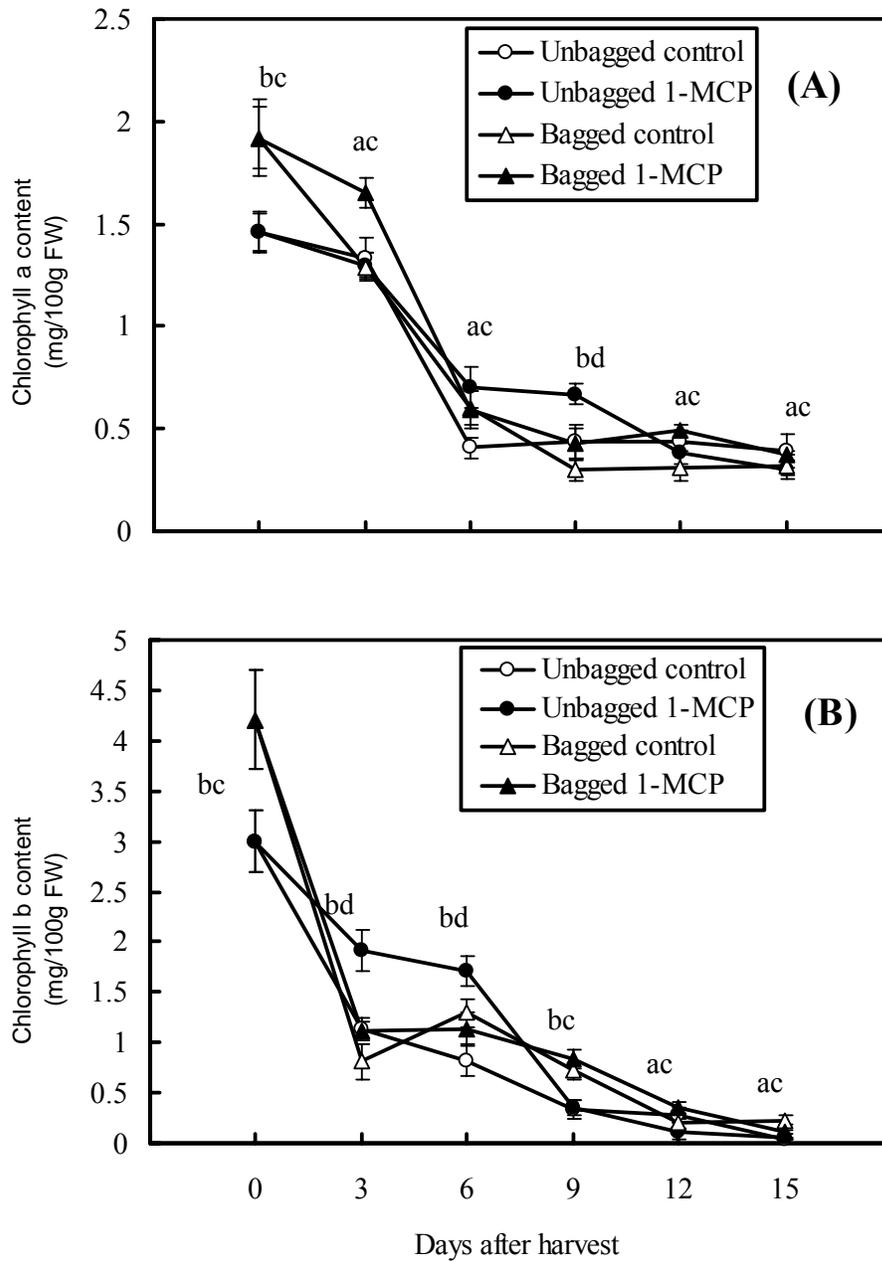


Fig. 21: Changes in chlorophyll a (A) and b (B) content of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

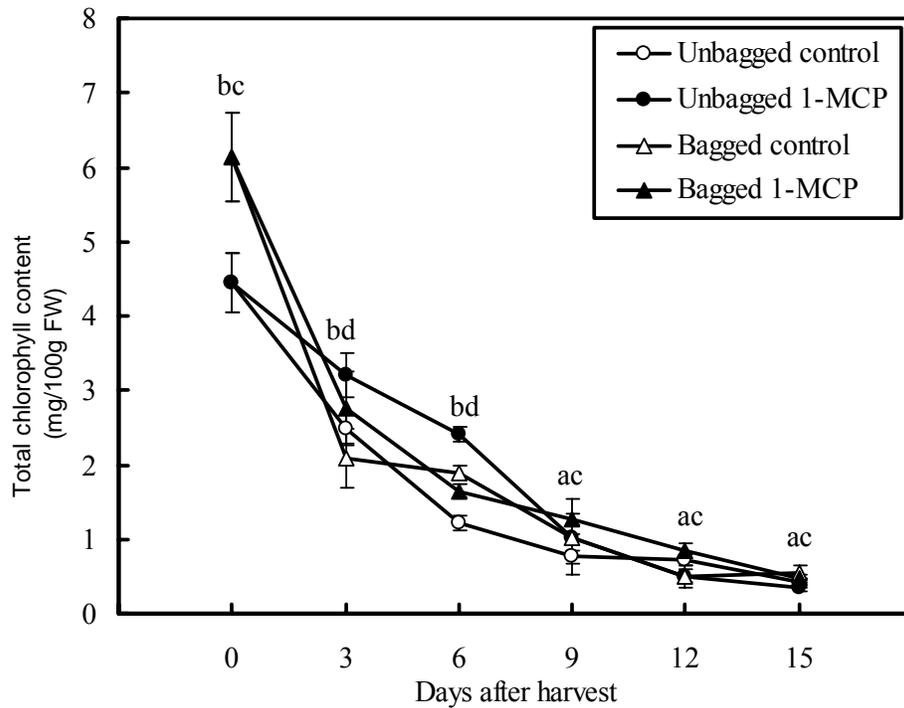


Fig. 22: Changes in total chlorophyll content of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ ,  $\text{RH } 60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

#### 4.2.2.5 Fruit $\beta$ -carotene and anthocyanins content

The  $\beta$ -carotene content of the fruit pulp increased, whereas anthocyanin content decreased with increase in storage time for all the treatments (Fig. 23). Increase in  $\beta$ -carotene content was reported in ripening of Dosehari (Rathore et al., 2007) and Tommy Atkins (Madigu, 2007) mangoes. The decrease in  $\beta$ -carotene content after 12 DAH could have been due to oxidation.  $\beta$ -carotene is highly unsaturated and susceptible to oxidation (Rodriguez-Amaya, 1999) when exposed to light.

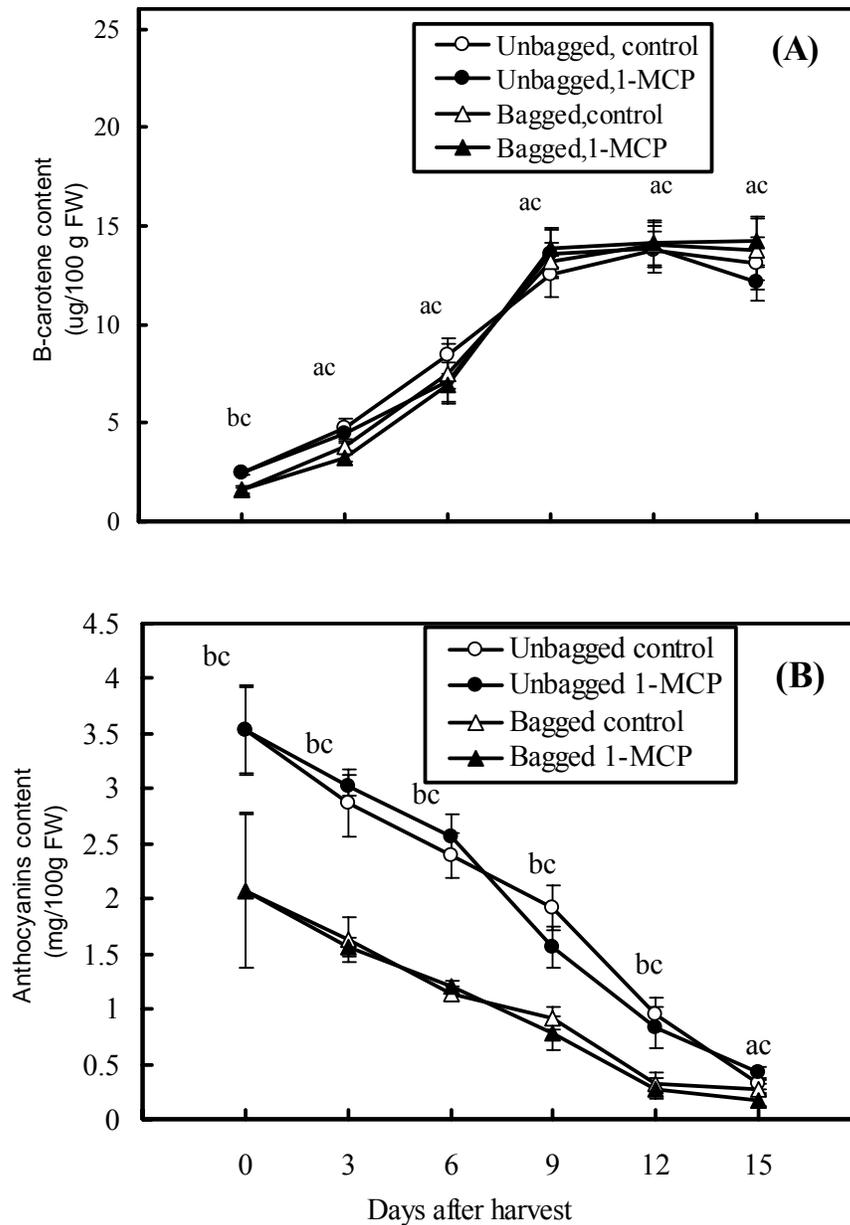


Fig. 23: Changes in  $\beta$ -carotene (A) and anthocyanins (B) content of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25 \pm 1^\circ\text{C}$ , RH  $60 \pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively whereas 'b' denotes a period of significant difference for bagging treatment.

There was no significant difference ( $p>0.05$ ) in  $\beta$ -carotene content between bagged and unbagged fruits, and also between fruits with and without 1-MCP treatment. Similar observations were made on 1-MCP 'Pedro Sato' guavas and attributed to lycopene biosynthesis being independent of ethylene production (Azzolini et al., 2005). In many fruits additional  $\beta$ -carotene and lycopene is synthesized during ripening. The pathway for  $\beta$ -carotene biosynthesis shows lycopene as the precursor of  $\beta$ -carotene (Lizada, 1993). Therefore,  $\beta$ -carotene biosynthesis in mango fruit in this study could also have been independent of ethylene and 1-MCP.

Anthocyanins content of the fruit peel decreased with increase in storage time in all the four treatments. Decrease in peel anthocyanin content during storage was also reported in Tommy Atkins mango fruit (Madigu, 2007). Unbagged fruits had significantly ( $p<0.05$ ) higher anthocyanin content at harvest and maintained the relatively higher contents throughout storage period. Treatment with 1-MCP had no significant ( $p>0.05$ ) effect on anthocyanin contents of the peel.

#### **4.2.2.6 Fruit ascorbic content**

Fruit ascorbic decreased with increase in storage time for all the treatments (Fig. 24), as also reported in Haden, Irwin and Keitt (Vazquez-Salinas and Lakshminarayana, 1985) and Tommy Atkins (Madigu, 2007) mango fruits. This decrease could have been due to oxidative degradation. Treatment with 1-MCP retarded degradation of ascorbic acid in both bagged and unbagged

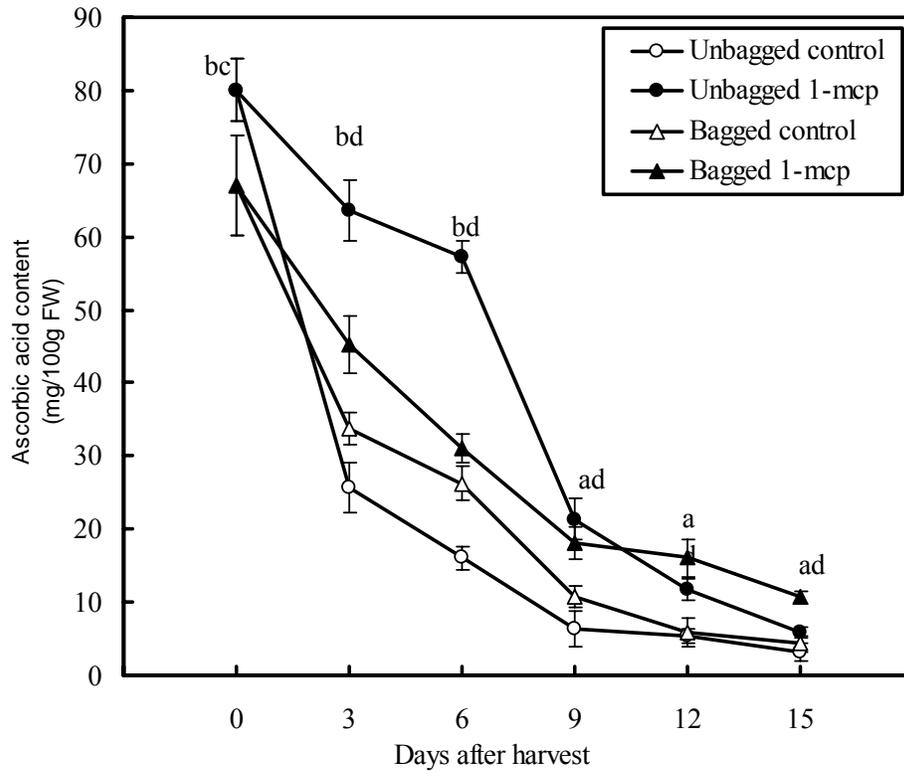


Fig. 24: Changes in ascorbic acid content of both bagged and unbagged mango fruits, each with and without 1-MCP treatment during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. ‘a’ and ‘c’ denotes a period of no significant difference for bagging and 1-MCP treatments respectively. ‘b’ and ‘d’ denotes a period of significant difference for bagging and 1-MCP treatments respectively.

fruits. Similar observations were made on sapodilla fruits (Quiping et al., 2006). However, unbagged fruits had significantly ( $p < 0.05$ ) higher retention of ascorbic acid when treated with 1-MCP. Bagging had a significant effect ( $p < 0.05$ ) on mango fruits ascorbic acid content during ripening. Unbagged fruits without 1-MCP treatment had significantly ( $p < 0.05$ ) lower ascorbic acid content up to 9 DAH. Higher rates of transpirational water loss and respiration

could have exposed the unbagged fruits to higher rates of oxidative processes leading to higher loss of ascorbic acid. Ascorbate oxidase has been proposed to be the major enzyme responsible for enzymatic degradation of ascorbic acid (Lee and Kader, 2000). Adverse physical, pathological and chemical stress increase ascorbate oxidase leading to increased degradation of ascorbic acid (Loewus and Loewus, 1987). Ascorbic acid is more stable in acidic conditions (Nagy, 1980) and fruits with higher titratable acidity will have slower ascorbic acid degradation. Treatment with 1-MCP caused fruits to have higher acidity, and may have been the reason of their higher ascorbic acid content.

#### **4.2.2.7 Changes in fruit minerals content**

Changes in calcium, magnesium, potassium and phosphorous fruit pulp and peel contents during postharvest storage are shown in tables 2 and 3. There were no significant changes in fruit peel and pulp calcium contents with increase in DAH. Bagging and 1-MCP treatments did not have significant effects on fruit peel and pulp calcium contents. The calcium content of plantain showed small and inconsistent increases during ripening (Ahenkora et al., 1996). Fruit peel and pulp magnesium content increased slightly during ripening, and bagging and 1-MCP treatments did not have a significant effect on its fruit levels. Changes in potassium and phosphorous fruit peel and pulp content due to ripening were insignificant and the trends were inconsistent. Potassium content of false horn plantain decreased by 1% with ripening, though significant but inconsistent increases were observed for iron, copper and zinc (Ahenkora et al., 1996).

Table 2: Calcium and Magnesium content in the peel and pulp of both bagged and unbagged fruits, each with and without 1-MCP during postharvest storage at 25±1°C, RH 60±5%.

Calcium and Magnesium content (mg/100 g FW)											
DAB	Mineral	Peel				Pulp					
		Unbagged Control	Unbagged 1-MCP	Bagged <sup>a</sup> control	Bagged 1-MCP <sup>b</sup>	Unbagged Control	Unbagged 1-MCP	Bagged control	Bagged MCP		
0	Calcium	2.3±0.04 <sup>c</sup>	2.3±0.04	2.2± 0.01	2.2±0.01	ac	1.1±0.01	1.1± 0.01	1.0±0.02	1.0±0.02	ac
	Magnesium	10.3±0.04 <sup>c</sup>	10.3±0.04	12.2± 0.02	12.2±0.02	bc	2.4±0.02	2.4± 0.02	2.1±0.01	2.1±0.01	ac
3	Calcium	2.3±0.01	2.3±0.01	2.3±0.01	2.3±0.01	ac	2.3±0.01	2.2 ±0.01	2.2±0.01	2.1±0.02	ac
	Magnesium	11.8±0.53	12.0±0.36	11.7±0.17	12.5±0.14	ac	2.5±0.01	2.5±0.02	2.5±0.03	2.4±0.28	ac
6	Calcium	2.4±0.01	2.4±0.01	2.4±0.01	2.4±0.01	ac	2.3±0.02	2.2±0.01	2.2±0.01	2.2±0.01	ac
	Magnesium	13.6±0.19	13.6±0.41	13.2±0.66	13.3±0.71	ac	2.7±0.02	2.7±0.01	2.6±0.01	2.5±0.06	bc
9	Calcium	2.3±0.02	2.3±0.03	2.2±0.01	2.3±0.03	ac	2.3±0.01	2.2±0.01	2.1±0.01	2.3±0.02	ac
	Magnesium	15.3±0.28	14.3±0.52	15.2±0.09	15.3±0.55	ac	2.8±0.01	2.6±0.03	2.5±0.01	2.5±0.02	bc
12	Calcium	2.3±0.04	2.4±0.05	2.3±0.01	2.2±0.02	ac	2.2±0.01	2.3±0.01	2.3±0.01	2.2±0.01	ac
	Magnesium	14.9±0.44	15.5±0.39	15.4±0.92	16.0±0.26	ac	2.7±0.16	2.7±0.01	2.7±0.01	2.7±0.02	ac
15	Calcium	2.4± 0.02	2.4±0.01	2.4±0.03	2.4±0.02	ac	2.4±0.01	2.4±0.01	2.2±0.01	2.3±0.01	ac
	Magnesium	15.9± 0.24	15.9±0.71	15.9±0.37	15.9±0.28	ac	2.7±0.01	2.7±0.05	2.6±0.08	2.7±0.04	ac

<sup>a</sup>Bagging treatment was done at 70 DAB.

<sup>b</sup>1-MCP treatment at 20 ppm was done immediately after harvest.

<sup>c</sup>Data values are means ±SE of six replicates.

a and c denotes not significantly different at p≤0.05 for bagging and 1-MCP treatments respectively while b denotes significantly different at p≤0.05 for bagging treatment.

Table 3: Potassium and phosphorous content in the peel and pulp of both bagged and unbagged fruits, each with and without 1-MCP during postharvest storage at 25±1°C, RH 60±5%.

		Potassium and phosphorous content (mg/100 g FW)									
DAB	Mineral	Peel				Pulp					
		Unbagged Control	Unbagged 1-MCP	Bagged <sup>a</sup> control	Bagged 1-MCP <sup>b</sup>	Unbagged Control	Unbagged 1-MCP	Bagged control	Bagged 1-MCP		
0	Potassium	63.3±5.40 <sup>c</sup>	63.3±5.40	65.0±7.10	65.0±7.10	ac	63.4±4.18	63.4±4.18	79.3±2.02	79.3±2.02	bc
	Phosphorous	22.4±1.04 <sup>c</sup>	22.4±1.04	22.2±1.41	22.2±1.41	ac	25.4±1.45	25.4±1.45	26.0±1.88	26.0±1.88	ac
3	Potassium	70.7±3.01	71.9±2.50	69.1±1.01	70.0±3.14	ac	92.1±5.55	93.1 ±4.71	90.6±10.32	91.6±3.02	ac
	Phosphorous	24.7±2.25	25.3±0.99	23.5±2.24	25.1±2.01	ac	29.7±0.91	29.3±2.58	27.4±2.01	28.0±2.02	ac
6	Potassium	80.9±4.08	78.2±1.21	76.0±3.51	75.3±4.09	ac	85.7±6.58	87.4±3.11	87.6±3.33	90.3±6.94	ac
	Phosphorous	27.4±1.01	26.7±2.58	30.5±1.17	31.0±1.81	ac	28.7±4.60	32.9±2.01	36.4±3.21	37.1±2.14	ac
9	Potassium	84.8±3.51	84.8±2.47	85.7±1.58	85.7±6.21	ac	94.0±6.21	93.0±8.26	93.7±5.31	90.7±8.87	ac
	Phosphorous	37.1±3.11	31.7±3.03	32.9±2.24	32.1±1.73	ac	35.3±3.31	31.5±2.01	30.6±3.21	32.8±3.25	ac
12	Potassium	83.0±4.44	83.0±5.05	82.7±8.28	82.2±2.92	ac	96.2±6.39	93.6±6.01	96.5±8.01	96.6±6.80	ac
	Phosphorous	26.4±1.40	27.4±1.50	25.5±1.14	28.6±2.22	ac	33.9±2.61	35.4±1.01	35.8±2.01	35.5±2.01	ac
15	Potassium	79.1± 4.73	80.9±1.51	78.2±5.68	78.2±6.62	ac	100.2±8.24	97.7±6.68	102.4±7.71	99.2±11.42	ac
	Phosphorous	25.3±2.02	26.4±3.01	24.5±2.29	24.5±1.52	ac	35.1±2.01	35.0±2.21	36.7±2.49	32.1±3.53	ac

<sup>a</sup>Bagging treatment was done at 70 DAB.

<sup>b</sup>1-MCP treatment at 20 ppm was done immediately after harvest.

<sup>c</sup>Data values are means ±SE of six replicates.

a and c denotes not significantly different at p≤0.05 for bagging and 1-MCP treatments respectively while b denotes significantly different at p≤0.05 for bagging treatment.

Generally, the minerals content of the fruits were low, and this could have reflected lack of fertilizer application on the mango trees. Low calcium levels in both pulp and peel could have been the cause of faster softening of the fruits.

#### **4.2.3 Changes in fruit physiological parameters**

##### **4.2.3.1 Fruit weight loss**

Percentage fruit weight loss increased with days of storage in all the treatments (Fig. 25). Postharvest weight loss in mango fruit is attributed to physiological weight loss due to respiration, transpiration and other biological changes taking place in the fruit (Rathore et al., 2007). Rate of weight loss in both bagged and control fruits was not significantly different up to 6 DAH, but significantly higher ( $p < 0.05$ ) losses were observed in control fruits thereafter. Unbagged fruits had shriveled by 9 DAH (Plate 6) as opposed to 15 DAH for bagged fruits, indicating a higher water loss in control fruits. Cuticle covers fruits surface and one of its functions is to restrict transpiration water loss. Bagged and unbagged fruits may have had different cuticle structures (Amarante et al., 2002a) that could have lead to differences in transpiration water loss. Unbagged fruits with low natural gloss and irregular layer of lignified epidermal cells have higher skin permeability to gases (Amarante and Banks, 2002). However, no difference in skin permeability to water, and therefore weight loss, was observed between bagged and unbagged pears during four weeks of cold storage (Amarante et al., 2002a).

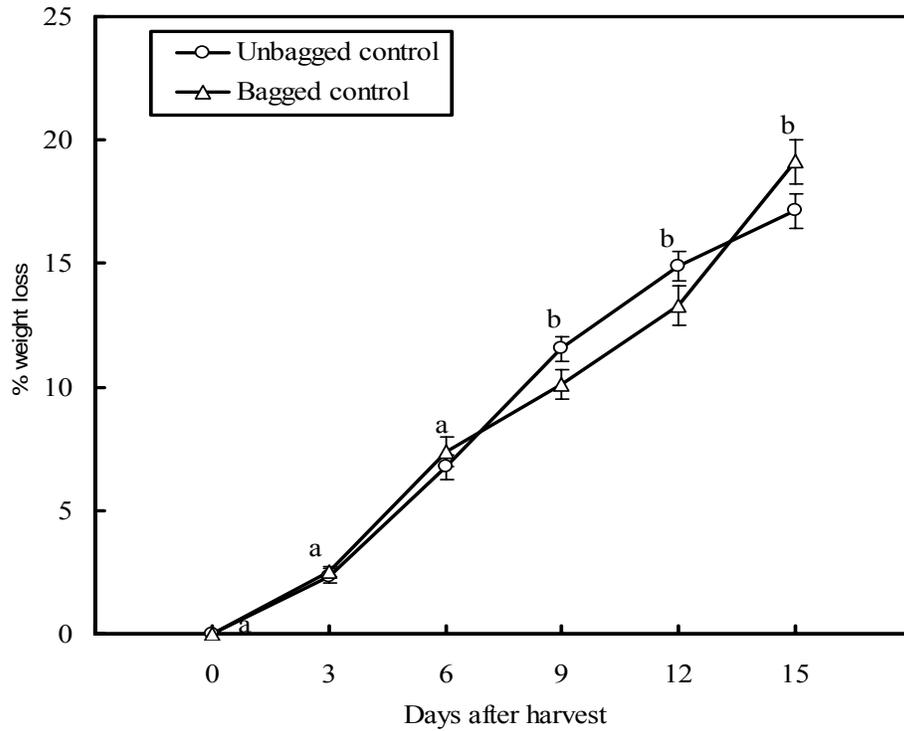


Fig. 25: Changes in percentage weight loss in bagged and unbagged mango fruits during postharvest storage at  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ . The vertical bars represent SE of the mean of 9 replicates. When absent, the SE fall within the dimensions of the symbol. 'a' denotes a period of no significant difference while 'b' is a period of significant difference between bagged and control fruits.



(A)



(B)

Plate 6: Visible effects of transpiration water loss on bagged (A) and unbagged (B) mango fruits at 9 DAH.

#### **4.2.3.2 Fruit respiration and ethylene production rates**

Fruit respiration rate increased with storage time up to the climacteric peak at 12 DAH in all the treatments (Fig. 26 A) whereas ethylene production was detected 9 DAH only, and assumed to be the peak ethylene production (Fig. 26 B). The increase in respiration rate exhibited a true climacteric pattern. In a typical climacteric fruit, a low respiration rate is observed before the onset of ripening, and then a surge in respiratory activity (Azzolini et al., 2005). This peak may correspond, precede or proceed optimum eating ripeness (Tucker, 1993). Optimum eating ripeness preceded respiratory climacteric and peak ethylene production in Apple mango fruit in this study. This indicates that once started, ripening associated changes in Apple mango may require very low ethylene levels that were not detectable in this study. Small amount of ethylene present in mango fruit at harvest is sufficient to initiate ripening (Mitra and Baldwin, 1997). Ripening mango fruit has a low level of climacteric peak (3  $\mu\text{l/l}$ ) of internal ethylene (Tucker, 1993). Bender et al. (1995) reported maximum levels of about 800 nL  $\text{C}_2\text{H}_4$  in ripe Tommy Atkins mango fruit stored in air at 20°C. Tommy Atkins mango fruit had ethylene detected only once during storage, though respiratory peak preceded peak ethylene production (Madigu, 2007). Peak ethylene production in mango fruit can occur before or after the climacteric peak (Mitra and Baldwin, 1997). This could be due to varietal differences.

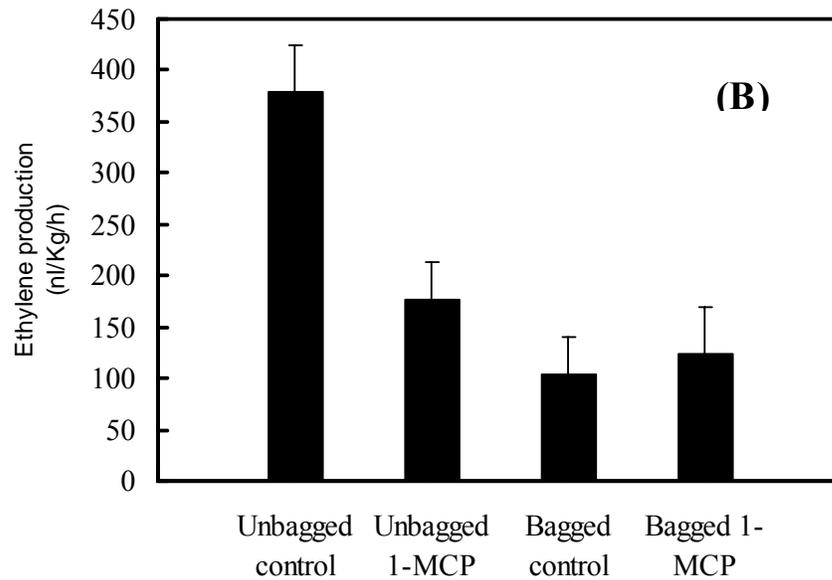
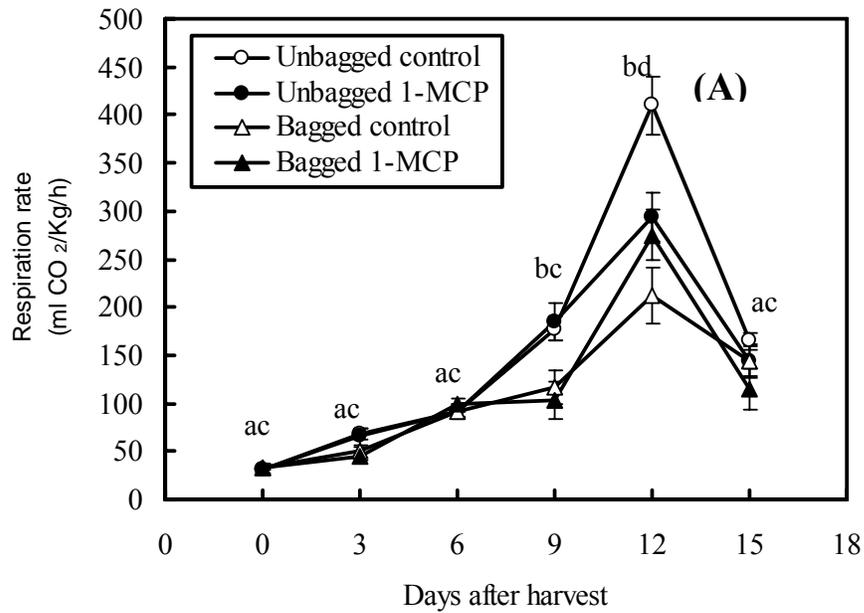


Fig. 26: Respiration rate (A) and peak ethylene production (B) of both bagged and unbagged mango fruits, each with and without with 1-MCP treatment during postharvest storage at  $25 \pm 0.5^\circ\text{C}$ , RH  $60 \pm 5\%$ . The vertical bars represent SE of the mean of six replicates. When absent, the SE fall within the dimensions of the symbol. 'a' and 'c' denotes a period of no significant difference for bagging and 1-MCP treatments respectively. 'b' and 'd' denotes a period of significant difference for bagging and 1-MCP treatments respectively.

The patterns of respiration and ethylene production, and consequently ripening behaviour of mango fruit vary among varieties, climatic conditions and harvesting date (Mitra and Baldwin, 1997). Late harvested mango fruits do not have a pre-climacteric phase (Mitra and Baldwin, 1997). This may have occurred in this study as the fruits were harvested at 168 DAB when ripening processes like loss of firmness, yellowing of the pulp and starch degradation had already commenced. Late harvested fruits have higher numbers (Trewavas, 1982) and more sensitive (Bron and Jacomino, 2006) ethylene receptors. It seemed that a basal level of ethylene and respiratory activity was sufficient to stimulate biochemical changes associated with mango fruit ripening.

Bagging had a significant effect on fruit respiration rate only at 9 and 12 (respiratory peak) DAH. This could have been due to more stress as a consequence of higher transpiration water loss during the period. Increase in respiration rates during ripening of climacteric fruits is considered to be a homeostatic response of the mitochondria caused by detrimental physical and chemical changes during ripening (Romani, 1984). Bagged mango fruit had reduced respiration rate probably due to lower O<sub>2</sub> and higher CO<sub>2</sub> internal partial pressure as reported in bagged pears (Amarante et al., 2002a), although, in their case respiration rate was not affected.

Treatment with 1-MCP had no significant ( $p>0.05$ ) effect on respiration and ethylene production rates, though control unbagged fruits had higher

respiration and ethylene peaks of 409.82 ml CO<sub>2</sub>/Kg/h and 378.62 nl/Kg/h, respectively. However, delay of on-set time and rates of respiration and ethylene production by 1-MCP has been reported for most of climacteric fruits. These include; bananas (Lohani et al., 2004), avocado (HersHKovitz et al., 2005), tomatoes (Mostofi et al., 2003), strawberries (Bower et al., 2002), guava (Azzolini et al., 2005) and apples (DeEll et al., 2001). Limited effect of 1-MCP on physico-chemical changes during ripening may have been due to late harvesting when the ripening process had already commenced. Yellowing of the fruit pulp and breakdown of starch and chlorophyll of Apple mango in this study had been initiated by the time of harvest, 168 DAB. Late harvested 'Kent' mango fruit was too ripe to respond to the 1-MCP treatment (Plotto et al., 2003). A single dose of 1-MCP treatment of peach fruit had little effect on ethylene biosynthesis, but multiple treatment delayed significantly the induction of ethylene biosynthesis (Mathooko et al., 2001). Limited fruit response to single treatment with 1-MCP with respect to ethylene biosynthesis could have been due to ethylene receptor sites generated within a short time (Mathooko et al., 2001). Treatment of strawberry fruits with 1.0 µL 1-MCP had little effect on fruits stored in air, though it protected the fruits from effect of external ethylene (Bower et al., 2002). The decrease in respiration rates during post-climacteric represents the loss of homeostatic ability of mitochondria, with the predominance of senescence during this period (Azzolini et al., 2005).

#### **4.2.4 Fruit sensory quality on ripening**

Bagged and unbagged fruits were fully ripe (peel completely turned yellow and pulp attained eating softness) at 7 DAH (Plate 7). Bagged fruits were rated superior in terms of general appearance, pulp and peel colour, and overall acceptance (Fig. 27). This could be attributed to bagged fruits being brighter as indicated by higher L values and lack of blemishes. The differences in aroma, taste and texture between bagged and unbagged fruits were not significant ( $p>0.05$ ) though bagged fruits had higher scores. Thus, there was no significant difference ( $p>0.05$ ) between bagged and unbagged fruits in terms of eating quality. It is the sugars/acids ratio that determines mango fruit eating quality (Rathore et al., 2007). Indeed, there were no significant differences ( $p>0.05$ ) in TSS and TTA contents between bagged and unbagged fruits during storage. Total soluble solids and total titratable acidity, and hence eating quality of Keitt mango fruit was also not affected by bagging (Hofman, 1997). Improved overall acceptance of bagged fruits could, therefore, be attributed to better visual appearance and not eating quality.



**(A)**



**(B)**



**(C)**



**(D)**

Plate 7: Unbagged (A) and bagged (B) peel, and unbagged (C) and bagged (D) pulp appearance of mango fruit on ripening, 7 DAH (Storage:  $25\pm 1^{\circ}\text{C}$ , RH  $60\pm 5\%$ ).

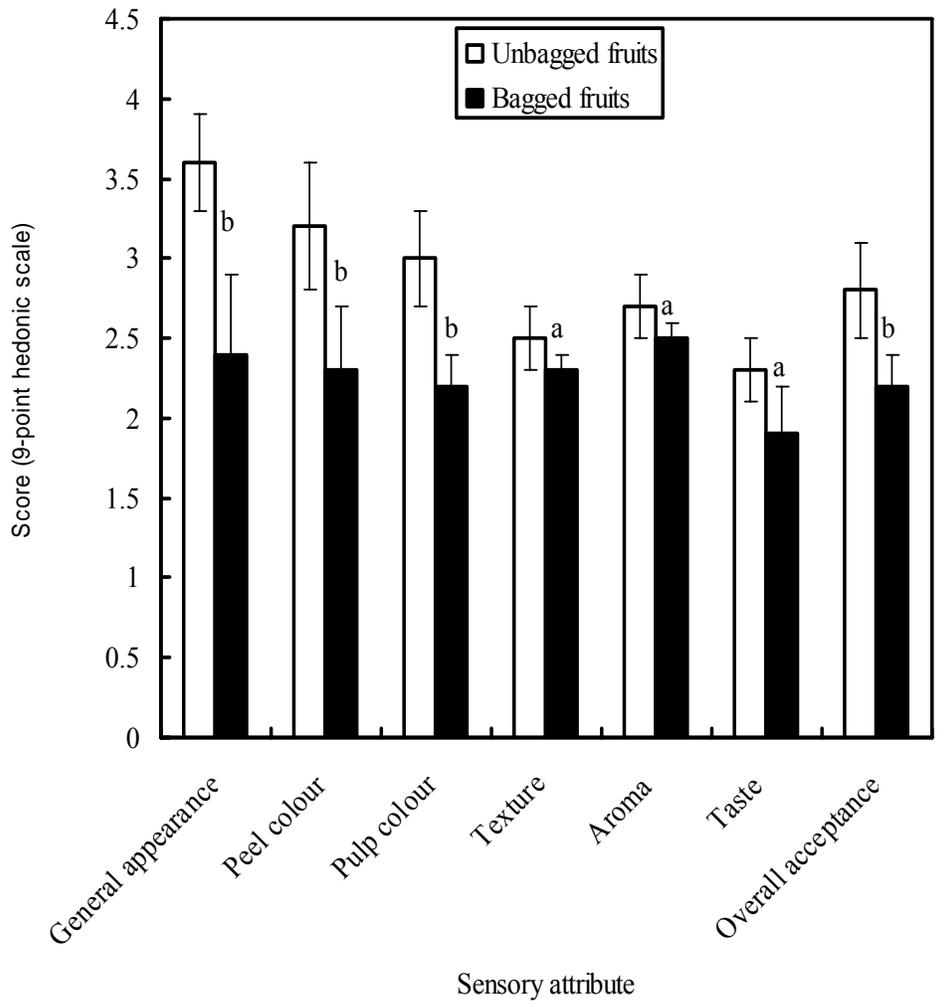


Fig. 27: Sensory quality scores of bagged and unbagged mango fruits on full ripening, seven DAH. Rating was done on a 9-point hedonic scale (1-like extremely and 9-dislike extremely). The vertical bars represent SE of the mean of 15 replicates. ‘a’ denotes a period of no significant difference while ‘b’ is a period of significant difference between bagged and control fruits.

## CHAPTER FIVE

### 5.0 CONCLUSION AND RECOMMENDATIONS

The main purpose of fruit bagging is to prevent mechanical damage and, physiological and pathological disorders. This study showed that bagging controlled development of 'rust' lesions on Apple mango fruit peel, and also improved the postharvest shelflife by six days. In addition, bagging did not affect the eating quality of the fruit, but improved visual appearance and acceptance. Therefore, preharvest bagging can be applied for production of high quality Apple mango fruits for export market. Postharvest 1-MCP (20 ppm) treatment did not retard ripening of Apple mango fruit harvested at 168 DAB. In addition, its effect on changes in fruit quality parameters during ripening was minimal. Cold storage of mango fruit is highly limited by its susceptibility to chilling injury, and failure by 1-MCP to slow Apple mango ripening presents another big setback in the attempt of improving its postharvest shelflife. However, retarded loss of firmness by 1-MCP treatment, though for a short duration, could be useful in minimizing mechanical damage during loading and shipping.

Preharvest bagging is however laborious, though safe and easy to apply. The viability of its application will depend on farm labour, which is cheap and available in Kenya. However, its economic viability in Kenya should be ascertained by investigations on cost-benefit analysis and practicability. Stakeholders in fruits industry should link with paper manufacturing industry so as to come up with suitable bagging materials and user friendly bags for

various fruits. Moreover, impact of preharvest bagging on fruit quality may be affected by duration of bagging and climatic conditions. More studies should be done on these two factors so as to find out the optimum bagging period for maximum results. Investigations on light penetrating bagging materials or unbagging the bagged fruits prior to harvest should be done as an attempt to mitigating the negative impact of bagging on the characteristic fruit peel colouration. The obvious benefits that can be accrued from successful use of 1-MCP on mango fruit warrants further investigations on factors such as maturity stage and mode of application as these may have an effect on fruit response. Therefore, investigations on the effect of different maturity stages without significantly compromising on fruit eating quality) and intermittent 1-MCP application during postharvest period on mango fruit response should be done.

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## APPENDICES

Appendix I: Tomatoes untreated (A) and treated (B) with 1-MCP (20 ppm) 7 days after harvest (DAH)



**A**

**B**

Appendix II: Sensory evaluation form

Sheet No..... Date: .....

Panelist name:..... Phone number.....

Test the provided two samples either by tasting or visually, and using the 9-point hedonic scale provided, indicate the score of the stated attribute.

9-point hedonic scale

- |                             |                       |
|-----------------------------|-----------------------|
| 1. Like extremely           | 6. Dislike slightly   |
| 2. Like very much           | 7. Dislike moderately |
| 3. Like moderately          | 8. Dislike very much  |
| 4. Like slightly            | 9. Dislike extremely  |
| 5. Neither like nor dislike |                       |

	<u>Sample code</u>	
	<u>459</u>	<u>667</u>
<b><u>At harvest</u></b>		
1. Peel appearance	_____	_____
2. Peel colour	_____	_____
3. Overall acceptance	_____	_____
<b><u>On ripening</u></b>		
1. General appearance	_____	_____
2. Peel colour	_____	_____
3. Pulp colour	_____	_____
4. Texture	_____	_____
5. Aroma	_____	_____
6. Taste	_____	_____
7. Overall acceptance	_____	_____

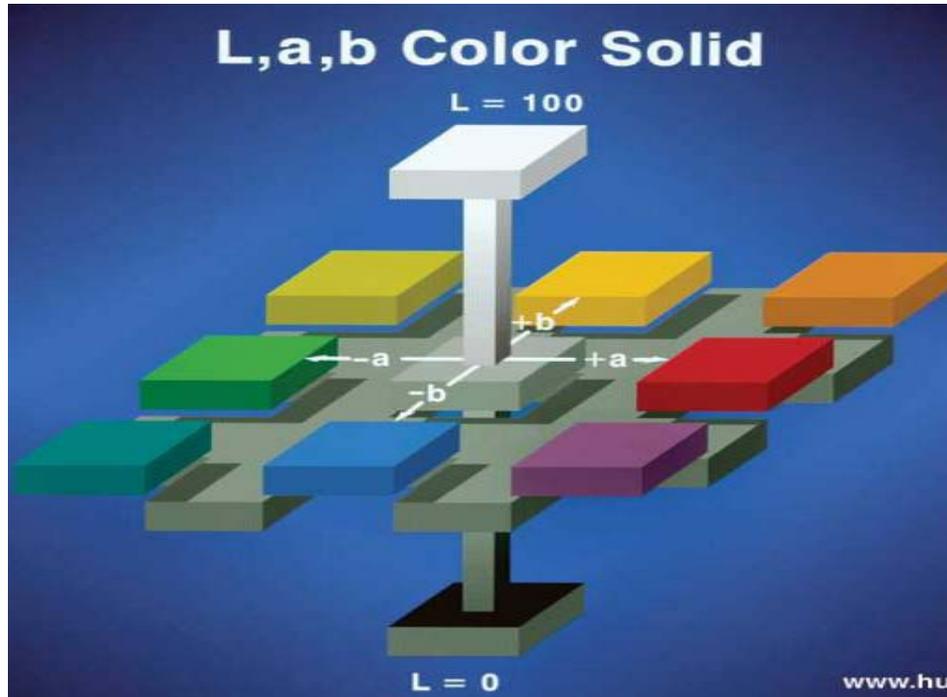
Comments:

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Appendix III: The Hunter's CIE (Commission Internationale de l'Eclairage)  
L\* a\* b\* system colour space.



The Hunter  $L^*$  (whiteness/darkness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness) CIE (Commission Internationale de l'Eclairage) system colour space. Hue describes a visual sensation according to which an area appears to be similar to one or proportions of two of the perceived colours, red, yellow, green and blue. The hue angle is thus the actual colour. An angle of  $0^\circ$  = red-purple hue,  $90^\circ$  =yellow hue,  $180^\circ$ = bluish green,  $270^\circ$ =blue. Total colour change measures the changes in the three colour components: Lightness  $L^*$ , red-green  $a^*$  and yellow-blue  $b^*$ . On a horizontal axis, positive ' $a^*$ ' indicates a hue of red-purple, a negative ' $a^*$ ' indicates bluish green, a positive ' $b^*$ ' indicates yellow and negative ' $b^*$ ' indicates blue.